Highly diversified crop systems can promote the dispersal and foraging activity of the generalist predator *Harmonia axyridis*

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Abstract: High plant biodiversity and landscape food provision stability is known to have a positive impact on biocontrol services provided by natural enemies. However, few studies have assessed how differential spatial and temporal crop richness actually impact biocontrol services, and notably how natural enemies may spill-over among crops. Within this framework, using a four-cage maze system under laboratory conditions, we evaluated the effect of a diversified crop system (four crops, namely cotton, tomato, squash and soybean) and low diversified crop systems (one single crop), on the generalist predator *Harmonia axyridis* (Pallas) and its predation capacity on aphids. The system varied food availability both in space, i.e. different cages, and in time, i.e. different dates of resources implementation. In general, the effects of crops on the natural enemies' traits observed in the diversified crop system resulted from the average effects of the individual low diversified crop systems; the impact of the predator was highly dependent on the plant. Low diversified crop systems actually proved to be either very suitable for the predator's development (tomato and squash), or not at all (soybean and cotton), but inversely suitable in reducing pest population with lower efficacy in tomato and squash and higher efficacy in soybean and cotton. The spillover of the ladybird was strongest in the squash low diversified crop system and lowest in the cotton one, other systems showing intermediate spillover values. In the diversified crop system, the ladybird presence was always closely related to plant presence, and aphid populations were maintained at a stable population increase. Still, the predator was also found in cages lacking plants, as opposed to the low diversified crop systems; this hinted at the potential of the highly diversified crop system to promote ladybird dispersal and increase foraging activity. We demonstrate that increasing crop diversity in agroecosystems may help promote biocontrol services provided by *H. axyridis* by promoting its spillover between crops (e.g. while the plants are senescing and/or when they are harvested).

Key words: polyculture, monoculture, resource diversity, ladybird, generalist predator, aphid.

Introduction

Modern agricultural practices, such as using chemical control on insect pest populations, have multiple side effects on other insect communities, including negative effects on natural enemies, thereafter named “NEs” (Kareiva 1987; Desneux et al., 2007; Lew et al., 2009; Hallmann et al., 2014; Tejada et al., 2015; Heimbach et al., 2017). By simplifying agricultural landscapes, modern agriculture leads to a reduction in habitat stability which is defined by the capacity of a habitat to maintain NEs, through the spatial and temporal continuum of its resources, a key to maintain NEs efficiency (Rand et al., 2006; Woodcock et al., 2016; Gurr et al., 2017). A growing body of evidence suggests that naturally occurring NEs could replace chemical inputs to control pest populations in many cases (Lu et al., 2012; Wratten et al., 2012; Jonsson et al., 2014; Karp et al. 2018; Jactel et al. 2019). The lack of habitat stability and/or food sources may lead to a decrease in arthropod biodiversity and could reduce biocontrol services provided by natural enemies (Veres et al., 2013; Martin et al., 2016). Continuity of nutritional resources is a key aspect for maintenance of food-web stability (Vasseur et al., 2013; Schellhorn et al., 2015). Habitat management to keep NEs in the fields and increase biocontrol is a key aspect used in Conservation Biological Control (Eilenberg et al., 2001). This technique consists in manipulating the habitats in order to make them suitable for NEs and therefore increase their presence and their biocontrol efficiency in the fields.

A management method often used in conservation biological control is the provision of semi-natural areas or ecological structures near or between crops (Rusch et al., 2016; Jankovic et al., 2017; Perovic et al. 2018; Hatt et al. 2019; Jaworski et al. 2019). Landscape complexity strongly enhances biocontrol through the provision of alternate food sources such as preys and hosts as well as floral resources, e.g. nectar and pollen, and includes shelter from intrusions (Rand et al., 2006; Rusch et al., 2013; Gagic et al., 2016; Rusch et al., 2016; Gurr et al., 2017).

However, natural areas are time-consuming for farmers to manage and can trigger the appearance of pests and weeds in adjacent fields and do not represent a direct source of income, which often discourages farmers from adopting these techniques (Bianchi et al. 2006; Burton et al. 2008; Brewer and Goodell, 2012).

Another concept of CBC that has proved efficient and does not use natural areas as the main driving factor for biodiversity enhancement, is ecological engineering by increasing crop diversity (Altieri 1999; Gurr et al. 2004; Letourneau et al., 2011). Ecological engineering focuses mainly on maintaining continuous food source diversity in time and space in order to enhance NEs performance and therefore reduce pest pressure (Altieri, 1999; Vasseur et al. 2013; Schellhorn et al. 2015). Studies undertaken on the effect on diversifying crop types on NEs have largely proved efficient on a field scale (Moreira et al., 2016; Letourneau et al., 2011). However, the underlying mechanisms leading to the positive interactions between natural enemies and the different type of crops have scarcely been evaluated. In order to complete their life cycle, some NEs may need continuous, diversified (Schellhorn et al., 2015) and complementary food sources such as pollen, nectar, hosts and prey (Altieri, 1999; Gurr et al., 2004; Langellotto and Denno, 2004; Letourneau et al., 2011; Rusch et al., 2013).

Natural enemies are very diversified in terms of needs. Plant diversity is known to have a positive impact on most of NEs life history traits and performance (Poveda et al., 2008; Letourneau et al., 2011) as majority of them may need alternative food sources to complete their life cycle or for fitness increase. For example, some parasitoids need proteins coming from preys at their larval stages and need pollen and nectar at their adult stages to increase their fitness (Géneau et al., 2103; Chan and Godfray, 1995). Other natural enemies like ladybirds also complete their diet with pollen and nectar which increase their longevity (De Clerck et al., 2005). Therefore, by providing diversified resources through plant diversification, biocontrol can be increased (Gurr et al., 2017).

Diversification of crops placed at close quarters and overlapping in time might therefore ensure (i) the presence of different plant and prey blends and food sources in the polycultural system might increase generalist predators' search behavior compared to monocultures and therefore increase their spillover between crop patches and consequently (ii) increase their offspring and survival rate, and (iii) the predation capacity of generalist predators by increasing their fitness.

