SHORT COMMUNICATION

Pest regulation and support of natural enemies in agriculture: Experimental evidence of within field wildflower strips

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Abstract

Restoring ecosystem services in agriculture is vital to reach a sustainable food production. More specifically, developing farming practices which enhance biological pest control is a main issue for today's agriculture. The aim of this study was to assess whether the two strategies of complicating the search of host plants by pests by increasing plant diversity, and of supporting their natural enemies by managing habitats, could be combined simultaneously at the field scale to restore biological pest control and reduce chemical insecticide use. In Gembloux (Belgium), wildflower strips (WFS) were sown within wheat crops in which pests (i.e., aphids), their predators (i.e. aphidophagous hoverflies, lacewings and ladybeetles) and parasitoid wasps were monitored for 10 weeks in the period of May through July 2015 as indicators of the ES of pest control. Aphids were significantly reduced and adult hoverflies favoured in wheat in between WFS, compared to monoculture wheat plots. No significant differences were observed for adult lacewings, ladybeetles and parasitoids. In all treatments, very few lacewing and ladybeetle larvae were observed on wheat tillers. The abundance of hoverfly larvae was positively correlated with the aphid density on tillers in between WFS, showing that increasing food provisions by multiplying habitats within fields, and not only along margins, can help supporting aphidophagous hoverflies in crops. By enhancing the ecosystem services of biological pest control, this study shows that increasing both plant diversity and managing habitats for natural enemies may reduce aphid populations, hence insecticide use. Future research should continue this vein of work by quantifying the link between agricultural practices and the delivery of ecosystem services in order to guide future measures of agricultural policies.

Keywords: agroecological engineering, conservation biological control, plant diversity, aphids, predators, parasitoids

1. Introduction

The intensification of agriculture in Europe, which was characterised by an increased use of external inputs (i.e., improved seeds, chemical fertilisers and pesticides), has led to a simplification of agricultural ecosystems, environmental damages and health issues (Robinson and Sutherland, 2002). This acknowledgement goes beyond scientific concerns, as attested by, among other, the European Biodiversity Strategy which clearly states the need to "increase the contribution of agriculture to maintaining and enhancing biodiversity" (Target 3). More specifically, the spread of large monoculture fields and the loss of natural habitats have increased the risk of pest outbreaks (Altieri and Nicholls, 2004) and led to a reduction of biodiversity imperilling the provision of ecosystem services (ES) (Flynn et al., 2009). Moreover, the harmful effects on human health and the environment of chemical insecticides used to control agricultural insect pests have been largely proved (Baldi et al., 2013; Devine and Furlong, 2007). The ever-tighter regulation on pesticides (Skevas et al., 2013) and the call from consumers for healthier food (Howard and Allen, 2010) encourage the development of innovative agroecological practices that would restore ES, which may allow farmers to reduce their reliance on these inputs. Among other strategies (Zehnder et al., 2007), two may be of particular interest: (i) complicate the search of host plants by pests, and (ii) provide habitats supporting pest natural enemies that may exercise predation and parasitism.

