**MSc Thesis**

**Farming System Ecology Group**

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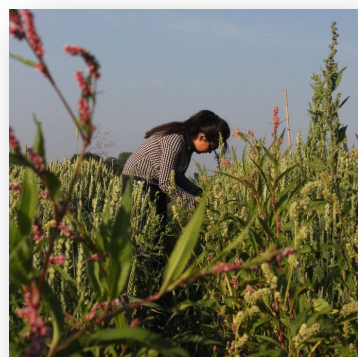

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# Population densities of pests and natural enemies in wheat (*Triticum aestivum*) and potato (*Solanum tuberosum L.*) in a diverse strip cropping system in the Netherlands

MSc Thesis, *Plant Sciences*

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## ABSTRACT

The aim of this research was to evaluate the effect of crop diversification (monocropping and intercropping) and soil disturbance (tillage, manuring and mulching) on population dynamics of aphid pests and natural enemies on different treatments of wheat and potato in a complex cropping system at Droevendaal Experimental Organic Farm of Wageningen University and Research Centre. This research was conducted on two strips of the strip cropping field (wheat and potato), a potato experimental strip, and a large monoculture wheat field.

By using three types of samplings (visual assessment, beat sampling and pitfall trapping), the populations of aphid pests and natural enemies were evaluated under three groups of study: aphid density, plant-dwelling natural enemies density, and ground-dwelling natural enemy density. Data analysis was done by fitting Generalized Linear Models (GLMs), and statistical significant differences between treatments were detected by a multi-comparison of pairwise treatments by post-hoc test with Tukey contrast.

In wheat, aphid pest's populations were significantly higher in some of the most diverse treatments. Parasitized aphid densities were lower in the most diverse treatments, with no significant differences. Plant-dwelling and ground-dwelling natural enemies densities tended to be higher in the treatments with more diversity, with no significant differences between treatments.

In potato, aphid pest's populations and parasitezed aphids were significantly lower in the crop diverse treatments compared with the soil disturbance treatments. Plant-dwelling natural enemies had no significant differences between any of the treatments, but crop diversity treatments presented higher densities than soil disturbance treatments. Ground-dwelling natural enemies were significantly higher in crop diversity treatments compared with some soil disturbance treatments.

Higher crop diversity of agroecosystems and soil management practices have influence over a better control of aphid pests populations and the enhancement of natural enemies populations.

Composition of the crop surroundings could benefit aphid populations with additional resources and offering a higher potential for the effective colonization of parasitoids populations and other beneficial arthropods. Suitable soil management practices could impact soil biointeractions, soil temperature and humidity creating a microecosystem that can influence natural enemies abundance.

# INTRODUCTION

## Background

Agricultural intensification has a great impact over the agricultural ecosystems and the interaction of the different elements within it. The use of intensive farming practices and simplification of the agricultural ecosystems had contributed to the loss of biodiversity, the weakening of ecosystem services (e.g. pollination, pest control), and a general deterioration of the natural ecosystem (Bianchi et *al.*, 2006). With the forecast of higher variation in climatic conditions and weather extremes, food production under mono-cropping systems is very vulnerable as it depends on an instable system where crop loss and low yield productivity are more likely to be present (Altieri et *al.*, 2015). Therefore, there is a great interest on re-designing the current mono-cropping systems into more resilient and sustainable diverse agricultural systems (Tilman et *al.,* 2011).

### *From intensive monocultures to diverse agroecosystems*

The transformation of mono-agricultural systems into crop and field diverse agroecosystems should consider the integration of the natural agricultural landscape elements, which can vary from a region to a field; and focus on the creation of agroecosystems that restore and conserve functional biodiversity, which can lead to promote a positive balance between food production, protection of the environment and profitability (Barberi et *al.,* 2010; Wezel et *al.*, 2014; Altieri et *al.*, 2015; Burgio et *al.,* 2015).

Diversification of agricultural systems goes beyond just genetic diversity, it should not be only considered as polycultures or varieties of mixtures. Spatial (e.g. field and landscape level) and temporal scales are two important factors that can bring a wider range of options and combinations in order to diversify the agroecosystem (Kremen and Miles, 2012; Altieri et *al.*, 2015). Moreover, the type of agricultural management practices can also have a direct or indirect impact over the diversity of the agroecosystems. For example, conservation practices (e.g. mulching and minimum tillage) aim to mitigate harmful conditions or enhance favourable ones, in order to benefit the proper development of natural enemies in terms of survival, fecundity and longevity (Landis et *al.,* 2000; Weibull et *al.,* 2003; Barberi et *al.,* 2010).

At the end, the main goal of diversification of agroecosystems is to maintain biotic interactions and functional complementarities of the agroecosystem elements that enhance the provision of ecosystem services, which are valuable due to the positive economic impacts they have over the agricultural production (Kremen and Miles, 2012; Jonsson et *al.,* 2013).

### *Functional biodiversity*

Functional biodiversity is an important factor when aiming for multifunctional agriculture. In multifunctional agriculture, the importance of biodiversity does not rely only in the nature conservation services, but also in other type of ecosystem services. This ecosystem services can be production and environmental services, that contribute to the development of more productive and sustainable agroecosystems (Barberi et *al.,* 2010). For example, functional biodiversity can aim to provide and enhance pest biocontrol as an ecosystem service by enhancing longevity and fecundity of natural enemies, and alter their behaviour trough the provision of a variety of resources (e.g. shelter, food) (Burgio et *al.,* 2015).

### *Pest biocontrol*

Pest biocontrol is an essential ecosystem service for sustainable crop production that has positive economic and environmental impacts (e.g. reducing yield loss and limiting the damaged caused by crop pests). Natural enemies, including predators and parasitoids, provide natural control of crop pests populations without the negative impact of chemical pesticides (Chaplin-Kramer et *al.,* 2013; Crowder and Jabbour, 2014).

More effective and efficient pest biocontrol has been attributed to diverse agroecosystems. This has been associated to the higher variety and availability of non-crop habitats and of resources (pollen, nectar), which may be able to sustain more stable natural enemies populations (Smith and McSorley, 2000). On the other hand, there are authors that discuss that more diverse agroecosystems may have negative impacts over the natural enemies populations (Bianchi et *al.*, 2006; Crowder and Jabbour, 2014).

According to the review of Andow (1991) there are some hypotheses that explain why the efficiency pest biocontrol may have negative results on diverse agroecosystems. 1) Negative relation between natural enemies and resource concentration (of pests in host plants) in diverse agroecosystems, supported by studies that show mortality of predators and parasitoids in polycultures due to resource concentration. 2) Polyphagous herbivores in polycultures, it can result on a relation between arthropod-plant host range the amount of pests densities is higher than natural enemies in polycultures. Smith and McSorley (2000) discussed that explaining arthropod response to vegetational diversity depends mainly on the arthropods adaptive characteristics and the ability to colonize the crop, which will depend on the characteristics of host-finding mechanisms, the range of diet, and relative mobility.

Crowder and Jabbour (2014) suggest that study of pest biocontrol should be done on more realistic biodiverse agroecosystems to better understand how pest biocontrol works in 'real-world' agroecosystems.

### *Pest biocontrol: spatial and temporal scales*

It has been demonstrated that both the structure of the crop system and the spatial composition, have a direct effect over the abundance and diversity of pests and natural enemies and the potential of the system to reach a suitable biological control (Bianchi et *al.,* 2015; Weibull, Östman and Granqvist, 2003). Therefore, the study of natural pest management should take into account both, spatial and temporal variations, which influence the dynamics of the system. By understanding the ecological principles of pests and natural enemies in space and time, more efficient and productive farming management systems can be designed (Bianchi, Schellhorn and Cunningham, 2013; Rusch et *al.,* 2013).

The spatial scale (arrangement on the field) has influence over the life cycle of different beneficial arthropods. Here the heterogeneity of the landscape elements (field margins, fallows, hedgerows and wood lots) are an important factor as they provide non-crop habitats that give access to shelter and extra resources. More complex types of landscapes can support more diverse and abundant communities of beneficial arthropods (Rusch et *al.,* 2013; Alignier, et *al.,* 2014). The temporal scale has influence over the arrival of natural enemies to crops. An effective pest management system will depend on the timing of colonization and the number of colonizers (Bianchi et *al.*, 2006; Bianchi et *al.,* 2015). Therefore, it is essential to have a good knowledge over the population dynamics of pests and natural enemies and their relationship with the habitat in order to design proper pest management strategies.

### *Aphids and natural enemies of wheat and potato*

Wheat and potato are two staple foods, which agricultural productivity account for about the 60% of the world's vegetables sources (Altieri et *al.*, 2015) and are considered part of the dietary requirements of the European diet. The Netherlands is among the 10 largest global producers of potato with almost 25% of the arable land (160 000 ha) (FAO, 2008). Around 140 000 ha are used for wheat production in the Netherlands (FAO, 2000).

Aphids are a major pest for both wheat and potato. In Europe aphids are responsible for annual losses of 700,000 tons of wheat and 850, 000 tons of potato (Dedryver et *al.,* 2010). They affect yield productivity by causing serious losses due to the direct consumption of phloem sap and indirectly by the transmission of viruses. They belong to the family Aphididae (Hemiptera). In their lifecycle, aphids produce two types of morphs apterous (wingless) and alate (winged). Winged aphids are considered to be the colonizers and have the ability to find new hosts (Dedryver et *al.,* 2010; Douglas, 2003; Saguez et *al,* 2013). For wheat, six species of aphids are the responsible for damage (*Rhopalosiphum padi, Schizaphis graminurn, R. Maidis, Metopoliphiurn dirhodum, Sitobion avenae and Diuraphis noxia*) (Kamran et *al.,* 2013). For potato, the aphids species related are non-specific, most of them are polyphagous and cosmopolitan (Saguez et *al,* 2013).

Yield losses caused by aphids are related to their ability of infestation and dispersal, their high reproductive potential and adaptability to local conditions. Aphid populations are affected by climate conditions (temperature, wind), natural enemies and plant quality (Leslie et *al.,* 2009; Dedryver et *al.,* 2010; Kamran et *al.,* 2013).