The well-known generalist predator, *Harmonia axyridis* Pallas, depends on different types of food sources, namely soft bodied insects such as aphids, their main food source, but also pollen and nectar from flowers (Spellman et al., 2006; Mathews et al., 2016; Wolf et al., 2018; Chen et al. 2019). Within this framework, under laboratory conditions, we targeted the possible direct effects of increasing functional crop diversity, both in space and time, on the population dynamics, the spillover, that we define in this study as the movement of the predator from one cage to another for food search, and the predation performance of *H. axyridis*. With regard to our small scale study, we compared a system with only one crop, so called "Monoculture" in this study, to a more diversified one composed by four crops, so called "Polyculture" in this study.

Material and methods

Biological Materials

Crop Plants

Four different crop plants were tested during the study: tomato (*Solanum lycopersicum*, Nano variety), soybean (*Glycine max*, Merrill variety), cotton (*Gossipium hirsutum*, Zongmian 49 variety) and squash (*Cucurbita moschata*, Butternut variety). These crop plants were chosen for their complementary functional traits, i.e. flowering periods, nectar and pollen availability

(Table S1, Supplementary Material) and plant structure, as well as for their economic importance in the agricultural world (source: FAO Statistic division, FAOSTAT 2018). Tomato and squash plants were 3 weeks old, and soybean and cotton plants were 4 weeks old when they were inserted into the experimental setup. As plants were too young to provide any flowers, artificial nectar was administered on squash and soybean using a mixture of water and honey with 1:4 honey for water (Hogg et al., 2011) applied on a cotton placed around the stem during the entire experimental period. Tomato and cotton plants were not provided with artificial nectar as the tomato plant does not produce nectar accessible to ladybirds whereas cotton produced its own extrafloral nectar (on the mid-rib of the leaves) available for the predator during the experimental period (Röse et al., 2006). All plants were grown under the same conditions in a growth chamber maintained at 25 ± 2°C; LD 15:9; 70 % RH.

Pests

The pests species were selected according to two main criteria: 1) pest type naturally found on the selected crops and 2) the pest best suited to the needs of the natural enemy. Therefore, all the pests were aphids, namely: *Aphis gossypii* Glover on cotton and squash, *Aphis glycines* Matsumura on soybean and *Macrosiphum euphorbiae* Thomas on tomato. The three aphid species were reared under laboratory conditions on their respective host crop plants (25 ± 2°C; LD 15:9; 50 – 70 % RH).

Predator

The predatory ladybird, *Harmonia axyridis* (Pallas), was reared under laboratory conditions and fed, at the same time, on both *Macrosiphum euphorbiae* and *Metopolophium dirhodum* on tomato and wheat plants, respectively. A mixture of 1:4 honey:water solution was added on small tubes in the cages (25 ± 2°C; LD 15:9; 50 – 70 % RH). Before starting the experiment, one sexually mature male and one sexually mature female were selected seven days after emerging from the pupae (Pervez and Omkar, 2006). After identifying males and females (McCornack et al., 2007), couples were placed in separate boxes in order to check that eggs were laid. When egg presence confirmed sexual maturity, one male/female couple was introduced into one of the four experimental cages at the beginning of the experiment. Therefore, one pair of male/female couple was present in one four-cage maze system.

Experimental design

The experimental system is composed by 4 inter-connected cages by means of mesh tunnels in order to allow the predator spillover. Each cage was linked to six tunnels: two per side (one up and one down) on three sides of the cages. This design facilitated predator spillover from one cage to another (see Fig.1 for pictures and schemes of the experimental design). One plant and its associated pests were placed in one cage following a chronological order.

In order to evaluate the impact of crop diversity and associated preys on the predator population and its activity, the following treatments were used: (i) the polyculture system consisting of four crop species, one crop per cages, in the following chronological order: the first plant was introduced into cage 1 on day 1 and cut on day 10; the second was introduced into cage 2 on day 5 and cut on day 15; the third was introduced into cage 3 on day 10 and cut on day 20; and the fourth was introduced into cage 4 on day 15 and cut on day 25 (Fig.1 B); (ii) monoculture systems with only one crop species in the four-cages system. In order to allow the aphids to colonize the plant before being exposed to the predators, 100 aphids were put on each plant 5 days before being introduced into the four-cage system. Simulation of the crop harvest was carried out by cutting one plant every 5 days and pest population was counted after plant cutting.

A couple of sexually mature male and female predators was introduced on the first day of the experiment. The plants were physically separated in order to assess the position of the predator individuals, i.e. adults, eggs, nymphs and larvae, at each sampling time throughout the 25 days, which corresponds to the life cycle of the predator from egg to immature adults. While cutting the plants, the predators on plants and cages, in each cage, were counted, thus enabling assessment of predator numbers and spillover.

The control consisted of the same experimental system but without predators, which enabled the evaluation of aphid population growth without predators in the tested systems. Each polyculture system was replicated 16 times, with each monoculture replicated 4 times (clustered Monocultures N=16).

Variables measured

During the experiment we sampled the number of eggs laid by the female predator, the number of larvae on every stages, the number of pupae and the number of young and old adults every 5 days in the entire four-cage system. The number of aphids was assessed when the plant was introduced in the cage (before exposure to the predator) and when the plant was cut (after exposure to the predator). Here is a short clarification for the example of polyculture: the number of aphids on tomato was assessed at d0 and at d10; on soybean the number of aphids was assessed on d5 and on d15; on cotton the number of aphids was assessed on d10 and on d20; and on squash the number of aphids was assessed on d15 and on d25.

In total, 3 variables were measured as a proxy of predator life history traits: (i) the fecundity, which was evaluated by assessing the cumulated number of eggs laid by the female predator, (ii) and two ways of measuring predator survival: the number of larvae, pupae and adults present the last day of the experiment and the survival rate of the predator on an entire experimental period (ratio between the number of young adults on day 25 and the number of laid eggs between day 5 and day 10).

The predator capacity to exert an efficient biocontrol on aphids was measured by calculating the aphid population growth rate in the treatments with the predator and the associated treatments without predator (controls). Aphid population growth rate per predator was calculated as follow: the difference in aphid number recorded *after* and *before* exposure to the predator was divided by aphid number recorded *before* this exposure; the value was then divided by the total number of predators (larvae and adults) per four-cage system.