According to the 'resource concentration' hypothesis of Root (1973), it is more difficult for specialist herbivores to find their host plant in diversified fields than in monoculture. In practice, intercropping and agroforestry systems (i.e., cultivating simultaneously several crops or crop and trees, respectively) are known to increase plant diversity at the field scale (Malézieux et al., 2009). Previous studies showed that, when applied in wheat fields, aphids (Hemiptera: Aphididae) were systematically less abundant in these systems compared to pure stands (Lopes et al., 2016; Muhammad et al., 2005). However, these studies reported inconsistent results regarding natural enemy support. One reason can be that through these systems, adult natural enemies – which exclusively (e.g., hoverflies [Diptera: Syrphidae]) or partly (e.g., ladybeetles [Coleoptera: Coccinellidae], lacewings [Neuroptera: Chrysopidae], parasitoid wasps [Hymenoptera]) depend on non-prey food (Wäckers and Van Rijn, 2012) – do not find the resources they need, such as proteins, various sugars, amino-acids, mineral ions, alkaloids (Lundgren, 2009). These resources can be made available through managing appropriate infrastructures in agricultural landscapes. For instance, wildflower strips (WFS) are known to be habitats for pest natural enemies (Haaland et al., 2011) as they potentially provide them the needed resources through nectar and pollen (Lu et al., 2014). Moreover, they may support additional prey for predators and hosts for parasitoids and be a shelter from adverse conditions (Landis et al., 2000). Several studies assessed the potential of sowing WFS along field margins to favour natural enemies and enhance pest control in the adjacent fields. Some recently showed a positive effect on pest reduction (Balzan and Moonen, 2014; Tschumi et al., 2016a, 2016b) but previous ones recall that it may not be systematic (Hickman and Wratten, 1996; Pfiffner et al., 2009).

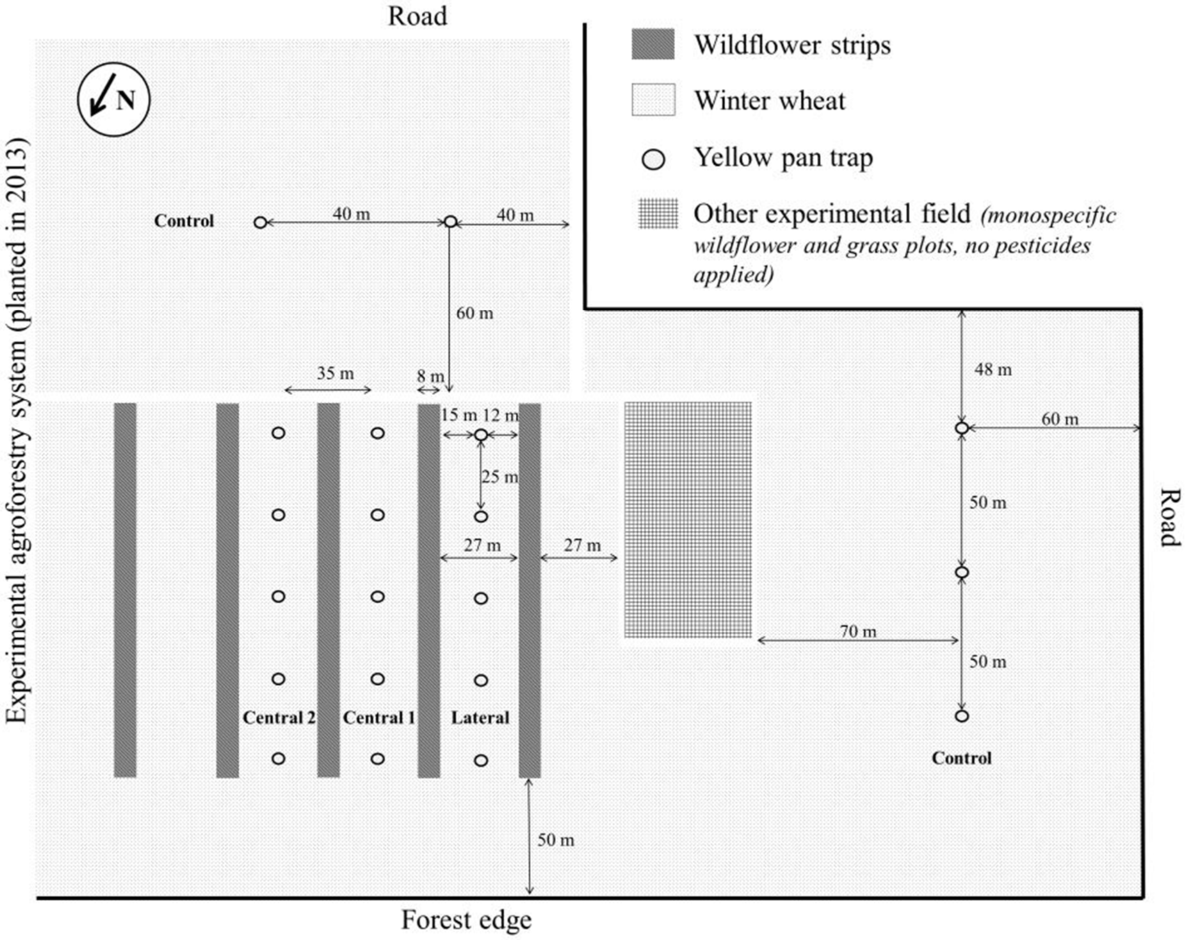
In the light of these results, the aim of this study was to assess whether the two strategies of complicating the search of host plant by pests and of supporting natural enemies could be combined simultaneously to restore biological pest control and reduce chemical insecticide use. To our knowledge, flowering habitats are almost always sown in strips at field margins. Only Sutherland et al. (2001) investigated whether WFS sown as one large patch or several smaller ones within fields better support hoverflies. However, the effect was assessed in the patches only, and not in the adjacent crops. In the present study, we tested whether sowing multiple WFS within fields could allow reducing pests by an increase of plant diversity and the support of natural enemies.