Chemical control of aphids has negative impacts both economically (high costs) and environmentally (effects over natural enemeies). Therefore, biological control has been considered as an potential solution (Östman et *al.,* 2001). There is a range of plant-dwelling and ground dwelling parasitoids and predators that are considered as natural enemies of aphids. Hoverflies (Syrphidae), lacewings (Neuroptera, Chrysopidae) and ladybeetles (Coccinellidae) are considered as important biocontrol as they are aphid-specific predators (Müller and Godfray, 1999; Rutledge et *al.,* 2004). Among other plant-dwelling natural enemies are parasitic wasps (Braconidae, Aphidiinae). Other generalist arthropod predators that help in the control of aphids populations are spiders (Araneae), harvestmen (Opiliones) and damsel bug (Nabidae) (; Alebeek et *al.,* 2005).

Moreover, there are other biotic and abiotic variables, such as climate conditions and crop development stage that have an influence over the distribution of arthropods and could affect the effectiveness of natural pest control strategies. So to better understand the population dynamics of pests and natural enemies measurements incorporating seasonal and interannual variations are needed (Wade et *al*., 2006; Chaplin-Kramer et *al.,* 2013).

## Aim, research questions and hypothesis

The aim of this research was to evaluate the effect of crop diversification (monocropping and intercropping) and soil disturbance (tillage, manuring and mulching) on population dynamics of aphid pests and natural enemies on different treatments of wheat and potato in a complex cropping system in the Netherlands.

In order to address the main objective of this research, the following research questions for each crop of study were formulated:

*Wheat*

1. What is the effect of the spatial scale of crop diversity on the population dynamics of aphid pests and their natural enemies?

*Potato*

1. What is the effect of crop genetic diversity on the population dynamics of aphid pests and their natural enemies?
2. What is the effect of soil management in terms of different tillage, manuring and mulching treatments on the population dynamics of aphid pests and their natural enemies?

For wheat, three treatments were defined regarding the type of cropping system: 1) *field* (single crop monoculture), 2) *strip* (single crop monoculture in strip cropping system), and 3) *plant* (faba-wheat intercropping in strip cropping system).

For potato, five treatments were defined regarding the type of tillage, manuring and mulching system: 1) *strip* (single variety and farm yard manure in strip cropping system), 2) *plant* (four varieties and farm yard manure in strip cropping system), 3) *mulching* (single variety with minimum tillage, straw mulching and grass-clover green manure), 4) *minimum tillage* (single variety with minimum tillage and grass-clover green manure), and 5) *tillage* (single variety with tillage and grass-clover green manure). In the following section (Methodology) the experimental design of the research will be explained in more detail.

It was expected to find differences in the population densities of aphid pests and natural enemies when comparing the different types of crop diversity and the different types of soil disturbance. Based on the background the hypothesis of this research was that 'more diverse and complex agricultural systems were able to sustain more stable and abundant populations of beneficial arthropods'. So it was expected that the population densities of natural enemies tend to be higher in the more diverse crop treatments. And consequently, population densities of pests were expected to be lower.

## METHODOLOGY

### Experimental site

The experimental site, a 250 ha field, was located at the Droevendaal Experimental Organic Farm of Wageningen University and Research Centre (51°59'28"N, 5°39'42"E) situated in Wageningen, The Netherlands. This research was part of a six-year strip-cropping project within an agro-ecological system, which started in 2014. Each year, a minimum of five different crops[1] are grown in a strip cropping system. In 2015, the second year of rotation, the growing crops selected were: grass-clover mixtures, wheat, maize, oilseed rape and potato. This research was carried out on two strips of the strip cropping field (wheat and potato), a potato experimental strip, and a large monoculture wheat field (Fig. 1).

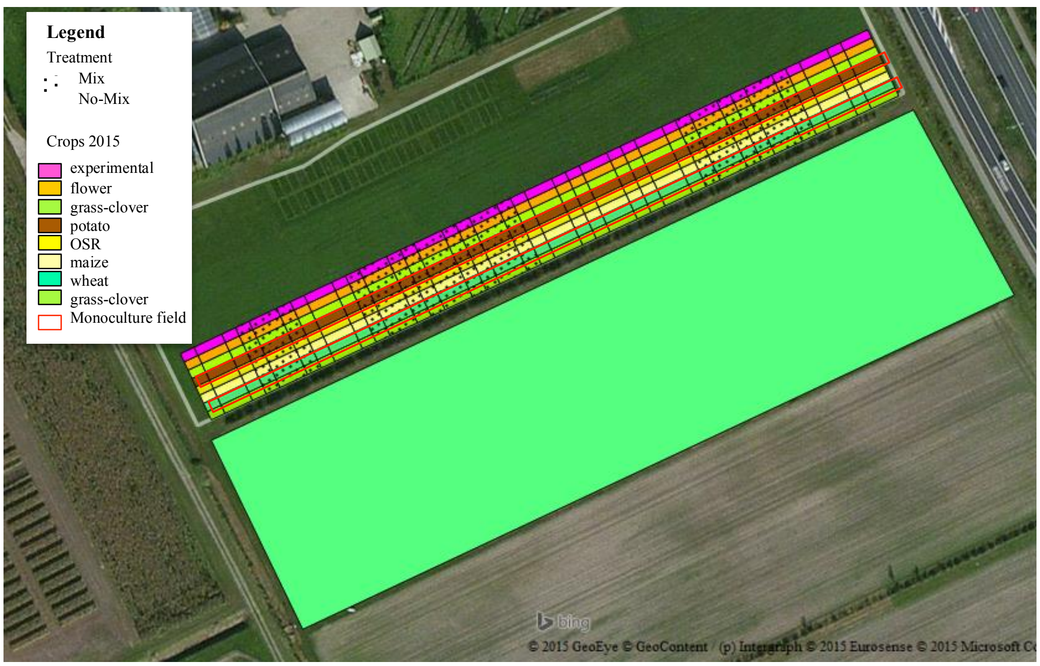


*Figure 1. Layout of the experimental site. Wheat and potato strips (outlined in red), potato experimental strip (pink strip), and monoculture wheat field (highlighted in green).*

### Experimental design

The experimental design of the strip cropping system had in total 6 crop strips and a flower strip of 250 meters long. Each crop strip had two different crop mixing treatments. No-mix treatment, with one single crop or limited mixing, identified in this research as *strip* scale crop diversification. And mix treatment, with two different crops or different varieties, identified in this research as *plant* scale crop diversification (Annex i). Each treatment had 6 replicates (plots), with a total of 12 plots per strip. In three blocks, two replicates of each treatment were randomly allocated. Each plot was 3 meters wide and 20 meters large, including a buffer area of 5 meters at the start and end of each plot. Leaving a total experimental area of 3 meters x 10 meters (Annex ii).

The experimental design of the potato experimental strip had the same dimensions and number of plots that the strips in the strip cropping system. It had only one variety of potato (Toluca) that was under 3 different types of manuring and mulching system, identified in this research as: *mulching* (single variety with minimum tillage, straw mulching and grass-clover green manure), *minimum tillage* (single variety with minimum tillage and grass-clover green manure), and *tillage* (single variety with tillage and grass-clover green manure).

The monoculture wheat field had a total area of 70 x 250 meters and was separated from the strip field by a ten-year old edge located some meters after the strip field border (grass-clover strip). The samplings sites were located in two strips, at the same distance from the hedge as wheat strip (6 meters) and 34 meters from the edge, respectively. The sampling site 34 m from the edge was located in the middle of the field. Each strip was sampled at the same location relative to the wheat *strip* treatment (Table 1).

**Table 1: Scale and crop diversification of the crops of study, wheat and potato**

| Crop | Treatment code | Crop/disturbance diversification |
|---|---|---|
| Wheat | Field | Monoculture field, *Triticum aestivum* (Lennox) |
| | Strip | Strip cropping, no-mix, *Triticum aestivum* (Lennox) |
| | Plant | Strip cropping, mix, *Triticum aestivum* (CCP), *Vicia faba* (Nile) |
| Potato | Strip[1] | Strip cropping, no-mix, *Solanum tuberosum* (Toluca), FYM |
| | Plant[1] | Strip cropping, mix, *Solanum tuberosum* (Toluca, Tiamo, Anabelle, Ditta), FYM |
| | Mulching[2] | Strip cropping, single crop, *Solanum tuberosum* (Toluca), minium tillage, green manure + straw+winter rye. No additional ridging for weed control |
| | Tillage[3] | Strip cropping, single crop, *Solanum tuberosum* (Toluca). Green manure |
| | Minimum tillage[4] | Strip cropping, single crop, *Solanum tuberosum* (Toluca), minimum tillage with green manure |

Manuring and mulching specifications: [1]Farm yard manure, [2]minimum tillage, straw mulching and grass-clover green manure, [3]tillage and grass-clover green manure, [4]minimum tillage and grass-clover green manure

## Sampling methods

In this research, the objective was to evaluate the effect of crop diversification and soil disturbance on population dynamics of aphid pests and natural enemies on different treatments of wheat and potato in a complex cropping system. The following table presents the specifics of the sampling groups:

**Table 2. Groups studied in wheat and potato, type of sampling and sampling season**

| Variable | Sampling | Sampling season | |
|---|---|---|---|
| | | Wheat | Potato |
| 1) Aphid density | Visual assessment | Growing season | Mid to end of growing season |
| 2) Plant-dwelling natural enemies density | Beat sampling | mid to end season | Mid to end of growing season |
| 3) Ground-dwelling natural enemies density | Pitfall trap sampling | Growing season | Mid to end of growing season |

All the samplings were conducted every two weeks throughout the corresponding sampling season of the crops. For wheat, visual assessment and pitfall sampling started once 50% of the plants from the strip had germinated. Beat sampling, started once the plants had grown enough to not suffer damage from the beating. For potato, samplings started around the mid of the growing season (Table 3, Figure 2). Due to the arrival of *Phytophtora,* the potato strips had to be burned which resulted on ending the samplings earlier than expected. The sampling sites were selected randomly from a list of pre-determined sampling sites for this project.