Difference in predator performance was assessed by comparing the aphid population growth rate per predator individuals regarding the different treatments except the control where no predators were present; difference of aphid population growth per treatment was assessed by comparing the aphid population growth rate per treatment without the predator, i.e. in the control treatments. The efficacy of biocontrol in each treatment was assessed by comparing the aphid population growth rate with and without predator, each treatment separately.

Finally, in order to estimate the spillover of the predators, the presence of female adults, who act as the main drivers of population spread (Evans, 2003), was recorded in each cage on each sampling day (d5, d10, d15, d20, d25) throughout the entire sampling period. An index of spillover was established. The spillover was considered effective when the female adult was found in a cage containing a plant, that is to say: in cage 1 on day 5, in cage 1 or 2 on day 10, in cage 2 or 3 on day 15, in cage 3 or 4 on day 20 and in cage 4 on day 25. If the adult was found in a cage where no plant was present, then the spillover was considered ineffective.

Statistical Analysis

Predator life history traits, biocontrol efficiency and spillover were assessed comparing both polyculture and monoculture treatments. The polyculture and monoculture systems were compared following two different approaches: 1) by analysing polyculture (N=16) vs. all monoculture systems clustered (N=16) , i.e. the average of each variable on cotton, tomato, squash and soybean monoculture; and 2) by analysing polyculture vs. separate monocultures (N=4), i.e. the actual results in each monoculture compared to polyculture in order to test the effect of each crop on the predator features.

The effect of crop diversity on the cumulated number of eggs laid and on the number of individuals was analyzed with a Generalized Linear Model (GLM) following a Poisson error distribution. The effect of crop diversity on the survival rate was evaluated with a Linear Model (LM) after verification of the Gaussian distribution of the data using a Shapiro test. Tukey's post-hoc analysis (multcomp package [Hothorn et al., 2017]) in order to test respective differences between polyculture and separated monocultures.

The effect of crop diversity on the aphid population growth rate per predator as well as the aphid population growth rate in control treatments were evaluated with a LM after verification of the Gaussian distribution of the data using a Shapiro test. A multiple comparison Tukey's post-hoc analysis (multcomp package [Hothorn et al., 2017]) tested the respective differences between polyculture and separate monocultures. Each treatment was compared with its control treatment with a one-way ANOVA followed by a Chi² test.

Concerning the spillover, an index of 1 was attributed when the spillover was considered effective, and an index of 0 was attributed when the spillover was considered ineffective. The indexes were analysed in a binomial model as variable to explain called "spillover index", regarding the explanatory variable "treatment". Spillover differences between the treatments were evaluated with a GLM following a Binomial error distribution. Additionally, differences between the presence of female adults per cage and per day were assessed with a GLM following a Binomial error distribution in order to clarify the movement of the individuals within each treatment.

Statistical tests were performed with the R 3.5.1 software (R Core Team, 2018).

Results

Predator life history traits

Predator fecundity was given with the cumulated number of eggs laid on an entire experimental period, i.e. 25 days. On tomato, soybean and squash monoculture, the number of eggs was always superior to 25. On the other hand, in polyculture and on cotton monoculture, the number of eggs laid in an entire period varied from 0 to more than 200 showing the potential unsuitability of cotton for the predator fecundity. The cumulated number of eggs laid by the predator was similar in both treatments when polycultural systems were compared to the clustered monoculture systems (Fig. 2 A; χ^2_1: 2.893, $P = 0.088$). However, when comparing the polycultural treatment to separate monocultures, the total cumulated number of eggs laid during the entire experiment was significantly higher in the squash monoculture compared to the others and the cumulated number of eggs laid was significantly lower in cotton monoculture (Fig. 2 B; χ^2_4: 2473, $P<0.001$). The polyculture, the tomato monoculture and the soybean monoculture showed a similar intermediate number of cumulated eggs laid (Fig. 2 B).

In terms of survival, the number of individuals present the last day of the experiment was first evaluated. Except in squash monoculture, in all other treatments the number of individuals present the last day of the experiment varied from 0 to 31 showing the high variability in crop suitability for predator survival. The number of individuals present in the polycultural system on the last day of the experimental period was similar to the one in the clustered monocultures (Fig. 3 A; χ^2_1: 2.42, $P=0.119$). However, when comparing polyculture to the separate monocultures, the squash monoculture showed a significantly higher number of individuals than the other treatments (Fig. 3 B; χ^2_4: 272.54, $P<0.001$). The polyculture had an intermediate number of individuals, followed by the tomato monoculture, the soybean and last of all, the cotton monoculture, which had a significantly lower number of individuals than the other treatments (Fig. 3 B; all $P<0.001$).

The other metric used to evaluate predator survival was the survival rate which, as reminder, is ratio between the number of young adults on day 25 and the number of laid eggs between day 5 and day 10. Similarly to the results showing the number of individuals present the last day of the experiment, except in squash which show a survival rate varying between 6.5 and 16, the survival rate varied in other treatments between 0 (no survival at all) and 15. The survival rate in cotton was the lowest with no survival at all. The survival rates of predators in the polyculture and the clustered monocultures were similar (Fig. 4 A; $F_{1,34}$: 2.468, $P=0.125$). However, when polyculture was compared to separate monocultures, there was a significantly higher survival rate in squash monoculture (Fig. 4 B; $F_{4,31}$: 7.459, $P<0.001$). The survival rate of predators was significantly lower in polyculture, as well as in soybean and cotton monocultures. The tomato monoculture rated second highest with high variability, but no differences between the squash monoculture and the other treatments were observed (Fig. 4 B).

Spillover

The spillover was evaluated by means of an index of movement (sp-index). The index was equal to 1 when the predator was found in a cage with a plant, and an index of 0 was given when it was found in a cage without plant, or in the corridor.

The spillover index was not significantly different when polyculture was compared to clustered monocultures, and the index revealed that the predator was present equally both in cages with plants and in cages without plants (Fig 5 A; χ^2_1: 0.42, $P=0.51$). When monocultures were compared separately to polyculture, the index of spillover was significantly different between the treatments (Fig. 5 B; χ^2_4: 14.7, P=0.005). The presence of the female predator in cages with plants in cotton monoculture was very low (mean sp-index: 0.2); on the other hand, in squash the female predator strongly followed the presence of plant in the cage (mean sp-index: 0.7). The female predator was equally present in both cages with and without plant in the polyculture treatment (mean sp-index: 0.5) with however no differences in spillover with the other treatments. More data about the female predator spillover within each treatment in each cage and on each sampling date has been given in the Supplementary figures (Fig. S1 for polyculture and Fig. S2 for monocultures). Discussion of differences in the behavior of the female predators has been provided hereunder.

