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Manipulating field margins to increase predation intensity in fields of winter wheat (*Triticum aestivum*)

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arthropod predation, artificial caterpillars, cereal aphids, conservation biological control, flower strips, sentinel prey

Abstract
The effectiveness of natural enemies to control pests can be enhanced through habitat manipulation. However, due to the differences in their ecology, generalist and specialist species may respond differently to the same manipulation. Moreover, interactions among natural enemies (i.e. cannibalism, intraguild predation, hyperparasitism) may complicate the assumption that a higher density of natural enemies would increase the level of biological control. We investigated the natural enemy guild composition and the predation rate along flower vs. grass margins at the edge of winter wheat (*Triticum aestivum*) fields in Denmark. Natural enemies were sampled by pitfall trapping and by suction sampling; predation intensity was measured using two different sentinel prey methods: artificial caterpillars made of plasticine, and sentinel aphid colonies. Specialist and generalist species responded differently to the two margin types: specialists (mostly parasitic wasps) were attracted by the flower margins, while generalists (ground beetles, rove beetles and spiders) were more active in grass margins. The number of artificial caterpillars attacked was significantly greater in grass margins (mean = 48.9%, SD = 24.3) than in flower margins (mean = 30.7%, SD = 17.4). We found a significant positive relationship between the number of artificial caterpillars attacked by chewing insects, and activity density for large (≥15 mm) ground beetles. Predation of sentinel aphids in wheat fields did not vary significantly in relation to margin type. Our results suggest that flowering margins may be beneficial for canopy-active specialist natural enemies, but grassy margins are more useful for ground-active generalist predators.

Introduction
Conservation biological control (CBC) intends to restore and sustain the activity of natural enemies that control crop pests (Eilenberg et al. 2001). Natural enemy communities can significantly decrease pest abundance (Symondson et al. 2002), especially in wheat monocultures that are mainly infested by indigenous herbivorous pests (Pedigo and Buntin 1993). Such natural enemy communities include generalist and specialist species that differ in their range of prey/hosts (Welch et al. 2012). Despite their different performance as biological control agents, both have important roles in controlling pest populations (Southwood and Comins 1976; Symondson et al. 2002; Welch et al. 2012). In winter wheat fields, parasitic wasps (Hymenoptera: Braconidae and Ichneumonidae) are effective natural enemies of aphids (Schmidt et al. 2003), and in this article, we label them ‘specialists’, while spiders (Araneae), carabids (Carabidae) and rove beetles (Staphylinidae) were considered generalist predators because they regularly prey on other organisms as well as aphids (Symondson et al. 2002; Welch et al. 2012).
Conservation biological control strategies often involve the manipulation of non-crop habitats surrounding fields to protect, enhance or recreate the conditions necessary for the persistence and activity of natural enemies (Barbosa 1998; Landis et al. 2000; Eilenberg et al. 2001). Field margins are areas of uncropped land at the edges of crops adjacent to their boundaries, and are sometimes specifically managed to make them flower-rich (Landis et al. 2005). They favour the establishment and survival of autochthonous beneficial arthropods by providing shelter with appropriate microclimatic conditions (Landis et al. 2000; Griffiths et al. 2008), or additional resources such as alternative prey, pollen and nectar (Marshall and Moonen 2002; Landis et al. 2005; Griffiths et al. 2008). The presence of flowers adjacent to crops can promote a higher level of pest control compared to grass (Blåauw and Isaacs 2012; Balzan and Moonen 2014). This is usually attributed to the presence of floral resources (pollen, nectar and sometimes extra-floral nectar) that may be necessary during particular life stages of natural enemies such as hoverflies, lacewings, ladybirds and some spiders (Wäckers et al. 2008; Lu et al. 2014).

As a consequence of supporting more natural enemies (Dennis and Fry 1992), manipulated field margins are expected to improve biological control of pests in the adjacent fields. However, studies examining the effect of habitat manipulation on the level of biological control often lack a quantitative assessment of predation intensity in the field (Jervis and Kidd 1996; Howe et al. 2009), limiting the investigation to the natural enemy community (e.g. predator:prey ratio) rather than quantifying their positive function with respect to pest control (Pedigo and Buntin 1993; Griffiths et al. 2008). Interactions among natural enemies (i.e. cannibalism, intraguild predation, hyperparasitism) may complicate the basic assumption that more predators will result in a higher level of biological control (Letourneau et al. 2009). Sentinel prey methods are promising approaches to measure the in-field predation rate of natural enemies. To date, only a few studies have investigated the impact of habitat manipulation or naturally occurring non-crop habitats around the crop fields on the natural regulatory activity of beneficial arthropods. These often use sentinel prey, such as egg clutches (Thomson and Hoffmann 2010; Balmer et al. 2013), aphids (Griffiths et al. 2008; Holland et al. 2008; Rusch et al. 2013) or artificial caterpillars (Howe et al. 2015) to estimate efficacy.