**Table 3. Starting and ending dates of sampling in wheat and potato for year 2015**

| Crop | Sampling | Starting date | Julian day | Ending date | Julian day |
|---|---|---|---|---|---|
| Wheat | Visual assessment | May 07 | 127 | August 12 | 224 |
| | Beat sampling | June 20 | 171 | August 12 | 224 |
| | Pitfall trap sampling | May 04 | 124 | August 24 | 236 |
| Potato | Visual assessment | July 03 | 184 | July 31 | 212 |
| | Beat sampling | July 03 | 184 | July 31 | 212 |
| | Pitfall trap sampling | July 13 | 192 | July 27 | 208 |

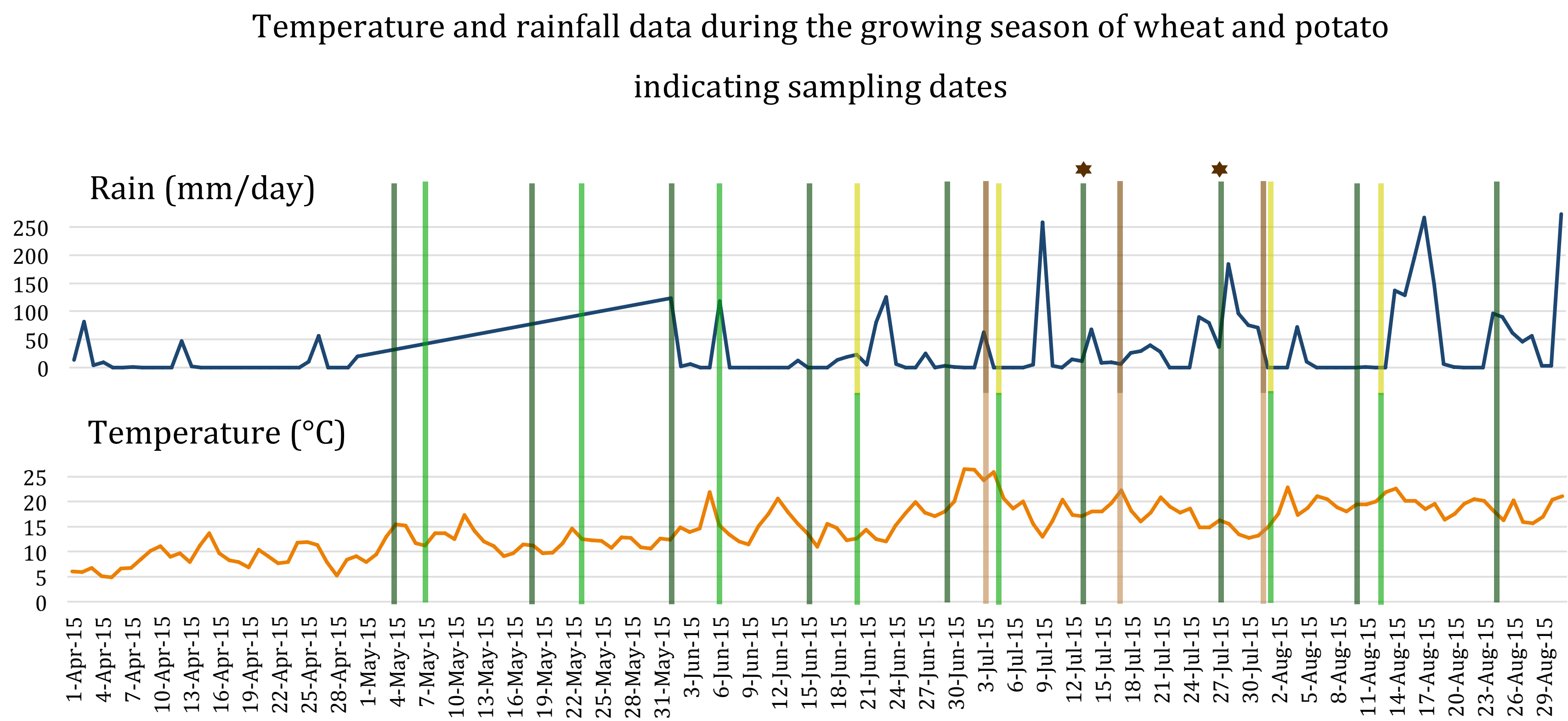


*Figure 2: Temperature (℃) and rainfall (mm/day) data from the weather station Veenkamp during the growing season of wheat and potato indicating sampling dates (pitfall trapping, visual assessment and beat sampling) as following: WHEAT ■ Pitfall sampling, ■ Visual assessment, ■ Beat sampling. POTATO ✶ Pitfall sampling, ■ Visual assessment, ■ Beat sampling.*

For wheat there were in total 6 replicates at the *strip* and *plant* level, and 6 replicates at the *field* level. For potato there were in total 6 replicates at the *strip* and *plant* level, and 4 replicates for the *mulching, minimum tillage* and *tillage* treatments.

### *Visual assessment*

Pest and natural enemy densities were estimated by assessing aphid and parasitized aphid densities. The estimation was done by visual observation of winged aphids, wingless aphids, and parasitized aphids (or mummies) on wheat and potato plants. For wheat, 10 plants were randomly selected on a square area of 0.5 m x 0.5 m of the sampling site (per plot). For potato, from the two rows adjacent to the sampling site, 10 plants were randomly selected and 5 random branches of each plant were used for the sampling (per plot). The sampling was done under dry weather conditions. For wheat, a total of 8 visual days of observations were completed, between 07th May and 12th August 2015, at 2-week intervals. For potato, 3 visual days of observations, between 03th July, 17th July and 31st July, 2015

### *Beat sampling*

Plant-dwelling natural enemies were recorded by beat sampling. Per plot, the sampling was done along the middle row, parallel to the length of the field. Wheat plants were beaten 10 times with a bamboo stick over a 1-meter row. Potato plants were shaken by hand 10 times (for potato) over a 1-meter row. The falling arthropods were collected in a 250 x 150 x 10 centimetre white box and counted *in situ*. Following Wade et al 2006 sampling was done under dry weather conditions (Wade et *al*., 2006). For wheat, a total of 5 beat days of sampling were carried out, between 20th June and 12th August 2015. For potato, 3 beat days of sampling, between 04th July and 31st July 2015.

The arthropods were subdivided in functional groups of natural enemies as followed: spiders, harvestmen, damsel bugs, ladybeetles (pupae, larvae and adult), lacewings (pupae, larvae and adult), and hoverflies (pupae, larvae and adult).

### *Pitfall sampling*

Ground dwelling natural enemies were recorded by pitfall trap sampling. Per sampling site, one roofed pitfall trap was placed. Each pitfall trap (Ø 8 ½ cm) was filled with 100 ml of preservative solution (propylene phenoxetol, propylene glycol, and water in the ration 1:9:90) and each pitfall trap was covered with a roof (Ø 12 ½ cm) to prevent flooding due to rain. In the strip cropping system, per sampling, there were 1 pitfall x 2 treatments (monoculture and intercropping) x 6 replicates = 12 pitfalls per strip. In the monoculture wheat field there were 1 pitfall x 2 distances from hedge x 1 treatment (monoculture) x 6 replicates = 12 pitfalls. And in the experimental potato strip there were 1 pitfall x 1 strip x 3 treatments (tillage, mulching and manuring) x 4 replicates = 12 pitfalls. The pitfalls were left in the field for two whole days. The arthropods collected were preserved in alcohol 70% for later identification. For wheat, a total of 9 pitfall trap day samplings were done, between 04th May and 24th August 2015. For potato, 2 pitfall trap samplings, between 13th July and 27th July, 2015. The arthropods collected were identified at different groups, levels and life stage as shown in Table 4.

**Table 4: Identification groups, levels and life stage of ground-dwelling arthropods collected by pitfall sampling in wheat and potato**

| Groups | Level of identification | Life stage |
|---|---|---|
| Spiders | Order: Aranea | Adult |
| Harvestmen | Order: Opiliones | Adult |
| Ground beetles | Family: Carabidae, Genus: *Agonum, Amara, Calathus, Clivina, Loricera, Poecilus, Pterostichus, Pseudophonus* | Larvae, adult |
| Rove beetles | Family: *Staphylinidae* | Larvae, adult |
| Hoverflies | Family: *Syrphidae* | Pupa, larvae |
| Lacewing | Family: *Chrysopidae* | Pupa, larvae, adult |
| Earwig | Order: *Dermaptera* | Adult |
| Centipede | Class: *Chilopoda* | Adult |
| Damsel bug | Family: *Nabidae* | Adult |
| Ladybeetle | Family: Coccinellidae | Pupae, larvae, adult |

### Statistical analysis

The statistical analysis for the three groups of study (aphid density, plant-dwelling natural enemies density, and ground-dwelling natural enemies density) was conducted with the statistical program R (R Core Team, 2015). The initial data exploration showed that the count data were not normally distributed (histogram frequency representation, R's package MASS), which is common when working with biological count data. Therefore, the data analysis was done by using Generalized Linear Models (GLMs) that allows to work with response variables that have non-normal error distributions, which are often common when working with ecological data (Guisan et *al.*, 2002). The distribution families used for the GLM analysis were Poisson or Negative Binomial Distribution. The selection of the best model was based on the Akaike information criterion (AIC) by selecting the model with the lowest AIC value. The explanatory variables used for the selection of the best model were: Plot (as integer), Treatment (as factor) and Julian day (as integer). Interactions between the variables were considered. From the selection of the best model (AIC) it was possible to determine if there were any significant interactions. If the best model selection did not present any significant interactions then in the final analysis the variables were consider as independent. The "dredge" function from the R's MuMin-package was used to rank the models according to the AIC value. The first analysis showed that there was no interaction between Plot and Julian day. So the base model used for the analysis was (treatment-1)*(plot+julian day). For comparison between treatments and more detailed information about the difference among treatments, a multi-comparison of pairwise treatments by post-hoc test with Tukey contrast was conducted from the best model previously selected. For both aphids and natural enemies the analysis was conducted using the mean values of the samplings per treatment per day. The R´s multcomp-package was used to run the post-hoc test.