Predation activity

Aphid population growth rate was calculated as a proxy of predation activity. The higher the growth is, the less efficient was the predation. When each treatment with predator was compared to its control without predator, the analysis showed in each case a significant reduction in the aphid population growth when the predator was present, implying that aphid control was effective in each treatment (Fig. 6; all P<0.001).

When the different treatments were compared in the presence of the predators, polyculture and clustered monocultures showed a similar aphid population growth rate per predator individuals ($F_{1,158}$: 0.63, P=0.42). However, when polyculture was compared to separate monocultures, significant differences between treatments were noted (Fig. 6; $F_{4,155}$: 7.07, P<0.001). The lower aphid population growth rates were observed in soybean monoculture, followed by cotton monoculture and polyculture then tomato and squash monocultures, showing therefore a higher control efficacy by the predators in these treatments.

Finally, when aphid population growth rate was assessed per treatment in the controls, aphid population growth rate was significantly more important in squash monoculture compared to the other treatments. On the other hand, the aphid population growth rate was significantly lower in cotton monoculture but not different from the one in tomato monoculture and polyculture (Fig. 5; $F_{4,155}$: 10.5, P<0.001).

Discussion

Crop diversification in space and time is likely to increase natural enemies' reproduction rates, survival and/or predation efficiency. In our experiment, results showed that in terms of population dynamics, the squash monoculture was systematically and significantly better for predator development, showing significantly higher numbers of cumulated number of eggs laid by the predator, higher numbers of predator individuals present on the last day of experiment and higher predator survival rates. Soybean and cotton monocultures showed significantly lower performance for these three variables and polyculture and tomato monoculture were almost similar. Predation performance, evaluated through the aphid population growth rate in the presence and in the absence of the predator, showed that predation was significantly higher in the presence of the predator. However, when treatments where compared, predation was significantly higher in cotton and soybean monocultures followed by polyculture whereas the lowest predation was found in tomato and squash monocultures. Spillover of female predators was found to be significantly higher in squash monoculture and significantly lower in cotton monoculture, while spillover in polyculture, tomato and soybean monocultures was similar but was not different from the one in squash monoculture or in cotton monoculture.

When assessing predator population dynamics, the predation efficiency and the spillover, our results suggest that polyculture is a result of the average effects of the clustered monocultures. The differences in performance of the predator in the various treatments considered may be explained by two main phenomena: firstly, the indirect effect of the plant modulating aphid nutritional value, and secondly prey availability to the predator (Kagata et al., 2005; Mooney et al., 2012).

As the number of aphids was higher in squash, as shown in the control treatment, the female predator may tend to lay more eggs on that plant (Koch, 2003) as it gives more food resources for the predator population to grow than the other treatments. Consequently, the number of eggs laid (Evans and Dixon, 1986) and predator survival are higher on this crop. A previous study showed that the presence of aphids boosts mating by olfactory and visual stimulation of the adult ladybirds (Obata, 1997). In the squash monoculture, the aphids were strongly aggregated on the leaves because the plants were adapted to their development. In so doing, ladybird larvae were able to search for their food intensively and easily find the aggregated aphids on the leaves. Therefore, this increased their chances in reaching the different development stages. However, the increased aphid population growth might have reduced the predation efficacy of the predator in comparison to the predation observed in cotton and soybean. Indeed, predators, unlike parasitoids, are limited by their satiation state (Symondson et al., 2002). The high number of aphids might not have been compensated by the number of predators too low to reduce the aphid population.

The reduced number of aphids in cotton relative to squash may have diminished the reproductive stimulation of the predator (Obata, 1997) and therefore reduced the number of eggs laid. In cotton monoculture the number of predator was sufficient to drastically reduce the number of aphids, which developed less efficiently on this last crop, and lead to increased predation efficiency. However, the nutritional value of the aphids developing on the cotton plant was not adapted for predator survival. Indeed, on the cotton plant, we observed that the aphids developed a small morph called the “dwarf morph” (Watt and Hales, 1996) which can be produced by the aphid population due to the presence of natural enemies (Mondor et al., 2004). This morph develops at a lower rate than other morphs (Watt and Hales, 1996) which explains the lower population growth in the control treatment. Interestingly, the same aphid species was used on squash and cotton crops, *Aphis gossypii*, but developed very differently on different crops. The direct influence of the crops on the aphid development underlined the indirect effect of crops on predator population development.

The aphid population growth in soybean was relatively high and associated control of aphids in the presence of the predator was also high. However, the predator population survival rate on soybean monoculture was low, showing that despite the high nutritional intake from predation on aphid, the ladybird larvae hardly reach the adult stage. This might be explained by one particular phenomenon that was observed during the experimental period. The eggs in soybean monoculture were systematically laid on the cages and not on the plant, which differed from the other treatments where eggs were laid both on cages and plants. This phenomenon could explain how difficult it was for the small larvae hatching on the cage to find food quickly enough to survive as the soybean plant at the plant's early stage of development was thin and barely touching the cage. Added to this fact, the thin branches of the soybean plant and its leaf softness might have reduced the capacity of the female ladybird to lay eggs on it. In the case of tomato and squash, which are wider than cotton or soybean plants and therefore touching the cage with their leaves, when eggs were laid on the cages, larvae were more likely to find the plant (Kareiva and Perry, 1989).

It is interesting to note that the aphid population growth rate was in average kept under zero in polyculture treatment, showing a good control of the aphid population. In agricultural systems based on biological control, it is important to keep a certain amount of pests in order to feed the natural enemies (Schellhorn et al., 2015). Monocultures, like the squash one in our experiment, might increase the presence of pests as this crop is suitable for their development. On one hand, the continuous predator satiation in monoculture of squash has allowed the predator population to grow and show higher life history traits performance than in other treatments but did not ensure a higher control of aphid populations. On the other hand, the polycultural system has allowed the predator to develop thanks to the presence of beneficial crops like tomato and squash, and at the same time has allowed a better control of aphids population than in these last two monocultures, thanks to the presence of less beneficial crops for pests development, namely soybean and cotton in the framework of our experiment. Defense mechanism of aphids and plant architecture are other mechanisms influencing the capacity of the predator to get some nutritional intake from their preys (Omkar and Sahu, 2009). These are other variables that will be interesting to measure in the future in the framework of a similar experimental protocol.