2. Material and methods

2.1 Field set up

This study was conducted at the experimental farm of Gembloux Agro-Bio Tech (University of Liège), Namur Province of Belgium (50°34'03''N; 4°42'27''E). In this region, a deep and loamy soil allows high crop productivity and the landscape is characterised by large crop fields and few non-crop habitats (in this region, 50-70% of the surface is dedicated to agriculture while 9% are wooded areas, respectively the highest and the lowest level in Wallonia, Service Public de Wallonie, 2014). On a surface of 9 ha, five replicated WFS (125 m. × 8 m.) were sown at a distance of 27 m. from each other (the field was surrounded by roads, a two-year old agroforestry system and a woodlot which edge was perpendicular to the WFS and the control plots, Fig. 1). Each WFS was composed of 17 perennial wildflower species and three grass species commonly found in Belgian grasslands (see Uyttenbroeck et al., 2015 for the list of the flower species and details on the sowing protocol) and available on the market (seeds were obtained from ECOSEM, Belgium). Based on this design, four treatments were considered related to the location of wheat plots: (i) plots surrounding the WFS were considered as the treatment 'control'; (ii) the plots between the two first WFS were termed 'lateral' treatment and from west to east, the plots with two and three WFS on each side were termed (iii) 'central 1' and (iv) 'central 2' treatment, respectively. WFS were sown the 6th June 2013 and mown twice each year. The winter wheat (variety 'Edgar') was sown the 23rd October 2014. No insecticides and no herbicides were used in the whole experimental area.

Figure 1. Experimental design.



2.2 Insect monitoring

As indicators of the ES of pest control, winged wheat aphids and their adult natural enemies were trapped for 10 weeks from 12 May to 29 July 2015 in wheat plots (excepting one week between the 30th June and 7th July which corresponded to the WFS mowing). Five yellow pan traps (Flora®, 27 cm diameter and 10 cm depth) were installed on a fibreglass stick in each treatment (Fig. 1). Traps were placed at a distance of 12 to 15 m from WFS and separated from one to another by 25 m. They were positioned at vegetation height, and filled with water containing a few drops of detergent (dish-washing liquid) to reduce the surface tension of water. Their position was adjusted during the growing season to follow wheat growth. Traps were emptied and refilled every seven days, and the trapped insects conserved in 70% ethanol.