## RESULTS

### Wheat

#### *Aphid density*

In total, 868 aphids were counted by visual observation on 5,040 standing wheat plants (10 plants x 12 plots strip cropping x 6 plots field x 7 sampling days). From those, 245 aphids were observed in *field-edge* treatment, 194 in *field-middle* treatment, 132 in *strip* treatment and 297 in *plant* treatment. The mean aphid density on wheat for all treatments tend to increase over the sampling season until reaching a maximum point around June 18th (Julian day 169) and from there it started to decrease getting to values close to zero after July 22nd (Julian day 203) (Fig. 3). At Julian day 169, aphid density was highest at *plant* level (faba-wheat intercropping) (2.73±0.449) while *strip* level (single wheat strip cropping) had the lowest mean aphid density (0.850±0.334).

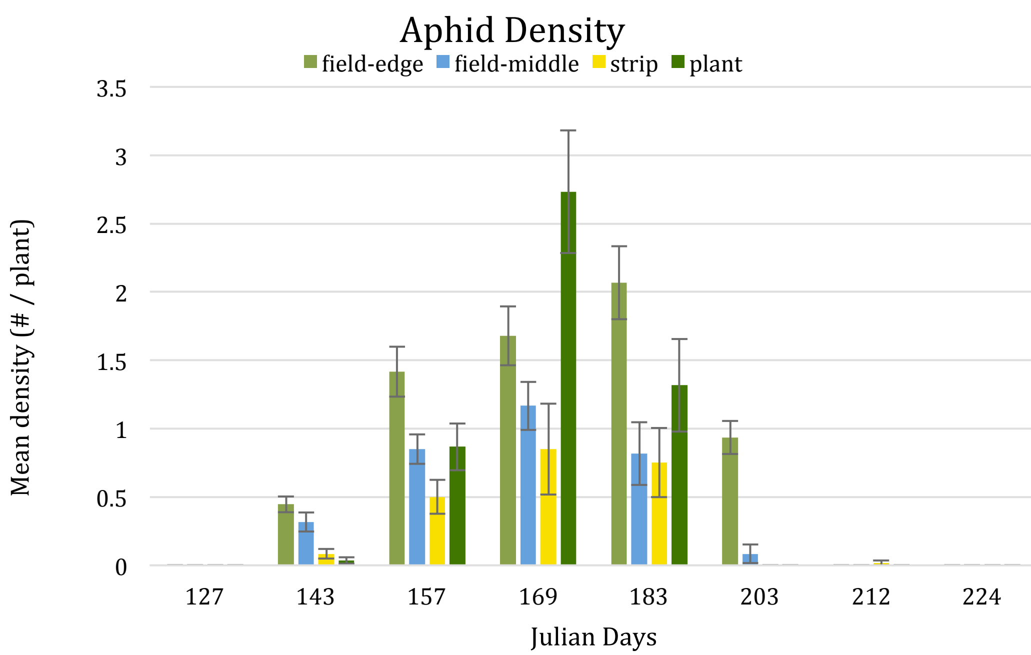


*Figure 3. Aphid mean density (# / plant) of four treatments in wheat during the sampling season (Julian Days). The bars in each column represent the standard error (SE).*

Regression analysis using a negative binomial error distribution indicated that Julian day and Treatment had a significant effect on aphid density (Table 5). Pairwise comparison indicated that aphid density was significantly higher at *plant* level than at *strip* level (p<0.001), and *strip* level was significantly lower than *field-edge* level (p=0.0115).

**Table 5: GLM: negative binomial distribution for wheat aphids. Best model results AICc = 2981.7, aphids = (Treatment-1) + Julian day.**

| Explanatory variable | Estimate | Std. Error | z value | Pr(>\|z\|) | Sig. |
|---|---|---|---|---|---|
| Treatment field-edge | 2.671 | 0.39 | 6.82 | 8.8e-12 | *** |
| Treatment field-middle | 2.419 | 0.39 | 6.19 | 6.2e-10 | *** |
| Treatment plant | 2.870 | 0.39 | 7.33 | 2.37e-13 | *** |
| Treatment strip | 2.069 | 0.39 | 5.29 | 1.22e-07 | *** |
| Julian day | -0.019 | 0.00 | -9.01 | <02e-16 | *** |

Significance codes: p<*0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

### *Aphids density: Parasitized aphids*

Parasitized aphids started to be present at June 18th (Julian day 169), but only in the *plant* treatment. A total of 124 parasitized aphids were counted by visual observation. Out of those, 44 parasitized were observed in *field-edge* treatment, 27 in *field-middle* treatment, 23 in *strip* treatment and 30 in *plant* treatment. At July 2nd (Julian day 183) the mean densities were at their maximum for all the treatments, followed by a drastic drop in the values for the next weeks (Fig. 4). At Julian day 183, the *field-edge* treatment had the highest parasitism rate (*0.633±0.116)*, followed by *field-middle treatment* (*0.416±00.023*), *plant* treatment (0.35±*0.085*) and at last *strip* treatment (*0.300±0.*085).

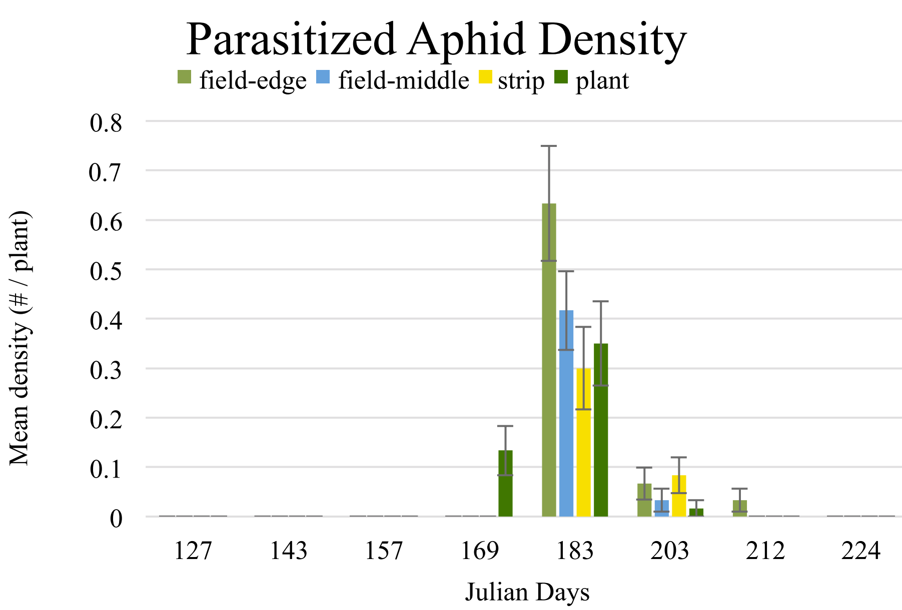


*Figure 4. Parasitized aphid mean density (# / plant) of four treatments in wheat during the sampling season (Julian days). The bars above each column represent the standard errors (SE).*

Regression analysis using a negative binomial error distribution indicated that Julian day and Treatment had a significant effect on parasitized aphid densities (Table 6). The estimate of parasitized aphids appears to decrease in time. And from the pairwise comparison it resulted in no significant differences between treatments.

**Table 6: GLM: negative binomial distribution for wheat parasitized aphids.**
**Best model results AICc = 891.42, pararasitized aphids = (Treatment-1) + Julian day.**

| Explanatory variable | Estimate | Std. Error | z value | Pr(>\|z\|) | Sig. |
|---|---|---|---|---|---|
| Treatment field-edge | -4.353 | 0.70 | -6.21 | 5.28e-10 | *** |
| Treatment field-middle | -4.834 | 0.71 | -6.76 | 1.37e-11 | *** |
| Treatment plant | -4.711 | 0.71 | -6.62 | 3.47e-11 | *** |
| Treatment strip | -5.006 | 0.72 | -2.94 | 3.90e-12 | *** |
| Julian day | 0.011 | 0.00 | 2.959 | 0.003 | ** |

Significance codes: p*<0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

Parasitized aphid density lagged two weeks from the (unparasitized) aphid density. By Julian day 183, when parasitized aphids were at their maximum density, in *field-edge* 59.4% of the observed aphids were parasitized, in *field-middle* 51.0%, in *strip* 40.0% and in *plant* 26.6%. For the four treatments parasitized aphids peaked when the aphid density was decreasing.

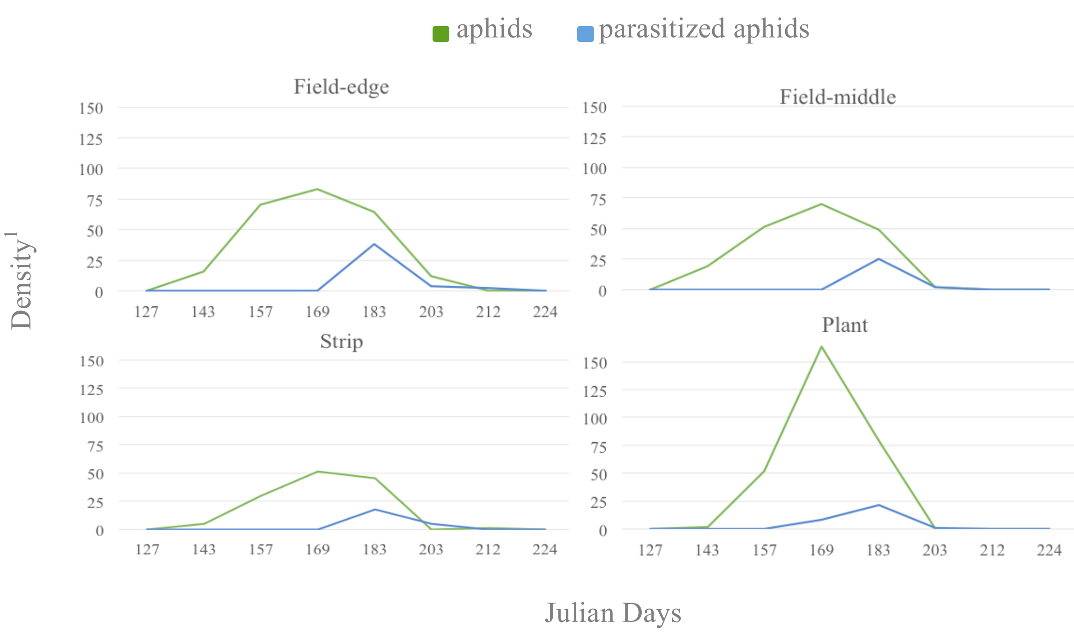


*Figure 5 Number of aphids and parasitized aphids observed in four treatments of wheat by visual assessment during the sampling season (Julian days). [1]Density estimated from the number of aphids and parasitized aphids found in 60 plants.*