The spillover of the predator was influenced by the systems we exposed it to. When measuring the spillover as defined by the presence of the female predator in cages where plants were, a higher spillover in squash monoculture was shown, with almost 70% of the females transferring to the new cage containing food sources. Transfers were also high on tomato with more than a 60% transfer and in polyculture with a 50% transfer. The reduced nutritional value of the aphids developing on the cotton crop as well as the low aphid population development rate might have reduced the presence of the female predator in the cages with cotton which was looking for other food sources. This has been confirmed by the assessment of females present in each cage and at each sampling time where no differences among the cages were observed throughout the whole experiment in cotton crops. However, in squash monoculture, the female adhered more accurately to the presence of plants in space and time, which can be explained by the attractiveness of the crop due to the increased presence of aphids. Interestingly, the effect of the plant was different on soybean compare to squash monoculture. Indeed, even if the aphids were developing well on soybean, biocontrol was still high in soybean monoculture compare to the one in squash. As in cotton monoculture, it is possible that the female, after eating most of the aphids, resulting in a low aphid population growth, might have look for some other source of food in the other cages. However, as no paper reported that *Aphis glycines* were of low nutritional value for the ladybird *Harmonia axyridis*, the hypothesis considering the lack of accessibility of the aphid resource for the larvae is the most suitable one to explain the low development of the predator on this monoculture.

The behavior of the female predator in polyculture was different from the one in monocultures. In the polyculture, the female predator was present in almost every cage, with a preference for the cage containing food sources. This may be due to the presence of different blends in different cages resulting from plant emissions as well as various aphid species. These factors induce the production of Herbivore Induced Plant Volatiles (HIPVs) (Xiu et al., 2019). Honeydew odors might also be cues for food location by *H. axyridis* (Obata, 1997; Evans, 2003; Leroy et al., 2012). The presence of the female predator in other cages than the one with food sources were lower in the monoculture treatments and might be due to reduced olfactory stimulation.

The present study assessing the performance of the *H. axyridis* ladybird and its spillover in a system offering varied food sources in space and time shows that predator efficiency is highly dependent on the plant because of indirect effect on the prey, i.e. the suitability of plant for prey development and its potential to feed the predator. Other indirect effects associated to plant location for efficient food intake by the predator might also have influenced the predator survival rate. The presence of multiple food source types present in the polycultural system tested might have stimulated the foraging activity of the ladybird. This resulted in an efficient predation activity, but with no more transfers than in simplified systems with plants adapted to the predator survival. Monocultures have been shown to be either very suitable for the predator development, i.e. in the case of squash and tomato monocultures, or increasing the predation capacity, i.e. in the case of cotton and soybean. In farming practices, increasing crop functional diversity might have a beneficial effect on natural biological control by the predatory ladybird only if plants suitable for predator development are present in the crop succession. The presence of plants beneficial for *H. axyridis* development, as it was the case in our study for squash, could be cultivated during the same period as a crop less beneficial for the ladybird development where however an efficient biocontrol could be exerted. The positive effect of the first crop could induce a predator population increase, which would therefore spill over into the adjacent field thanks to volatile attractiveness and provide efficient biological control for the second crop. The maintenance of such crop layouts would have to be ongoing in space and time in order to stabilize the natural enemy population and increase the efficiency of biocontrol (Schellhorn et al., 2015; Vasseur et al., 2013).

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Figures legends

Figure 1. Picture of the experimental design on the top and in front with the fours cages of the system as well as the tunnels visible (A), and schematic explanation of the system plant succession in space and time, where the introduction of predators and pests are represented by a logo corresponding to each species, and where C1 = cage of the system where the first plant + aphids are introduced as well as the male/female predator couple, C2 = cage of the system where the second plant + aphid are introduced, C3 = cage of the system where the third plant + aphids are introduced, and C4 = cage of the system where the fourth plant + aphids are introduced (B). Each polycultural system was replicated 16 times, with each monoculture replicated 4 times.

Figure 2. Boxplots of the cumulated number of eggs laid during the entire experimental period (25 days) in the polyculture treatment (N=16) compared to clustered monocultures (N=16) (A) and compared to separate monocultures (N=4 for each crop) (B). The different letters indicate significant differences in the number of eggs laid ($p<0.001$, GLM with Tukey's post-hoc analysis).

Figure 3. Boxplots of the number of individuals (larvae, pupae and adults) present the last day of the experiment in the polyculture treatment (N=16) compared to clustered monocultures (N=16) (A) and compared to separate monocultures (N=4 for each crop) (B). The different letters indicate significant differences in the number of individuals ($p<0.001$, GLM with Tukey's post-hoc analysis).

Figure 4. Boxplots of the survival rates of the predator population on an entire experimental period in the polyculture treatment (N=16) compared to clustered monocultures (N=16) (A) and compared to separate monocultures (N=4 for each crop) (B). The different letters indicate

significant differences in the number survival rates ($p<0.001$, LM with Tukey's post-hoc analysis).

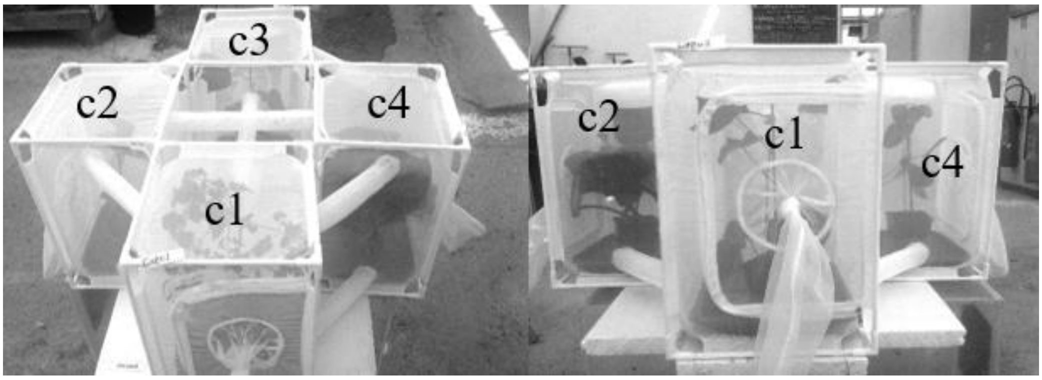
Figure 5. Bar plots of the female predator spillover index in polyculture (N=16) compared to clustered monocultures (N=16) (A) and compared to separate monocultures (N=4 for each crop) (B). The different letters indicate significant differences in the number of individuals ($p<0.001$, GLM with Tuckey's post-hoc analysis).