Wheat aphids, adult hoverflies, lacewings and ladybeetles, whose larvae are aphidophagous, were identified to the species level following Taylor (1981), van Veen (2010), San Martin (2004) and Roy et al. (2013) respectively. Keys from Tomanović et al. (2003) and Rakhshani et al. (2008) were used to identify parasitoid wasps of wheat aphids to the species level. Moreover, aphids and larvae of hoverflies, lacewings and ladybeetles were counted on wheat tillers during the same period. Around each traps, 20 tillers were randomly chosen every week. Rainy days were avoided and no distinction between larval stages was made.

2.3 Statistical analysis

Generalised linear mixed effect models (package 'lme4', function 'glmer', Bates et al., 2014) with Poisson error distribution (log-link function) were fitted to test whether the location of wheat plots with respect to WFS (i.e., treatments) affected the density of aphids and their natural enemies, both trapped and observed. The four treatments were analysed as fixed effects and trapping or observation dates (10 dates) were included as random effects as measurements were repeated each time in the same plot. Replications (five replications per treatment) were also included as random effects, nested into the effect of dates, in order to integrate their dependent relationship (i.e., pseudo-replications). The effect of the wheat plot location on insect abundance was tested using a likelihood-ratio test ($p<0.05$) and means were compared between the different treatments using a post-hoc test of Tukey ($p<0.05$, package 'multcomp', function 'glht', Hothorn et al., 2008). After a $\log(x+1)$ transformation, the linear relation between observed aphids and both adult predators and larvae (i.e., abundance of each predators at each observation point, polled from all observation dates) was tested through a linear regression ($p<0.05$). The statistical analyses were performed using R Core Team (2013).

3. Results

The presence of WFS significantly affected the aphids observed (df = 3; χ^2 = 93.1; p-value < 0.001) and trapped (df = 3; χ^2 = 13.9; p-value = 0.003) as well as hoverfly larvae observed (df = 3; χ^2 = 16.1; p-value = 0.001) and adults trapped (df = 3; χ^2 = 16.3; p-value < 0.001). These results suggest that combining the strategies of increasing plant diversity and managing habitats allows regulating pest abundance and supporting natural enemies. However, this pattern was not observed for the trapped ladybeetles (df = 3; χ^2 = 4.15; p-value = 0.246), lacewings (df = 3; χ^2 = 7.06; p-value = 0.07) and parasitoids (df = 3; χ^2 = 5.41; p-value = 0.144).

Significantly less winged aphids were trapped in the two central wheat plots compared to the control (Fig. 2a). Apterous aphids were also significantly less abundant on wheat tillers of the second central plot compared to the other treatments (Fig. 2b). As for natural enemies, significantly more hoverfly adults were trapped in the two central wheat plots compared to the control (Fig. 2c) and their larvae were significantly more abundant on tillers of the lateral plot compared to the control and the second central plot (Fig. 2d).

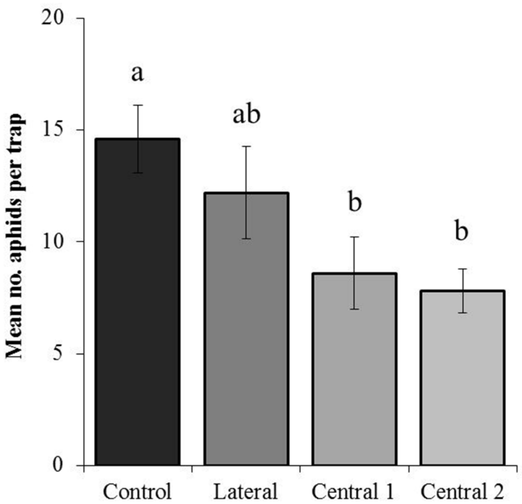
Hoverflies were by far the most predominant group (Table 1). Roughly ten times less lacewings, ladybeetles and parasitoid wasps were identified. A total of 67 hoverfly larvae, but almost none of ladybeetles and lacewings, were observed on wheat tillers. Aphids (both trapped and observed) as well as hoverfly larvae peaked simultaneously between the 23rd and 30th June, and hoverfly adults peaked the 15th July. The abundance of their larvae was positively correlated with the number of aphids on wheat tillers in between WFS (i.e. in all treatments except the control) (R^2 = 0.38; P = 0.015; y = -1.264 + 0.998x) while it was not the case for the adults (R^2 < 0.01; P = 0.89). The linear relations between aphids and adult ladybeetles, lacewings and parasitoids were not tested as these natural enemies were not affected by the treatments. No statistical analyses were performed on their larvae as very few of them were observed.