*Plant-dwelling natural enemies*

In total, 143 plant-dwelling natural enemies were counted by beat sampling on wheat plants. From those, 23 natural enemies were observed in *field-edge* treatment, 20 in *field-middle* treatment, 42 in *strip* treatment and 58 in *plant* treatment. The density of plant-dwelling natural enemies increased over time and there was an early arrival to the strip cropping system in comparison to the monoculture wheat field. *Plant* treatment had the highest mean density in comparison to the other three treatments, reaching its maximum (*2.50±0.806*) at July 31st (Julian day 212) and maintaining it until August 12th (Julian day 224). The densities of the rest of the treatments were as followed: *strip* (*1.50±0.0.500*), *field-middle* (*1.33±0.0.333*) and *field-edge* (*0.83±0.0.307*) (Fig. 6).

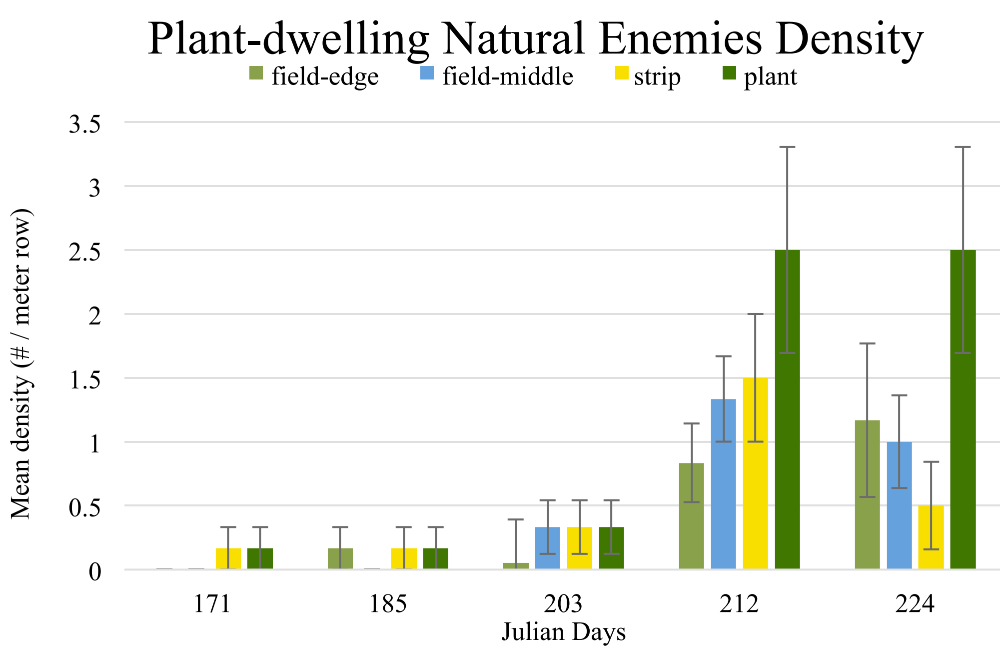


*Figure 6. Plant-dwelling natural enemies density (# / plant) of four treatments in wheat during the sampling season (Julian days). The bars above the columns represent the standard error (SE).*

Regression analysis using a negative binomial error distribution indicated that plant-dwelling natural enemies density were influenced by Julian day and Treatment. The *plant* treatment appears to have a higher density of plant-dwelling natural enemies in comparison with the other treatments. However, after testing for the pairwise comparison it showed that there were no significant differences between treatments.

**Table 7: GLM: negative binomial distribution for wheat plant-dwelling natural enemies. Best model results AICc = 238.72, Plant-dwelling natural enemies = (Treatment-1) + Julian day**

| Explanatory variable | Estimate | Std. Error | z value | Pr(>\|z\|) | Sig. |
|---|---|---|---|---|---|
| Treatment field-edge | -12.015 | 1.97 | -6.08 | 1.1e-09 | *** |
| Treatment field-middle | -12.020 | 1.97 | -6.08 | 1.17e-09 | *** |
| Treatment plant | -11.275 | 1.95 | -5.75 | 8.46e-09 | *** |
| Treatment strip | -11.974 | 1.97 | -6.06 | 1.32e-09 | *** |
| Julian day | 0.054 | 0.01 | 5.97 | 2.3e-09 | *** |

Significance codes: p<*0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

From the total count of above ground enemies, 4 natural enemy groups where identified in *strip* treatment, 5 groups were identified in *field-edge* treatment, and 6 groups were identified in *plant* and *field-middle* treatments. From the beat samplings, neither hoverfly nor lacewing were found in *strip* treatment (Fig. 7).

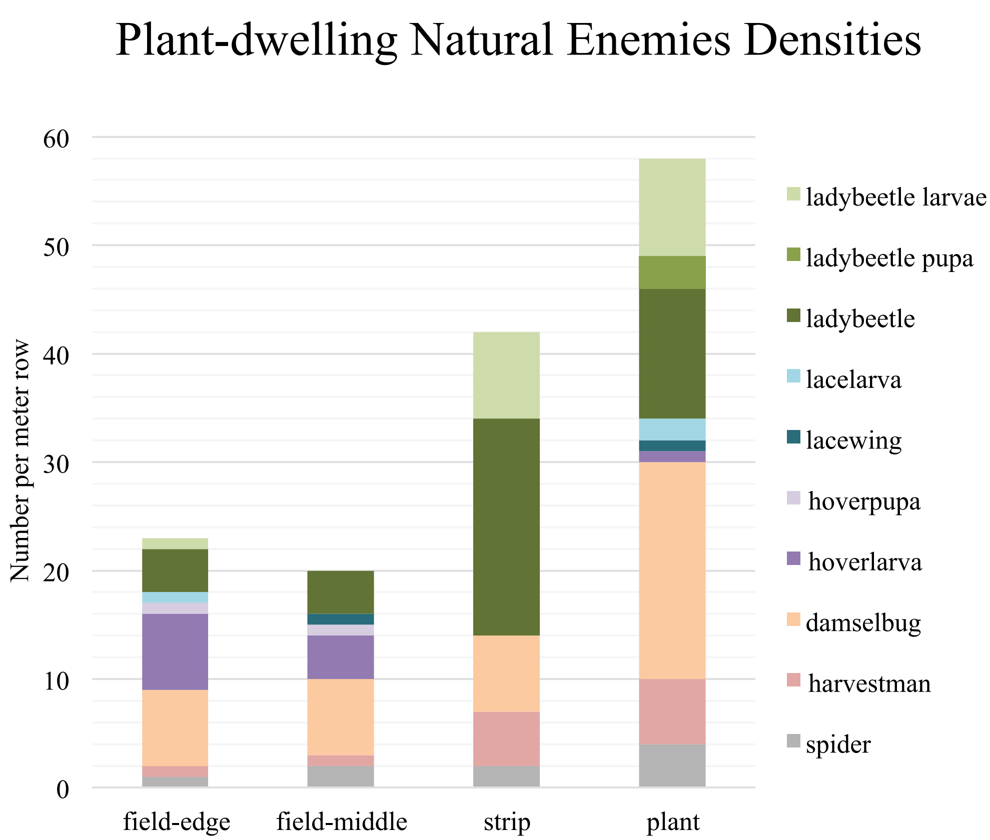


*Figure 7: Plant-dwelling natural enemies activity density (# / 1m$^2$) of four treatments in wheat, during the sampling season. In total 6 groups of plant-dwelling natural enemies were identified from the whole beat sampling.*

### *Ground-dwelling natural enemies*

In total, 3382 ground-dwelling natural enemies were captured by pitfall sampling. Out of those, 678 natural enemies were observed in *field-edge* treatment, 857 in *field-middle* treatment, 894 in *strip* treatment and 953 in *plant* treatment. The ground-dwelling natural enemies present variability on the mean densities along the sampling season (Fig. 8).