Figure 6. Boxplots of the aphid population growth rate in the polyculture treatment (N=16) compared to separate monocultures (N=4 for each crop). The aphid population growth rate per predator individuals (adults and larvae) is represented in solid color, and aphid population growth rate in control treatment, i.e. without predator, is represented in spotted color. The different letters indicate significant differences in the aphid population growth rate with the predator in normal character ($p<0.001$, LM with Tukey's post-hoc analysis), without the predator in italic character ($p<0.001$, LM with Tukey's post-hoc analysis). The stars indicate significant differences in the aphid population growth rate between one treatment and its control (*** $p<0.001$, one-way ANOVA with Chi² post-hoc analysis).

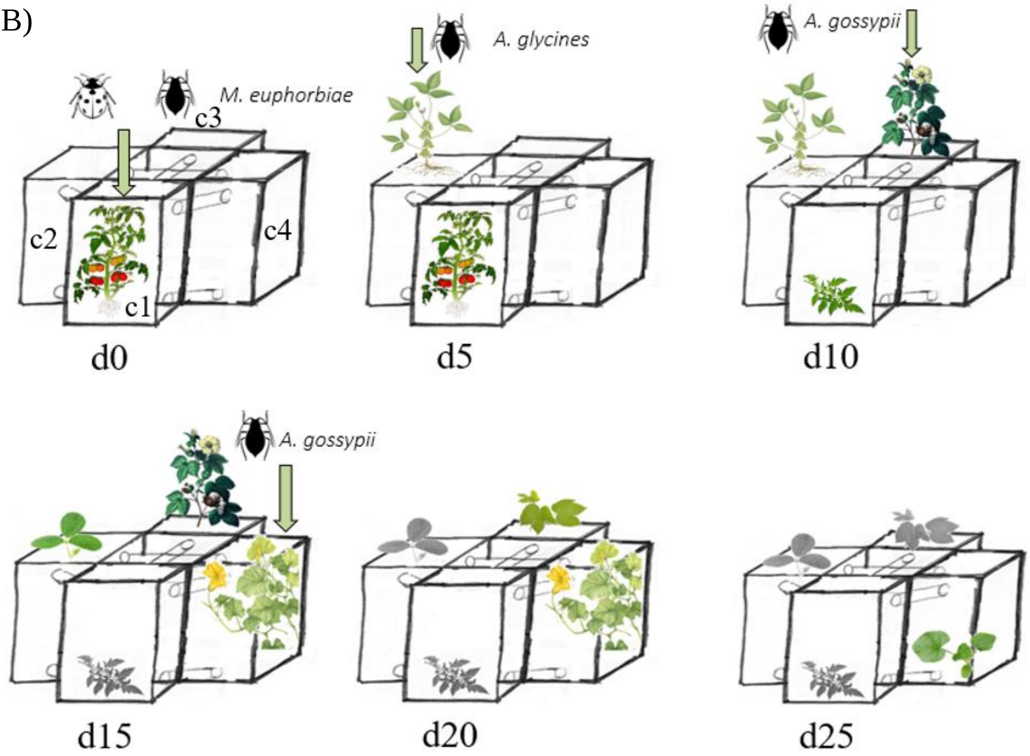
Figures

Figure 1.

(A)



(B)



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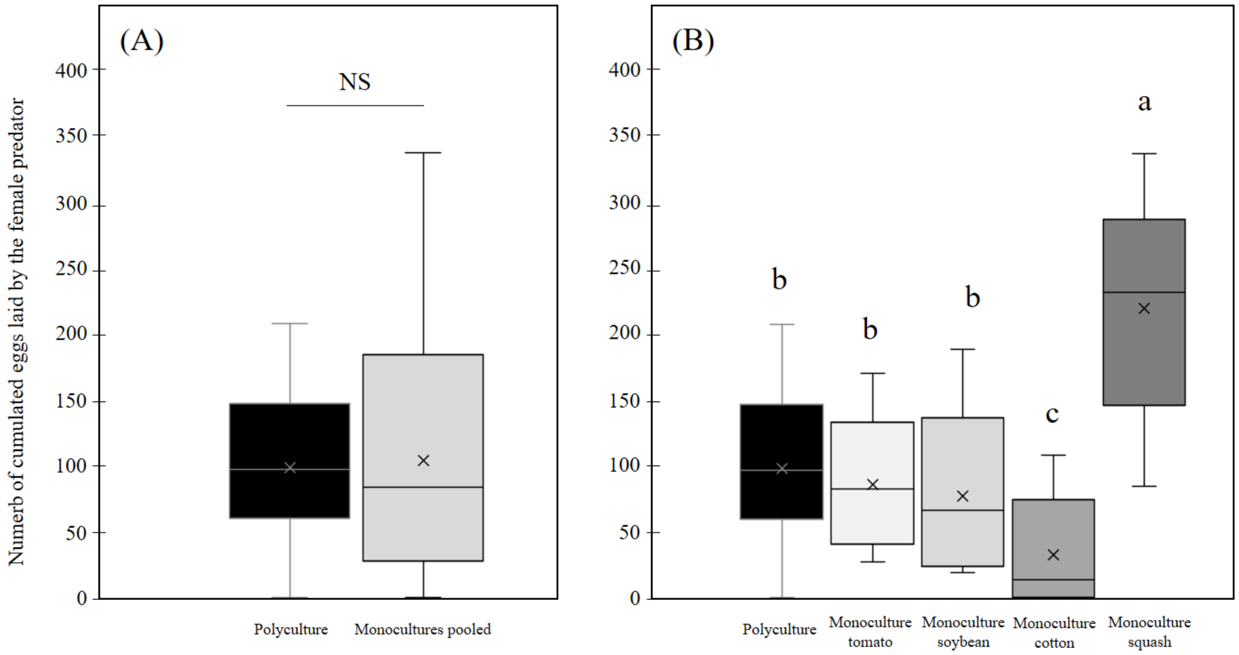

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698 Figure 3.