The aim of this study was to characterize assemblages of natural enemies to obtain quantitative estimates of their effect in cultivated fields adjacent to different field margin types (flower or grass). We hypothesized that (H1) a higher abundance of alternative food subsidies in sown flower margins would support a higher abundance of natural enemies in those margins than in grass margins. We further hypothesized that (H2) immigration of natural enemies from the flower-rich margins would be greater than that from grass margins resulting in larger populations of natural enemies in the crop in the former compared with the latter. Finally, we tested the hypothesis that (H3) predation pressure would be greater in the areas of the crop close to flower margins than in areas close to grass margins.

**Materials and methods**

**Study site and field experimental design**

The study was carried out at the Flakkebjerg Research Station Experimental Farm (Aarhus University), in the Vestsjaelland Region of Denmark. In the autumn of 2013, a seed mixture composed of an equal proportion of two cruciferous plants (*Brassica rapa* var. *rapa* and *Raphanus sativus* var. *oleiformis*) was sown along a 50 m long, 2.5 m wide strip at the edges of five winter wheat fields (mean = 6.7 ha, SD = 3.3 ha) (Figure S1). We chose these species as previous studies showed a differential in flowering time (*B. rapa* flowering early, and *R. sativus* flowering relatively later, S. Cook, unpublished data), and they attract a range of natural enemies, including aphid parasitoids (Cook et al. 2013), while they support no herbivorous pests of wheat. Grass margins were selected on the opposite side of the same fields, away from other flowering areas, and were regularly cut, to ensure no flower resources were present. During the 2014 season, the flower margins, grass margins and the crop strips were sampled to compare the effect of the margin type on predation rates/intensity and on the composition of natural enemy communities. In one field, only the grass margin was sampled due to the failure of the flower margin to establish.

**Arthropod sampling methods to determine natural enemy community composition**

The composition of the arthropod natural enemy community was evaluated using two complementary sampling methods. Epigeal predators were sampled using four pairs of pitfall traps (500 ml volume and 10 cm diameter, filled with 100 ml ethylene glycol 70%) per field; two pairs 5 m apart in the crop strip.
sampled adjacent to the grass margin, and two pairs 5 m apart in the crop sampled adjacent to the flower margin. The trap pairs were placed 12.5 m from the field edge, at the tips of a plastic fence arranged in a zig-zag pattern (one side 30 cm long, 20 cm tall, dug into the soil 10 cm deep; one part of the fence was open in a V-shape towards the crop centre, and the other towards the field margin, see Figure S1b). Barrier pitfall traps in general are more effective sampling devices than single pitfalls (Hansen and New 2005), but our arrangement had an additional advantage: one of the pitfalls caught mostly arthropods moving into the crop from the margin (immigrating), while the other mainly caught arthropods presumably leaving the crop. Each of the five fields therefore had eight traps in total except for the field in which the flower margin failed (36 traps per sample in total). To reduce the bycatch, each trap was covered by a square of galvanized iron (10 × 10 cm), supported by pegs. The traps were open for three periods of 7 days each, separated by 1 week (21–28 May, 3–10 June, 18–25 June and 2–9 July 2014). A total of 144 samples were collected using this method.

Arthropods active in the crop and margin vegetation were collected using a vacuum suction sampler constructed from a modified portable leaf blower (Husqvarna® 125BVx). The vacuum tube was 85 cm long and 12.5 cm diameter. Suction samples were collected by walking along a 15 m transect within each margin and the crop strips (Figure S1b). Every 3 m, the vacuum tube was placed onto the soil surface and kept there for 10 s before continuing the walk. After finishing one transect, the collected material (i.e. from five positions) was transferred into a large plastic bag, placed in a cooler box and transferred to the laboratory. Samples were stored at −20°C before sorting. Suction sampling was carried out fortnightly on 3 June, 16 June and 1 July 2014, resulting in a total of 54 samples (crop area associated with the failed margin was not sampled). All collected arthropods were identified to order or, in the case of beetles (Coleoptera), to family using the identification keys of Choate (1999) and Unwin (1981). We considered predatory beetles, spiders and harvestmen generalist natural enemies of aphids, while parasitoids, syrphid larvae, lacewings and coccinellids were categorized as aphid specialists.