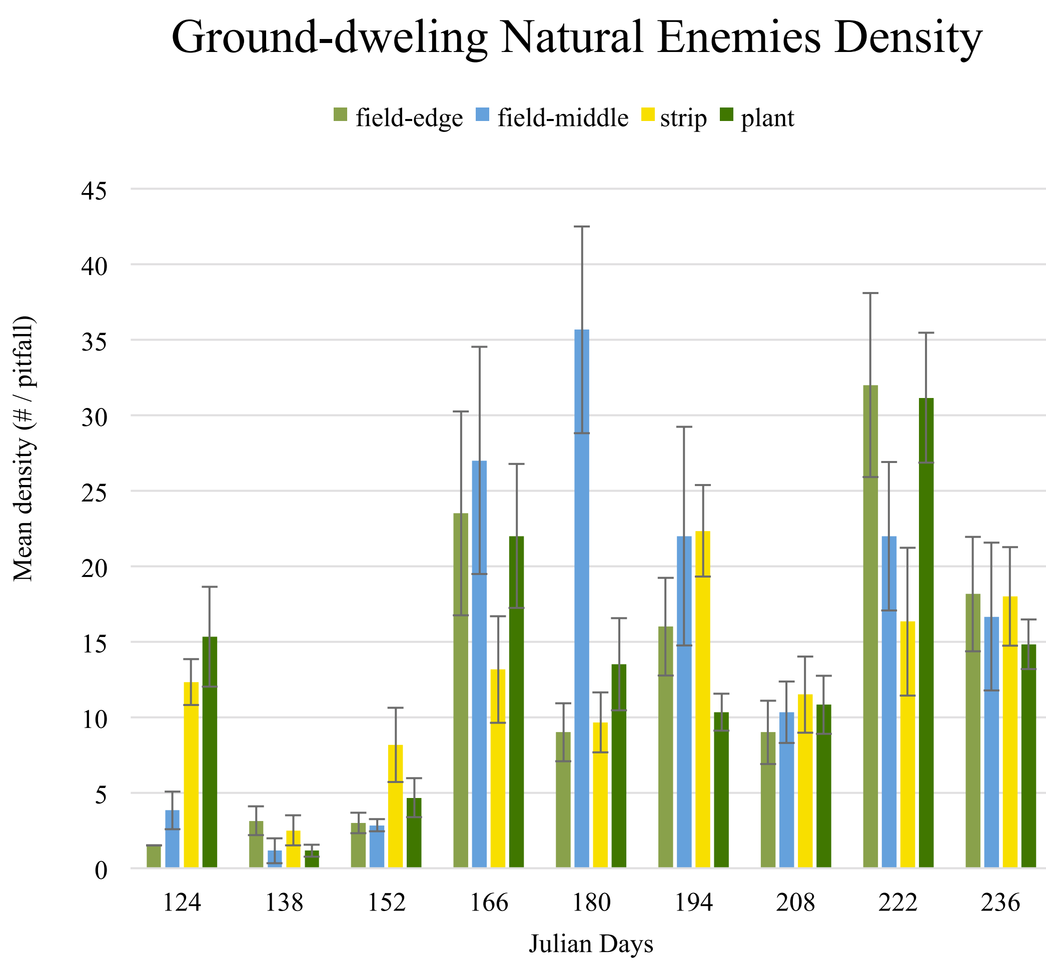


*Figure 8. Ground-dwelling natural enemies density (# / plant) of four treatments in wheat during the sampling season (Julian days). The bars above each column represent the standard error (SE).*

Regression analysis using a negative binomial error distribution indicated that densities of ground-dwelling natural enemies were influenced by Julian day (Table 8). However, after making a pairwise comparison it showed that there were no significant differences between treatments.

**Table 8: GLM: negative binomial distribution for wheat ground-dwelling natural enemies. Best model results AICc = 1548.3, Ground-dwelling natural enemies = Julian day**

| Explanatory variable | Estimate | Std. Error | z value | Pr(>\|z\|) | Sig. |
|---|---|---|---|---|---|
| (Intercept) | 0.447 | 0.304 | 1.46 | 0.142 | |
| Julian day | 0.011 | 0.001 | 7.07 | 1.5e-12 | *** |

Significance codes: p*<0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

From the total count of ground-dwelling natural enemies, 6 natural enemy groups were identified in *field-middle* treatment, 7 groups were identified in *field-edge,* 8 groups were identified in *plant* treatment, and 9 groups were identified in *strip* treatments (Fig. 9).

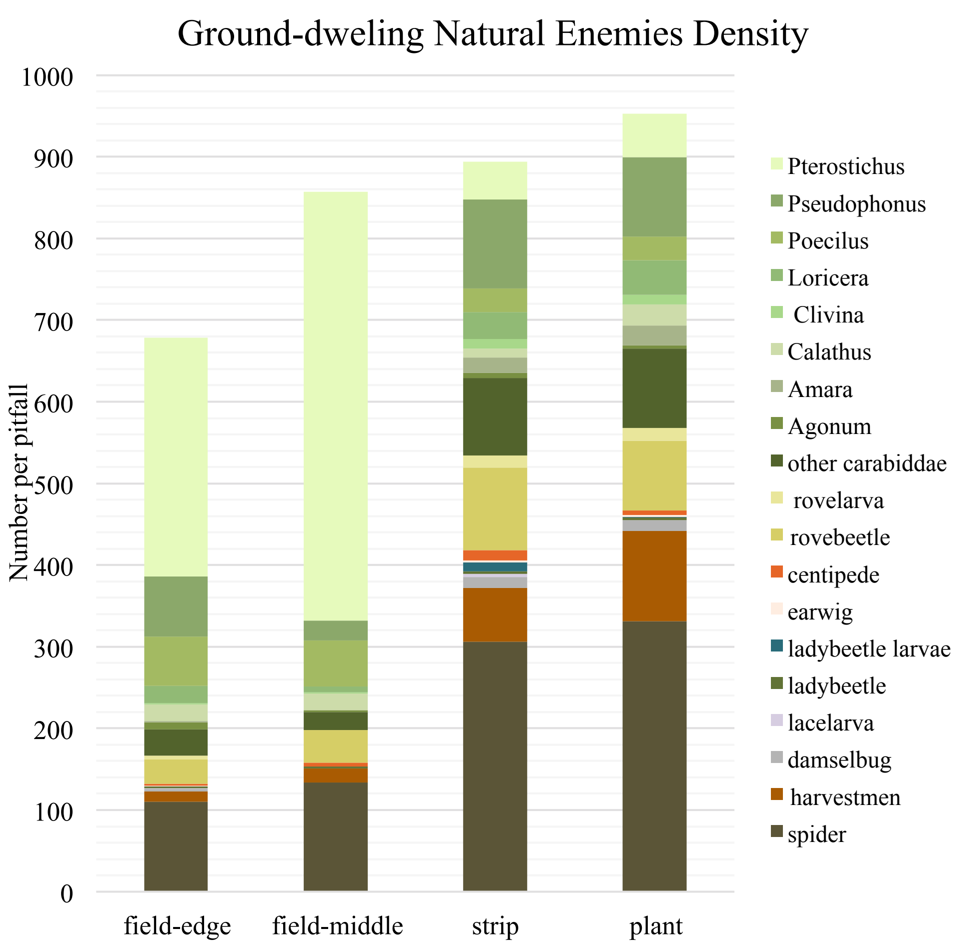


*Figure 9: Ground-dwelling natural enemies activity density (# / pitfall) of four treatments in wheat, during the sampling season. In total 6 groups of plant-dwelling natural enemies were identified from the whole beat sampling.*

## Potato

### *Aphid density*

The samplings on potato started around half way of the growing season on July 3rd (Julian day 184). In total, 8509 aphids were counted by visual observation on potato plants. From those, 3513 aphids were observed in *strip* treatment, 1834 in *plant* treatment, 1239 in *tillage* treatment, 1106 in *minimum tillage* and 817 in *mulching* treatment. The mean aphid density showed its peak for all treatments on the first day of samplings (Julian day 184) and from that point it started to decrease on the following Julian days (Fig. 10). The statistical analysis showed that *strip* treatment presented the highest mean density of aphids (*49.116±5.889*), while *mulching* had the lowest mean aphid density (*19.125±3.422*).

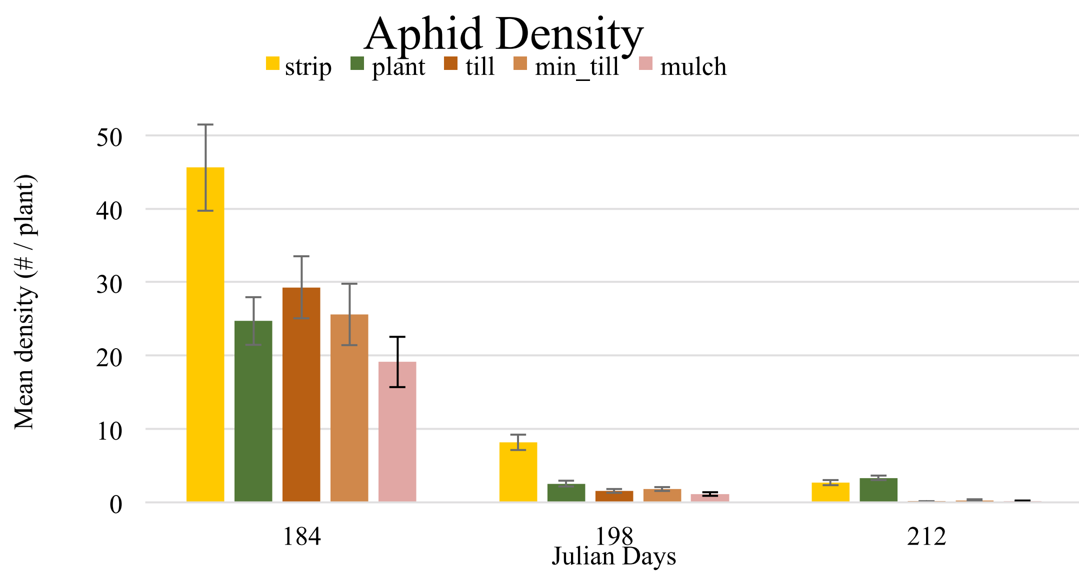


*Figure 10. Aphid mean density (# / plant) of five treatments in potato during the sampling season (Julian Days). The bars above each column represent the standard error (SE).*

Regression analysis using a negative binomial error distribution indicated that the mean aphid densities in potato are influenced by Plot and the interaction between Treatment and Julian day (Table 9). From the pair wise comparison test, both *plant* and *strip* treatments were significantly different from the treatments *tillage, minimum tillage* and *mulching*. Both p*lant* and *strip* aphid densities tended to decrease in comparison with *tillage, minimum tillage* and *mulching* aphid densities. Between *plant* and *strip* treatments there was a very low significant difference and *plant* aphid densities tend to be lower than *strip* aphid densities (Table 10).