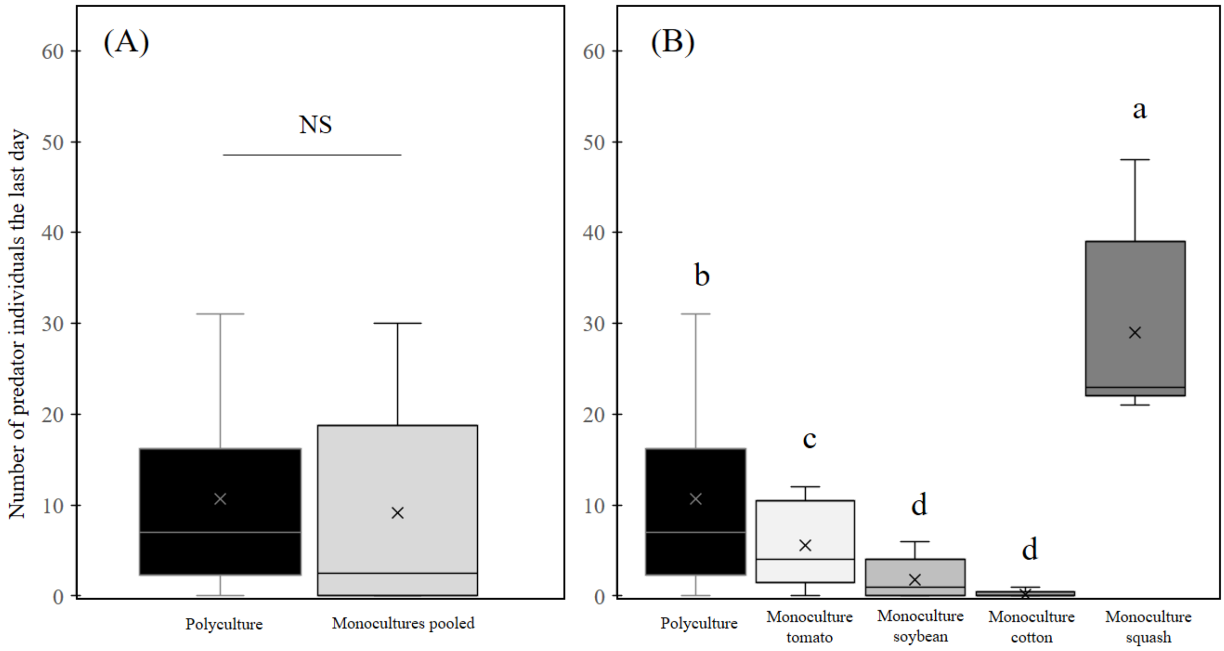

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Figure 4.

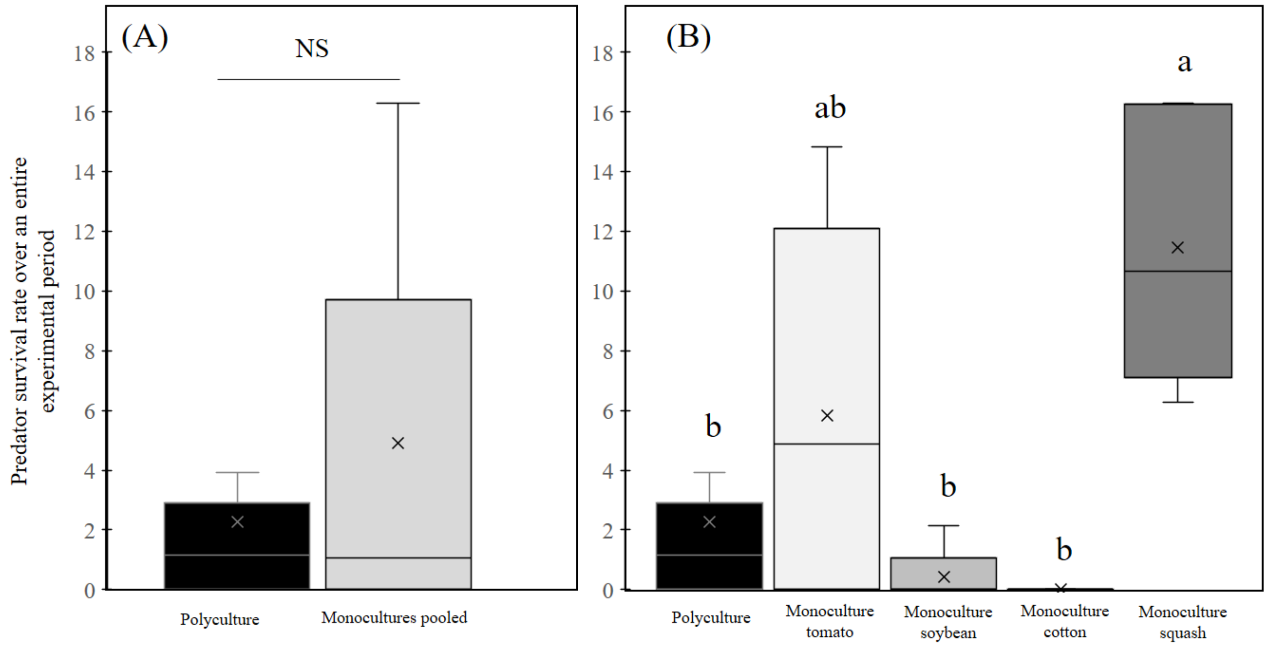


Figure 5.