Quantifying predation intensity

Predation pressure was quantified using two kinds of sentinel prey: populations of the grain aphid (Sitobion avenae) acting as live sentinel prey and artificial caterpillars made of green plasticine (Howe et al. 2009; Ferrante et al. 2014). Live aphid prey patches were established in three different types of exclusion cage per margin: open, partially closed and totally closed. They were placed in a random sequence in the crop strips, 5 m from each other. Cages were cylindrical (31.5 cm diameter, 50 cm height) with a solid plastic frame and a mesh cover. The open cage consisted of a completely uncovered frame, with no mesh, allowing access to the sentinel prey by all natural enemies. The partially closed cage was covered by a plastic mesh 2 × 2 cm size, left uncovered at the bottom, which excluded large potential natural enemies such as rodents or birds. The total exclusion cage was fully covered with muslin mesh (<1 mm) designed to exclude all natural enemies and was therefore used as the control. To ensure that no natural enemies could enter the total exclusion cage, two muslin mesh covers were used. The ‘top cover’ was glued to the frame of the cage, and a ‘bottom cover’ mesh was placed on the ground and brought up to overlap the top cover. We considered a set of three cages as one replicate. Each cage contained a pot with ca. 20 greenhouse-grown winter wheat plants, 10 cm tall, infested with ten grain aphids of mixed age (nymphs and adults). Aphids were transferred onto a single wheat leaf using a paintbrush (while in the greenhouse). The pots were transferred to the field and dug into the soil so that ground-active organisms had level access to the plants (except for the total exclusion cages, where the pot was put on the mesh at the base). The fate of these aphid colonies was followed during the flowering period of the flower margin, with non-destructive counting of living aphids twice each week. If a population of aphids did not establish (after being transferred to the field) in the totally or partially closed cage, a new replicate was performed; that is, the set of the three different cages was re-installed and restarted for each margin on that field. In total, 25 such replicates were run in the five experimental wheat fields from 7 June to 7 July 2014.

Artificial sentinel prey consisted of light green plasticine (Smeedi plus, V. nr. 776609, Denmark) ‘caterpillars’ 15 mm long and 3 mm thick (Howe et al. 2009). This method allows the identification of up to 14 different types of predators (Low et al. 2014; Lövei and Ferrante, 2017). Each caterpillar was glued onto a small piece of reed or bamboo, to be handled without touching the plasticine. We placed 15 caterpillars on the ground along the margins and also along the crop strips (Figure S1b; i.e. a total of 60 caterpillars per field per sampling event), and these were observed after 24 h for signs of predation attempts. Artificial
caterpillar experiments were run weekly (26 May, 4 June, 12 June, 18 June, 25 June, 3 July 2014), covering the period when the pitfall traps were active.

Statistical analysis

Community composition of natural enemies
We tested for differences in natural enemy abundance between flower margins and grass margins using Student’s paired $t$-tests. All data were normalized using a log$_{10}(x + 1)$ transformation. For each sampling method, taxonomic orders representing $<1\%$ of the total catch (Diptera, Neuroptera, Chilopoda, Coccinellidae and Cantharidae, see Tables S1, S2) were excluded from the analysis. The remaining natural enemies collected were divided into two guilds: generalists (Coleoptera, Araneae, Opiliones) and specialists (Hymenoptera).