Table 1. Abundance and diversity of aphids and natural enemies trapped in the different treatments.

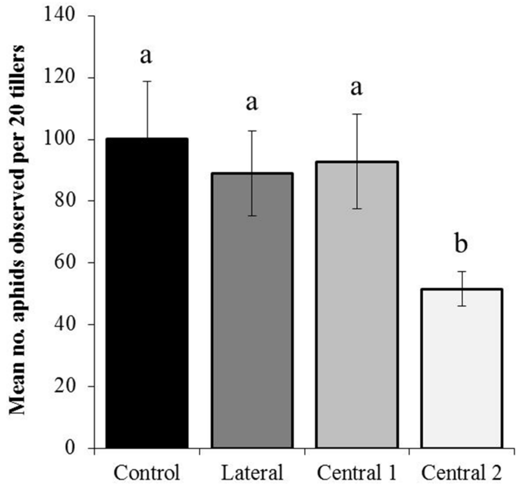
		Wheat between flower strips				
	Control	**Lateral**	**Central 1**	**Central 2**	**Total**	**%**
Aphid (Aphididae)	**73**	**61**	**43**	**39**	**216**	
Metopolophium dirhodum	5	11	6	7	29	13.4
Rhopalosiphum padi	61	42	26	30	159	73.6
Sitobion avenae	7	8	11	2	28	13.0
Hoverflies (Syrphidae)	**226**	**243**	**289**	**308**	**1066**	
Episyrphus balteatus	135	162	168	172	637	59.8
Eupeodes corollae	7	8	6	8	29	2.7
Melanostoma mellinum	28	19	28	40	115	10.8
Scaeva pyrastri	0	1	3	0	4	0.4
Sphaerophoria scripta	54	51	83	87	275	25.8
Syrphus ribesii	2	1	0	1	4	0.4
Syrphus vitripennis	0	1	1	0	2	0.2
Lacewings (Chrysopidae)	**31**	**33**	**34**	**17**	**115**	
Chrysoperla carnae	31	33	34	17	115	100.0
Ladybeetles (Coccinellidae)	**26**	**37**	**39**	**31**	**133**	
Coccinella 7 punctata	21	22	25	22	90	67.7
Harmonia 4 punctata	0	2	0	0	2	1.5
Harmonia axyridis	1	2	4	1	8	6.0
Hippodamia variegata	0	1	1	0	2	1.5
Propylea 14 punctata	4	10	9	8	31	23.3
Parasitoid wasps (Braconidae)	**41**	**53**	**51**	**34**	**179**	
Aphidius eadyi	8	6	4	4	22	14.0
Aphidius ervi	2	4	7	0	13	8.3
Aphidius matricariae	0	0	0	1	1	0.6
Aphidius rhopalosiphi	1	2	7	2	12	7.6
Aphidius salicis	0	1	0	0	1	0.6
Aphidius urticae	3	5	7	3	18	11.5
Aphidius uzbekistanicus	8	19	12	6	45	28.7
Diaeretiella rapae	0	1	0	0	1	0.6
Ephredus plagiator	11	6	5	8	30	19.1
Praon volucre	8	8	9	9	34	21.7
Trioxys auctus	0	1	0	1	2	1.3

Figure 2. Effect of wheat location treatment on aphids (a) trapped and (b) observed on tillers, (c) adult hoverflies trapped and (d) hoverfly larvae observed on tillers. The letters indicate the significant differences (p<0.05) of means using a post-hoc test of Tukey.