**Table 9: GLM: negative binomial distribution for potato aphids. Best model results AICc = 3868.7, aphids = Plot + (Treatment-1) * Julian day.**

| Explanatory variable | Estimate | Std. Error | z value | Pr(>\|z\|) | Sig. |
|---|---|---|---|---|---|
| Plot | -0.035 | 0.01 | -2.63 | 0.009 | ** |
| Treatment mulch | 37.518 | 2.84 | 13.18 | <2e-16 | *** |
| Treatment plant | 16.549 | 1.44 | 11.47 | <2e-16 | *** |
| Treatment strip | 22.010 | 1.42 | 15.41 | <2e-16 | *** |
| Treatment min tillage | 34.700 | 2.47 | 14.03 | <2e-16 | *** |
| Treatment tillage | 40.252 | 2.78 | 14.43 | <2e-16 | *** |
| Julian day | -0.170 | 0.01 | -13.26 | <2e-16 | *** |
| Treatment mulch:Jday | -0.016 | 0.02 | -0.82 | 0.411 | |
| Treatment plant:Jday | 0.097 | 0.01 | 6.59 | 4.1e-11 | *** |
| Treatment strip: Jday | 0.072 | 0.01 | 4.89 | 1.0e-06 | *** |
| Treatment mintil:Jday | -0.028 | 0.02 | -1.49 | 0.135 | |

Significance codes: p*<0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

**Table 10: Pairwise comparison for aphid mean densities of five different treatments on potato plants. Post -hoc with Tukey´s test, on the GLM: negative binomial distribution.**

| Pairwise comparison | Estimate | Std. Error | z value | Pr(>\|z\|) | 95% CI lwr | 95% CI Upr | Sig. |
|---|---|---|---|---|---|---|---|
| mulch - mintill == 0 | -1.85 | 3.16 | -0.58 | 0.976 | -10.43 | 6.73 | |
| plant - mintill == 0 | -16.25 | 2.66 | -6.10 | <0.001 | -23.48 | -9.02 | *** |
| strip - mintill == 0 | -10.91 | 2.65 | -4.10 | <0.001 | -18.12 | -3.70 | *** |
| tillage - mintill == 0 | 3.66 | 3.28 | 1.11 | 0.794 | -5.26 | 12.58 | |
| plant - mulch == 0 | -14.40 | 2.66 | -5.39 | <0.001 | -21.64 | -7.15 | *** |
| strip - mulch == 0 | -9.06 | 2.66 | -3.40 | 0.005 | -16.28 | -1.84 | ** |
| tillage- mulch == 0 | 5.51 | 3.29 | 1.67 | 0.442 | -3.43 | 14.45 | |
| strip - plant == 0 | 5.33 | 2.04 | 2.61 | 0.066 | -0.20 | 10.88 | . |
| tillage- plant == 0 | 19.94 | 2.81 | 7.06 | <0.001 | 12-26 | 27.56 | *** |
| tillage - strip == 0 | 14.57 | 2.81 | 5.18 | <0.001 | 6-94 | 22.20 | *** |

Significance codes: p*<0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

### *Aphids density: Parasitized aphids*

A total of 300 parasitized aphids were counted by visual observation on plants of potato. Out of those, 93 were found on *strip* treatment, 90 on *plant* treatment, 17 on *tillage* treatment, 40 on *minimum tillage* treatment and 39 on *mulching* treatment. Parasitized aphids showed to be at its highest value on July 3rd (Julian day 184) for all treatments, followed by a drop of the values on the next days. For the *tillage, minimum tillage* and *mulching* treatments the drop in the mean densities appeared to be drastic, getting to values close to zero. For *plant* and *strip* treatments the drop went more gradually (Fig. 11).

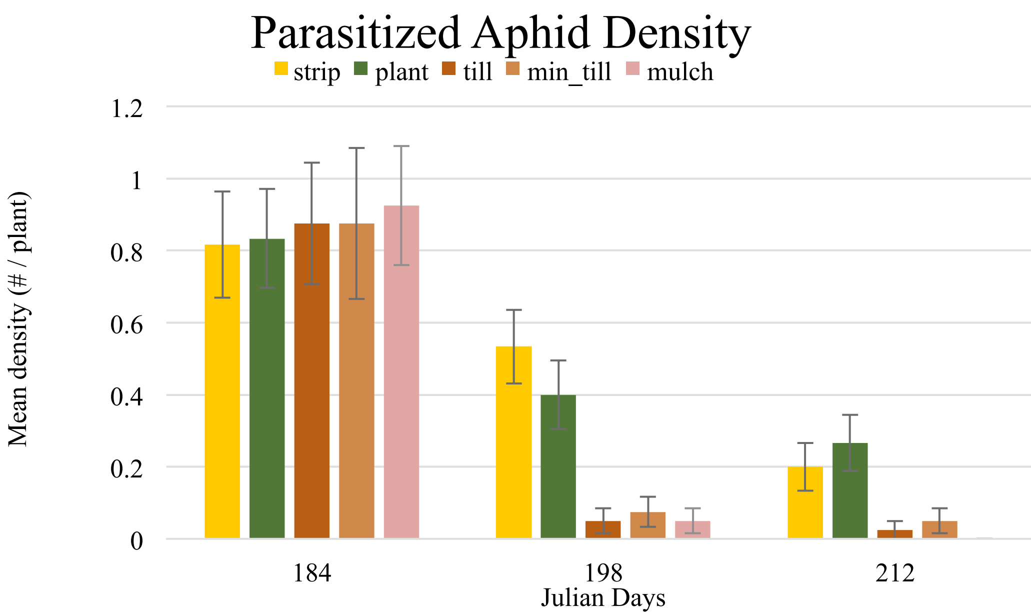


*Figure 11. Parasitized aphid mean density (# / plant) of five treatments in potato during the sampling season (Julian days). The bars above each column represent the standard error (SE).*

Regression analysis using a negative binomial error distribution indicated that parasitized aphid density was influenced by Plot and the interaction between Treatment and Julian day (Table 11). From the pair wise comparison, *plant* treatment showed to be significantly different from treatments *tillage, minimum tillage* and *mulching*. *Strip* treatment presented significant differences when compared with *tillage* and *mulching* treatments, but was just barely significantly different from the treatment *minimum tillage*. Between *plant* and *strip* treatments there were no significant differences (Table 12).

**Table 11: GLM: negative binomial distribution for potato parasitized aphids.**
**Best model results AICc = 1068.9, Parasitized aphids = Plot + (Treatment-1) * Julian day.**

| Explanatory variable | Estimate | Std. Error | z value | Pr(>\|z\|) | Sig. |
|---|---|---|---|---|---|
| Plot | -0.082 | 0.02 | -3.93 | 7.39e-05 | *** |
| Treatment mulch | 40.886 | 9.48 | 4.31 | 1.63e-05 | *** |
| Treatment plant | 8.021 | 2.22 | 3.60 | 0.00031 | *** |
| Treatment strip | 9.177 | 2.22 | 4.12 | 3.78e-05 | *** |
| Treatment min tillage | 24.073 | 5.13 | 4.68 | 2.77e-06 | *** |
| Treatment tillage | 30.695 | 6.67 | 4.60 | 4.22e-06 | *** |
| Julian day | -0.129 | 0.02 | -4.74 | 2.1e-06 | *** |
| Treatment mulch:Jday | -0.089 | 0.06 | -1.59 | 0.124 | |
| Treatment plant:Jday | 0.087 | 0.03 | 2.96 | 0.003 | *** |
| Treatment strip: Jday | 0.082 | 0.03 | 2.76 | 0.006 | *** |
| Treatment mintil:Jday | -0.035 | 0.045 | -0.77 | 0.439 | |

Significance codes: p*<0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

**Table 12: Pairwise comparison for parasitized aphid mean densities of five different treatments on potato plants. Post -hoc with Tukey´s test, on the GLM: negative binomial distribution.**

| Pairwise comparison | Estimate | Std. Error | z value | Pr(>\|z\|) | 95% CI lwr | 95% CI Upr | Sig. |
|---|---|---|---|---|---|---|---|
| mulch - mintill == 0 | 19.88 | 10.27 | 1.93 | 0.272 | -7.59 | 47.36 | |
| plant - mintill == 0 | -13.06 | 4.67 | -2.79 | 0.035 | -25.57 | -0.55 | * |
| strip - mintill == 0 | -11.91 | 4.67 | -2.54 | 0.070 | -24.42 | 0.59 | . |
| tillage - mintill == 0 | 3.66 | 6.40 | 0.57 | 0.976 | -13.48 | 20.80 | |
| plant - mulch == 0 | -32.94 | 9.67 | -3.40 | 0.005 | -58.82 | -7.07 | ** |
| strip - mulch == 0 | -31.80 | 9.67 | -3.28 | 0.007 | -57.67 | -5.93 | ** |
| tillage - mulch == 0 | -16.22 | 10.61 | -1.52 | 0.514 | -44.62 | 12.17 | |
| strip - plant == 0 | 1.148 | 3.14 | 0.36 | 0.995 | -7.26 | 9.56 | |
| tillage - plant == 0 | 16.72 | 5.39 | 3.10 | 0.014 | 2.29 | 31.15 | * |
| tillage - strip == 0 | 15.57 | 5.39 | 2.88 | 0.027 | 1.15 | 30.00 | * |

Significance codes: p*<0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

Aphid's density and parasitized aphids density showed to have a similar pattern of decrease in their values. From the total count of aphids and parasitized aphids, in *strip* 1.45% of the observed aphids were parasitized, in *plant* 4.90%, in *tillage* 3.06%, in *minimum tillage* 3.61%, and in *mulching* 4.77% of the total observed aphids were parasitized.

### *Plant-dwelling natural enemies*

In total, 55 plant-dwelling natural enemies were counted by beat sampling on standing potato plants. From those, 21 natural enemies were observed in *strip* treatment, 16 in *plant* treatment, 6 in *tillage* treatment, 7 in *minimum tillage* treatment, and 5 in *mulching* treatment. The densities of plant-dwelling natural enemies presented different patterns for each treatment. At July 3rd (Julian day 184) *strip* treatment presented the highest mean density of all treatments (1.83±0.307), in the following days it gradually decreased. *Plant* and *mulching* treatments had an increase in their mean densities after Julian day 184. *Minimum tillage* and *tillage* densities drop to zero on July 17th (Julian day 198) and increased again by July 31st (Julian day 212) (Fig.12).