Sentinel aphids
Of the total of 25 aphid cage replicates, four (two for each of the two margin types) were excluded from the analysis because the control population (aphids in the total exclusion cage) also went extinct. As the replicates were run until the population of aphids went extinct in both open and partially closed cages (or one being extinct and the other one reaching a very low number of 1 or 2 aphids), the length of the observation periods varied. Therefore, when necessary, we interpolated the number of aphids after 2 and 5 days from the start of each replicate by connecting data points with a straight line, and reading the resulting value. To determine how the provision of the biological control service was affected by the margin treatments (flower or grass margins), the degree of aphid suppression was calculated in each margin by expressing the change in aphid numbers in open and partial exclusion cages as a proportion of aphid abundance compared to numbers reached in the absence of predators (numbers in the total exclusion cages). The resulting Biocontrol Service Index (BSI, Gardiner et al. 2009) is defined as

$$BSI = \frac{(A_C - A_o)}{A_C}$$

where $A_C$ is the number of aphids on the caged plant (total exclusion cage) after 2 or 5 days from inoculation, and $A_o$ is the number of aphids on the open plant (open or partial exclusion cage) on day 2 or day 5. Values of BSI can range from 0 to 1, with values increasing as the level of aphid predation increases. As suggested by Gardiner et al. (2009), cases with negative BSI values indicate a lack of effective biocontrol.

To assess the influence of the nature of the margin on the biological control service, BSI values were compared between margin types and among cages within the same margin of the same field, using the Wilcoxon signed-ranks test ($W$). The absence of aphid predation was compared using the odds ratio (Rita and Komonen 2008). The lifetime of the aphid colonies, represented by the number of days before the colony went extinct, was analysed using the Wilcoxon–Mann–Whitney U-test.

Artificial caterpillars
Predation rate was analysed as a response in a linear mixed model including three fixed factors, type of margin (grass vs. flower), position in the field (crop vs. margin), phenological period (before vs. after flowering of the margin (flowering: 26 May, 4 and 12 June vs. after flowering: 18, 25 June, 3 July)), and all their interactions; field was used as a random factor. As the random factor explained $<1\%$ variance, it was excluded and the model was simplified to a linear model including the aforementioned factors and their interactions. The best model was decided using backward selection and by comparing Akaike Information Criteria and included the three fixed factors and the interaction between margin and phenological period, and margin and position. Model residuals were checked to verify that the parametric assumptions of normality and homoscedasticity were satisfied. Model validation was carried out graphically. Missing caterpillars were considered lost and were excluded from the analyses. Finally, we tested the relationship between predation rate on artificial caterpillars by chewing insects and the activity density of carabids $\geq 15$ mm in length, using a simple linear regression. Ground beetles $<15$ mm were removed from the analyses, as it was assumed that they would not attack prey larger than themselves. Artificial caterpillars were always set up while pitfall traps were active, but as the two sampling methods had a different number of runs (six and four, respectively), we used the mean predation rate percentage in two cases. As we only had data for the grass margin in one field, this field was not considered in the analysis. Ground beetle activity density was log-transformed to meet the parametric assumptions. All statistical analyses were performed using the statistical program R, version 3.1.1 (R Core Team, 2014).

Results

Arthropod community composition
A total of 9640 (Table S1) and 1875 (Table S2) natural enemies were collected by pitfall trapping and by suction sampling, respectively. Generalist natural
enemies of aphids represented the majority of the catch from pitfall traps, with beetles (Coleoptera: 66.3%) and spiders (Araneae: 23.6%) being the most numerous, while specialist natural enemies of aphids (Hymenoptera) represented only 9.2% of the arthropods in pitfall traps. Hymenoptera represented the majority of the catch (62.5%) from vacuum suction sampling, in which the generalist natural enemies represented 37.0%, including Coleoptera (20.8%), Araneae (13.7%) and Opiliones (2.2%). Predatory Diptera collected (by both methods) were robber flies (Asilidae), and predatory Neuroptera were lacewings (Chrysopidae). Robber flies, lacewings and centipedes (Chilopoda) represented <1% of the catch.

The abundance of ground beetles and spiders collected by pitfall traps was not correlated (Pearson’s r = −0.07). However, spider abundance was negatively related to ground beetle abundance except on the first sampling occasion (fig. 1), although it was never statistically significant. This is because under low carabid abundance, spider abundance varied greatly. It is noticeable, though, that high carabid abundance was usually accompanied with low spider abundance, and high spider abundance was only found when carabid abundance was low (the slope of the upper envelope of fig. 1 is steeply negative).