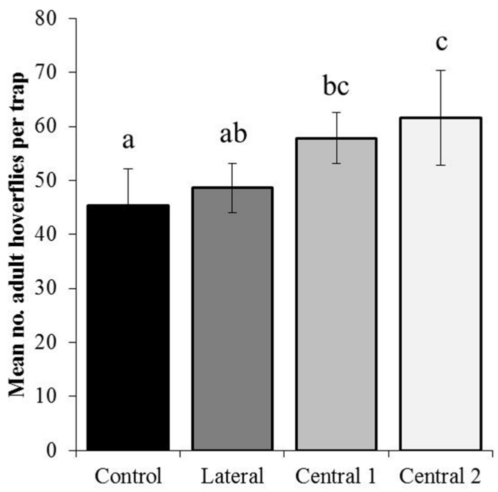
a)



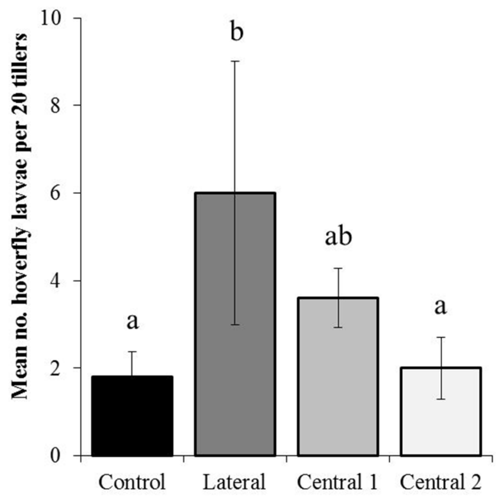
b)



c)



d)



4. Discussion

Increasing plant diversity at the field scale by sowing WFS within wheat fields allows regulating pest as the abundance of winged aphids and potentially apterous ones can be reduced when compared to wheat grown in monoculture. As expected, our results on winged aphids follow the 'resource concentration' hypothesis of Root (1973). Poveda et al. (2008) reviewed the several mechanisms that may increase pest control in diversified cropping systems, compared to simplified ones. In our case, it is known that aphids use visual cues (i.e., colours, contrast between target and background, target shape) when searching for their host plants. WFS may have masked wheat plants, while also creating a physical barrier of non-host plants. Moreover, aphids use olfactory cues (i.e., plant volatiles, other aphid pheromones) to find their host plants (Döring, 2014). WFS may have released volatiles that acted as odour-masking substances confusing aphids in their host plant search. However, the density of apterous aphids was not significantly different between the control and two out of the three wheat plots in between WFS, showing that few winged aphids can still allow the development of important populations on plants. We hypothesise that, because WFS were sown every 27 m., wheat plots were still large enough to allow apterous aphids to spread and develop. In other diversified systems such as strip-intercropping, crop strips are rarely that wide (rarely more than 5 m.), hence giving few opportunities for apterous aphids to spread from plant to plant (Lopes et al., 2015).