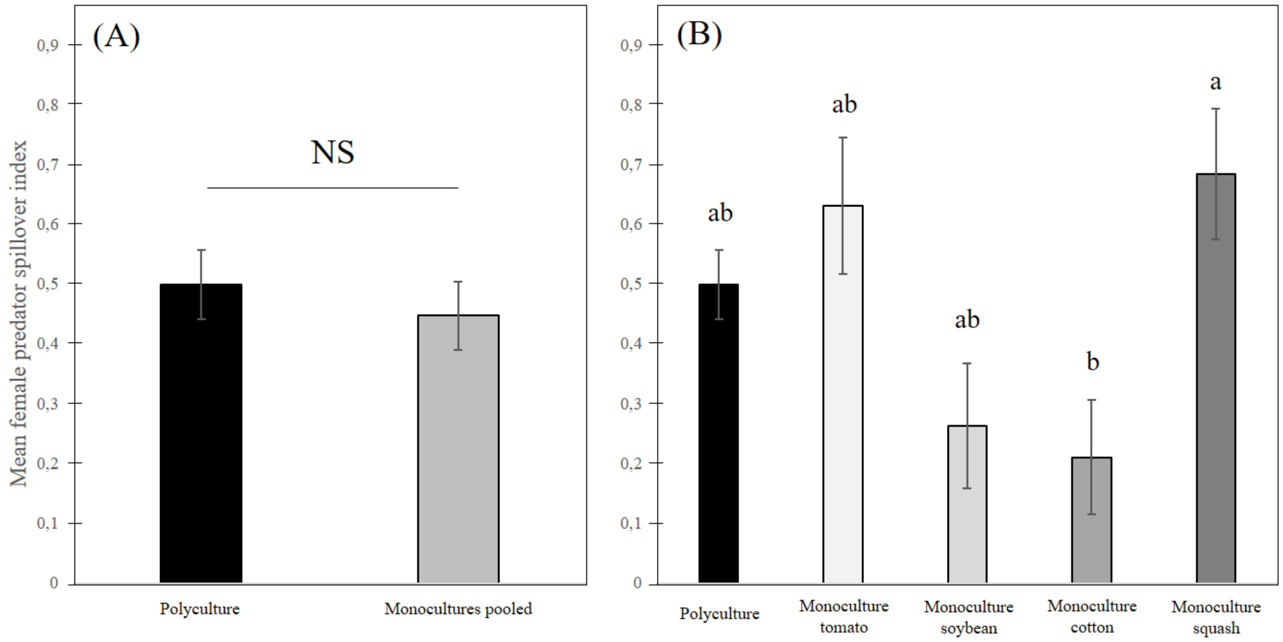
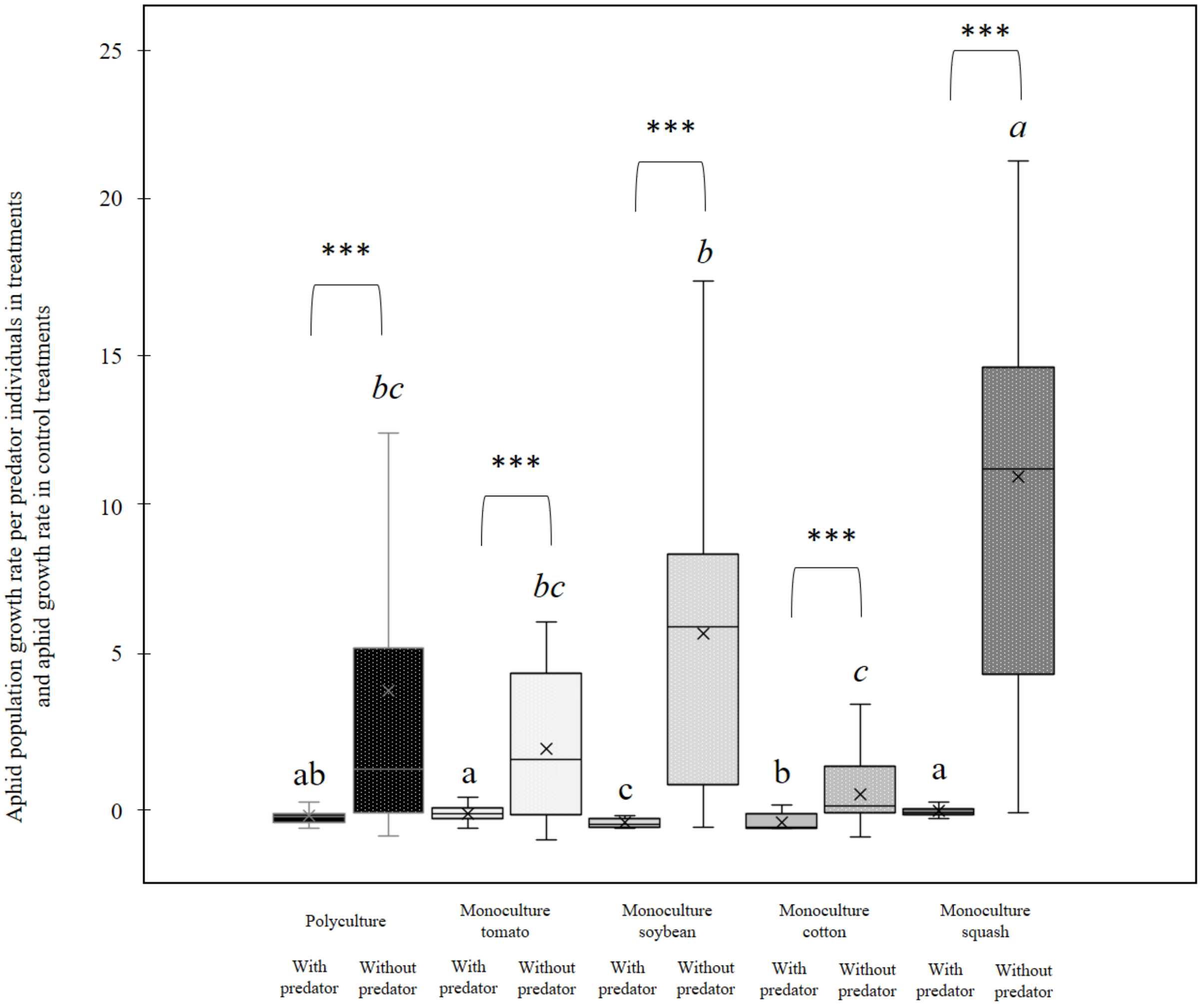


Figure 6.


Aphid population growth rate per predator individuals in treatments and aphid growth rate in control treatments
25
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ab
bc
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c
b
b
c
a
a
Polyculture
Monoculture tomato
Monoculture soybean
Monoculture cotton
Monoculture squash
With predator
Without predator
With predator
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