Effect of flower vs. grass margins on the abundance of natural enemies

Overall, the flower margins supported a significantly greater abundance of specialist natural enemies (collected by vacuum suction sampling) compared to the grass margins (Student’s t-test, t = 2.42, d.f. = 11, P = 0.03; fig. 2). Grass margins supported a significantly greater abundance of generalist natural enemies (measured by pitfall trap catches) compared to

![Graph](image.png)

**Fig. 1** Relationship between ground beetle and spider abundance (no. individuals per trap) in winter wheat crops with grass and flower margins during the 2014 field season in Flakkebjerg, Denmark.
the flower margins over the whole season (Student’s t-test, \( t = -4.33 \), d.f. = 11, \( P = 0.001 \)); this difference mainly driven by significantly greater numbers of generalists found in grass than flower margins in mid-June (Student’s t-test, \( t = 7.37 \), d.f. = 3, \( P = 0.005 \); fig. 2). This difference between margin types did not translate into a significant difference in the abundance of natural enemies between the crop strips of the fields for either generalists (Student’s t-test, \( t = -1.10 \), d.f. = 11, \( P = 0.30 \)) or specialists (Student’s t-test, \( t = -0.41 \), d.f. = 11, \( P = 0.69 \)). The activity density of generalist and specialist natural enemies in the pitfall traps in the crop was also not influenced by the type of margin (Student’s t-test, \( t = -0.02 \), d.f. = 61, \( P = 0.99 \) and \( t = 0.08 \), d.f. = 61, \( P = 0.94 \), respectively) except on two occasions. More generalists were found in pitfall traps in late May in the crops near flower margins compared with those near grass margins (Student’s t-test, \( t = 2.02 \), d.f. = 15, \( P = 0.06 \)), while in July, the opposite was found (Student’s t-test, \( t = -3.25 \), d.f. = 13, \( P = 0.006 \); fig. 3). Specialists caught by pitfall traps were low in abundance and did not differ according to the direction of pitfall traps (fig. 3).

**Influence of margin type on the distribution of natural enemies**

The data from the suction sampling indicated that in both types of margins, the abundance of specialist natural enemies was significantly greater in the margin than the crop throughout the whole season (Student’s t-test, \( t = 4.38 \), d.f. = 26, \( P = 0.0002 \); fig. 2). The abundance of the generalist predators did not significantly differ between crop and margin for either margin type (Student’s t-test, \( t = 0.24 \), d.f. = 26, \( P = 0.81 \); fig. 2). Generalists tended to be more abundant in the crop associated with flower margins (significant only in July, Student’s t-test, \( t = 3.85 \), d.f. = 3, \( P = 0.03 \)) and in the grass margins after mid-June (fig. 2). Directional pitfall traps showed no significant immigration or emigration of natural enemies except in late May, with significantly more individuals caught in the emigration than immigration traps near the flower margin (Student’s t-test, \( t = 3.25 \), d.f. = 7, \( P = 0.01 \); fig. 3). There was also a marginally significant sink effect of the grass margin on generalist predators at the beginning of July (Student’s t-test, \( t = 2.09 \), d.f. = 9, \( P = 0.07 \); fig. 3).

![Fig. 2](image-url) Abundance (mean ± SE) of generalist and specialist natural enemies of aphids collected by vacuum suction sampling in flower margins, grass margins and the adjacent wheat crop, during the 2014 field season in Flakkebjerg, Denmark.
Influence of field margin composition on aphid predation intensity

The average lifetime of the aphid colonies was significantly longer in the grass margin treatment (9.9 days) compared with the flower margin treatment (5.8 days) (Wilcoxon–Mann–Whitney test, U = 75, P = 0.017), but there were no differences between the open vs. partial exclusion cages (flower margins: Wilcoxon–Mann–Whitney test, U = 5.5, P = 0.224, grass margins: W = 9, P = 0.815). The value of the BSI significantly increased between day 2 and day 5 (Table S3, Wilcoxon signed-rank test, W = 129, P = 0.001). The type of margin did not influence the provision of the biological control service, with no difference in the BSI values when the same type of exclusion cages were compared adjacent to the grass vs. flower margins, either after 2 days (Table S3, Wilcoxon signed-rank test, W = 59, n = 14, P = 0.706), or after 5 days (Wilcoxon signed-rank test, W = 53, n = 14, P = 0.625). Open and partially closed cages showed a similar BSI after 2 days (grass margin treatment, Wilcoxon signed-rank test, W = 19, P = 0.093, flower margin treatment, W = 16, P = 0.834) and after 5 days (grass margin treatment W = 47, P = 0.23; flower margin treatment, W = 13, P = 1.0).