When plant diversification comes with the management of flowering habitats, it can additionally allow supporting pest natural enemies. In the present study, significantly more adult hoverflies were trapped in both central wheat plots compared to the control ones. However, this was not observed for parasitoids, ladybeetles and lacewings. These results are surprising regarding their dependence on sugar and/or protein sources provided by nectar and/or pollen (Lundgren, 2009). To our knowledge, the present study is the first assessing the abundance of aphid parasitoids in wheat crop adjacent to WFS. As for predators, Tschumi et al. (2015) also reported no effect of WFS on ladybeetles in wheat crops while lacewing abundance was increased. However, in our study, few individuals were trapped and almost no predatory larvae were observed in all treatments, indicating that they were generally few abundant in the field in 2015. Concerning adult hoverflies, they are also highly dependent on flower resources as all of them feed on nectar and pollen (Wäckers and Van Rijn, 2012). Nectar is their source of energy, while pollen provides them proteins. Their availability increases hoverfly longevity (Van Rijn et al., 2013) and fecundity (Laubertie et al., 2012), respectively. The presence of Apiaceae as well as some Asteraceae (e.g., *Achillea millefolium*, *Leucanthenum vulgare*) in the WFS may have supported them (Carrié et al., 2012; Wäckers and Van Rijn, 2012). Several studies showed that managed habitats providing floral resources benefit hoverfly populations (Haenke et al., 2009; Jönsson et al., 2015; Sutherland et al., 2001). However few assessed the effect on adjacent crops, compared to fields without WFS. Hickman & Wratten (1996) found inconsistent results between years while more recently, Tschumi et al. (2016b) found no differences of adult hoverfly abundance in crops adjacent to WFS, compared to control fields. We hypothesise that sowing WFS at field margins solely may not be enough to support hoverfly populations into adjacent crops. The present study suggests that increasing food provisions by multiplying habitats within fields, and not only along margins, can help support their presence in crops.

Even if hoverfly adults were found more abundant in the central wheat plots, their larvae were mainly observed on the lateral one. Their abundance was positively correlated with the density of aphids on tillers in plots in between WFS. Furthermore, their abundance peak was observed one to two weeks later than aphids' one. As hoverfly larvae feed on aphids, adult search for oviposition sites is guided by prey abundance on plants (Tenhumberg and Poehling, 1995). Cues such as aphid pheromones (namely (E)-β-farnesene) and plant secondary metabolites (such as (Z)-3-hexenol) released by plants when attacked by aphids are strong drivers for hoverfly adults to locate prey for their larvae (Verheggen et al., 2008). Whereas wheat control plots were more infested by aphids than the one in between WFS, few hoverfly larvae were observed on tillers. This indicates that hoverfly adults were first attracted by WFS in order to fulfil their need of proteins and energy, and then oviposited on adjacent wheat tillers if they were infested by aphids, which is consistent with the description given by Almohamad et al. (2009) on hoverfly behaviour.

As this study was conducted over a single season in one field, further research is needed in order to confirm the preliminary results obtained, that both increasing plant diversity and managing flowering natural habitats within fields enhance the ES of biological pest control by simultaneously creating barriers to pests while providing food resources and living sites for natural enemies. Moreover, longer term experiments are needed in order to assess whether such observations are valid on a variety of crops in a context of crop rotation as pests – and so their natural enemies – change with the rotating crops (which is actually a practice in itself for controlling pests - Oerke, 2006). Additionally, we can wonder whether the "barrier effect" provided by the increased plant diversity have a similar effect on other pests than aphids, and if perennial WFS can support natural enemies of a variety of pests.

Nevertheless, the present results are in the continuity of previous research (among others, Balzan and Moonen, 2014; Tschumi et al., 2016a, 2016b), showing that implementing WFS in agricultural landscapes can benefit farmer's activities. In Europe, it can even be subsidised though the agri-environmental schemes of the Common Agricultural Policy (Haaland et al., 2011). However, the present agri-environmental policies were developed in order to "reduce environmental risks associated with modern farming on the one hand, and preserve nature and cultivated landscapes on the other hand", using subsidies in order to compensate a loss of productivity farmers may face (European Commission, 2005). Our study shows that WFS introduced within fields could no longer be presented as a loss but as a potential for farmers to reduce their reliance on pesticides thanks to an increased pest regulation. Shifting toward measures that acknowledge the increased ES provision may encourage farmers to adopt diversified practices that will benefit their production (Ekroos et al., 2014). While biological diversification of farming systems is widely acknowledged to support critical ES to agriculture (Kremen and Miles, 2012), research remain to be done to quantify the link between specific agricultural practices and the delivery of ES. The present study fits within this recent vein of work by providing an estimation of the potential of within field flowering habitats on pest regulation and support of natural enemies.

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