In the flower margin treatment, the ratio of cages that showed no biocontrol effect (BSI = 0) after 2 days was 17%, and after 5 days, this increased to 28%. In the grass margin treatment, the opposite was found: an absence of aphid biocontrol effect was recorded in 33% of the cages after 2 days and only in 25% after 5 days. The absence of aphid biocontrol effect was not significantly different by margin treatment (odds ratio after 2 days = 2.00, CI95% = 0.46–8.62, P = 0.35 and odds ratio after 5 days = 0.90, CI95% = 0.24–3.42, P = 0.88).

Influence of field margin composition on predation on artificial caterpillars

Forty-six per cent (n = 756/1636) of the artificial sentinel prey were attacked after 24 h, mostly by chewing insects (88%, n = 665/756 of the bites), followed by small mammals (13.2%), and birds (1.3%). Fourteen caterpillars (0.79%) were lost. Predation rate by chewing insects was higher in grass than in flower margins (48.9%, SD = 24.3, n = 30 vs. 30.7%, SD = 17.4, n = 25, respectively) and was also higher in the margins than within the crop (45.3%, SD = 27.3, n = 30 vs. 35.9% SD = 19.3, n = 30, respectively). In the flower margin treatment,
predation was similar between the crop and the margin (30.9%, SD = 18.1, n = 25 vs. 30.6%, SD = 23.2, n = 25, respectively), while in the grass margin treatment, it was higher in the margins than in the crop (57.6% SD = 31.9, n = 30 vs. 40.1% SD = 24.5, n = 30, respectively). The multiple linear regression (adj. R² = 0.33) indicated that total predation was significantly higher in grass than in flower margins (Table 1) and was also significantly higher after flowering than during flowering (Table S1). Moreover, the interaction between flowering period and margin type was significant (Table S1) as predation in the grass margin was significantly lower during than after flowering (Table S1, fig. 4). We found a significant positive relationship (t = 5.616, P < 0.01, adj. R² = 0.50) between the activity density of large (≥15 mm) ground beetles and the attack rate on artificial caterpillars (fig. 5).

Discussion

Field margin manipulation is one of the most promising practices to enhance biological control in cultivated crops (Landis et al. 2000). However, the influence of the various groups within the natural enemy community on pest populations in adjacent crops still requires clarification (Pfiffner and Wyss 2004). In our study, grass and brassica-containing flower margins had different impacts depending on the natural enemy groups. Our hypothesis 1 (that a higher abundance of alternative food subsidies in sown flower margins would support higher numbers of natural enemies compared with grass margins) was only partially supported, as flower margins increased the abundance of specialist but not generalist natural enemies. Apparently, the structurally complex grass edge provided sufficient attraction to ground-active generalist natural enemies, to which flowers added little attraction. For ground-active predators, ground cover structure (possibly by influencing humidity) may be more important (Frank and Reichhart 2004; Woodcock et al. 2005) than other factors, at least in the first part of the growing season (note wheat is harvested in early July, that is the middle of the northern summer, when the activity period of the natural enemies is far from its end). The increase of specialists in flower margins did not translate to an increased abundance in the crop, so our second hypothesis (that immigration of natural enemies from the flower-rich margins would result in larger populations of natural enemies compared with crops adjacent to grass margins) predation was not supported, at least under Danish conditions. In a similar study, the abundance of aerial natural enemies (including specialists such as Aphidiinae) in winter wheat fields in the UK significantly increased in the presence of flower margins, while grass margins had only a small effect (Ramsden et al. 2015). In our study, generalist arthropods were more abundant in grass than in flower margins, and grass margins may represent a source of generalists migrating towards the crop. However, at the beginning of the experiment, generalist predator abundance was higher in flower margins than in grass

### Table 1

| Estimate  | Std. error | t value | P(>|t|) |
|-----------|------------|---------|--------|
| (Intercept) | 0.46202 | 0.05478 | 8.435 | <0.001 |
| Grass     | 0.30582 | 0.07371 | 4.149 | <0.001 |
| Crop      | -0.02320 | 0.06239 | -0.372 | 0.7107 |
| Flowering period | -0.14619 | 0.06244 | -2.341 | 0.0211 |
| Grass: flowering period | -0.17815 | 0.08451 | -2.108 | 0.0374 |

Fig. 4 Probability of predation on artificial caterpillars during the 2014 cropping season in Flakkebjerg, Denmark, estimated using a linear model. Circles and bars indicate fitted values and confidence intervals, respectively.
margins, suggesting that a positive effect may exist only until alternative optimal prey becomes available. Despite studies describing an increase in arthropod species diversity and abundance (Haaland et al. 2011), studies comparing flower margins with grass margins also indicate that carabids and spiders do not show a particular preference for any kind of margin (Meek et al. 2002; Pfiffner and Wyss 2004).

The positive BSI obtained using the sentinel aphid method suggests effective control of aphids in fields with either type of margin. The positive influence of the flower margin can be seen in the fate of aphid colonies: aphid survival was shorter adjacent to flower than grass margins. This response is in line with our third hypothesis, predicting a higher level of biocontrol near flower than grass margins. However, there were more generalist predators in grass than flower margins, and artificial prey were more attacked by generalists there, indicating higher predation pressure by generalists near grassy margins. These data do not support our third hypothesis. Predation on artificial caterpillars may reflect the activity of only a part of the natural enemy community, as not all will attack such potential prey (the size of the sentinel prey may discourage small predators (Lövei and Ferrante 2017), and parasitoids rarely attack such artificial prey (Howe et al. 2009)). Nonetheless, our results confirm the applicability of the method to European agroecosystems, which to date have been used only in tropical agroecosystems (Gray and Lewis 2014; Howe et al. 2015; Maas et al. 2015). An increase in predator abundance in crops neighbouring flower strips does not necessarily translate to increased biological control (Markó et al. 2012; Cox et al. 2014). The effectiveness of flower margins as a source of parasitoids for aphid control in adjacent crops may be strongly limited by the margin-crop distance. Distances over 2 m may already show almost no effect on the abundance of parasitoids (Bianchi and Wäckers 2008) or their parasitism (Tylianakis et al. 2004) in the crop. Other groups of natural enemies, such as hoverflies, show greater dispersal activity (Lövei et al. 1998) and can be linked to increased biocontrol (Hickman and Wratten 1996).

Predation impact did not differ between open (all predators had access to live sentinel aphids) and partial exclusion cages (only invertebrate natural enemies have access), indicating that invertebrate predators were mostly responsible for aphid control in winter wheat fields. The significant relationship between the abundance of large carabids and attack rates on the artificial caterpillars indicates that the natural enemy role of ground beetles can be important. The negative relationship between carabids and spiders may have been caused by different seasonal phenologies, but the more probable explanation is intraguild predation (Lang 2003; Schmidt et al. 2003). This also points to the complexity of the natural enemy impact, underlining that abundance measures only may not provide the true picture of such impact.

Conclusions
Flowering margins may benefit natural enemies, but our results suggest that they influence generalist and specialist natural enemies differently. As both may be important in pest regulation, agro-environments should be managed as a mosaic of different habitats, which may include flower margins and grassy strips, to provide abiotic and biotic resources to the whole natural enemy community. To date, predation rates in agroecosystems have been measured using artificial caterpillars only in tropical areas (Lövei and Ferrante 2017). Our results confirm the applicability of the method in European agroecosystems. The partial overlap between abundance patterns in the natural enemy community and the measures of their functionality suggests that both live and artificial sentinel prey are informative and ecological studies focusing on biological control should include both. To evaluate the impact of habitat manipulation on biological control, we recommend measuring predation intensity together with traditional investigations of natural enemy densities and community composition.
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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (a) Schematic representation of the field experiment design; the shaded box represents a grass margin; the stippled box represents a flower margin, white areas represent areas of crop sampled. (b) Spatial locations and types of sampling in crop and field margins.

Table S1. Mean activity-density (no. of individuals trap−1 day−1 ± SE) collected by directional pitfall traps, representing emigration (out) and immigration (in) in wheat fields with adjacent flowe (n = 8) and grass margins (n = 10) on the four sampling events during the spring-summer season, 2014 in Flakkebjerg, Denmark.

Table S2. Mean number of individual arthropods (±SE) caught by suction sampling in flower margins (n = 4), grass margins (n = 5), and in adjacent areas of
the wheat crop during the spring-summer season, 2014 in Flakkebjerg, Denmark.

**Table S3.** Descriptive characteristics of the Biocontrol Service Index (BSI) in the aphid sentinel prey cages with grass or flower margin treatments, 2 days and 5 days after the sentinel aphid colony establishment.