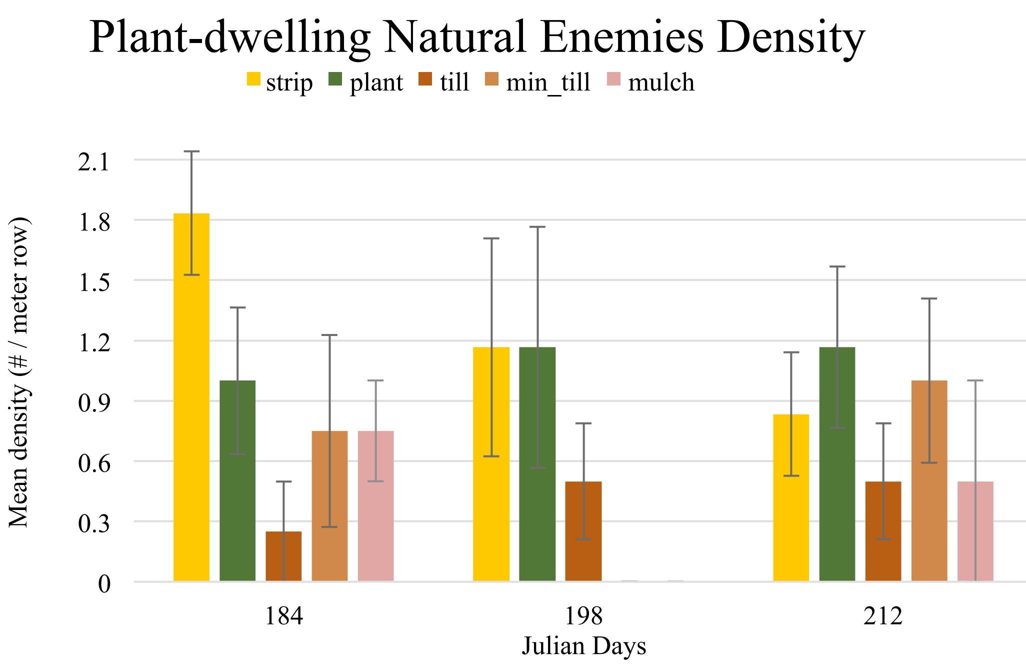


*Figure 12. Plant-dwelling natural enemies mean density (# / plant) of five treatments in potato during the sampling season (Julian days). The bars in each column represent the standard error (SE).*

Regression analysis using Poisson error distribution indicated that plant-dwelling natural enemies density was influenced only by Treatment (Table 13).

**Table 13: GLM: poisson distribution for potato plant-dwelling natural enemies. Best model results AICc = 172.5, Plant-dwelling natural enemies = Treatment-1**

| Explanatory variable | Estimate | Std. Error | z value | Pr(>\|z\|) | Sig. |
|---|---|---|---|---|---|
| Treatment mulch | -0.875 | 0.44 | -1.95 | 0.050 | . |
| Treatment plant | 0.105 | 0.22 | 0.47 | 0.637 | |
| Treatment strip | 0.245 | 0.20 | 1.17 | 0.239 | |
| Treatment min tillage | -0.875 | 0.45 | -01.95 | 0.050 | . |
| Treatment tillage | -0.539 | 0.37 | -1.42 | 0.153 | |

Significance codes: p*<0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

From the total count of plant-dwelling enemies, 3 natural enemy groups where identified in *strip* treatment, 6 groups were identified in *plant* treatment, 2 groups were identified in *minimum tillage,* and 4 groups were identified in *tillage* and *mulching* treatments. From the total count *plant* treatment showed to have the higher representation of natural enemy groups, however, *strip* treatment presented the highest total number of plant-dwelling natural enemies. *Tillage, minimum tillage* and *mulching* treatments remain below half of the maximum value counted. Yet, after testing the pairwise comparison showed that there were no significant differences between any of the treatments (Fig. 13).

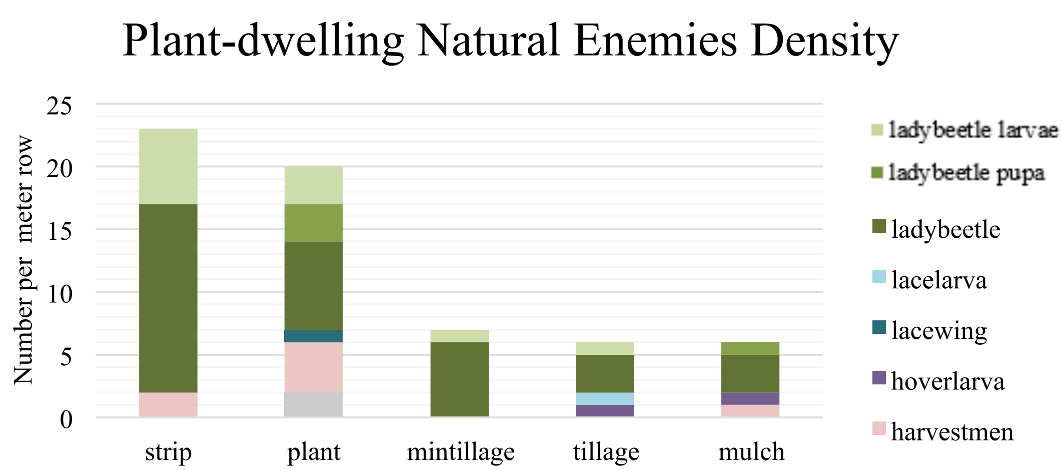


*Figure 13: Plant-dwelling natural enemies activity density (# / 1m$^2$) of five treatments in potato, during the sampling season (Julian days). In total 5 groups of Plant-dwelling natural enemies were identified from the whole beat sampling.*

### *Ground-dwelling natural enemies*

In total, 617 ground-dwelling natural enemies were counted by pitfall sampling. Out of those, 161 natural enemies were observed in *strip* treatment, 184 in *plant* treatment, 77 in *tillage* treatment, 103 in *minimum tillage* treatment, and 92 in *mulching* treatment. The mean density of ground-dwelling natural enemies was variable for each treatment on the two sampling dates. *Mulching* and *tillage* treatments showed an increase, *strip* and *plant* had a small decrease, while *minimum tillage* treatment showed an increase on its mean density (Fig. 14).

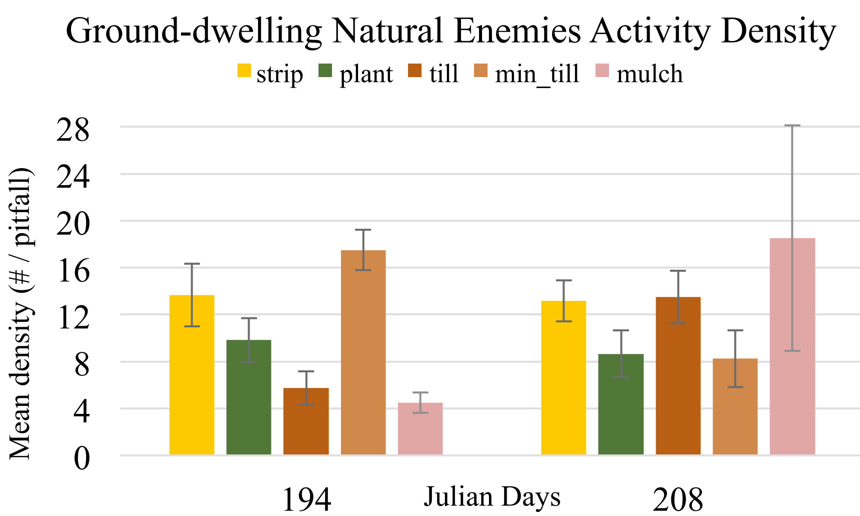


*Figure 14. Ground-dwelling enemies mean density (# / pitfall) of five treatments in potato during the sampling season (Julian days). The bars above each column represent the standard error (SE).*

Regression analysis using a poison error distribution indicated that the densities of ground-dwelling natural enemies are influenced by Plot, Julian day, and the interaction between Treatment and Julian day (Table 14).

**Table 14: GLM: Poission error distribution for potato ground-dwelling natural enemies. Best model results AICc = 1068.9, ground-dwelling nat. enemies = Plot + (Treatment) + Julian day.**

| Explanatory variable | Estimate | Std. Error | z value | Pr(>\|z\|) | Sig. |
|---|---|---|---|---|---|
| Plot | 00.03 | 0.02 | 2.43 | 0.047 | * |
| Treatment mulch | -18.33 | 3.85 | -4.75 | 1.9e-06 | *** |
| Treatment plant | 03.83 | 2.72 | 1.40 | 0.160 | |
| Treatment strip | 02.91 | 2.26 | 1.28 | 0.198 | |
| Treatment min tillage | 13.12 | 2.99 | 4-38 | 1.1e-05 | *** |
| Treatment tillage | -10.30 | 3.62 | -2.84 | 0.004 | ** |
| Julian day | -0.05 | 0.01 | -3.56 | 0.0003 | *** |
| Treatmentmulch:Jday | 0.15 | 0.02 | 6.42 | 1.33e-10 | *** |
| Treatment plant:Jday | 0.05 | 0.02 | 2.20 | 0.0276 | * |
| Treatment strip: Jday | 0.05 | 0.01 | 2.71 | 0.006 | ** |
| Treatment mintil:Jday | 0.11 | 0.02 | 4.92 | 8.76e-07 | *** |

Significance codes: p*<0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

From the pair wise comparison test it showed that *plant* and *strip* treatment were significantly different from *mulching* and *tillage* treatments. And *minimum tillage* was significantly different when compared with *strip* and *mulching* treatments (Table 15).

**Table 15: Pairwise comparison for ground-dwelling mean densities of five different treatments on potato plants. Post -hoc with Tukey´s test, on the GLM: Poisson error distribution.**

| Pairwise comparison | Estimate | Std. Error | z value | Pr(>\|z\|) | 95% CI Lwr | 95% CI Upr | Sig. |
|---|---|---|---|---|---|---|---|
| mulch - mintill == 0 | -31.48 | 4.88 | -6.45 | <0.001 | -44.75 | -18.22 | *** |
| plant - mintill == 0 | -9.32 | 4.05 | -2.303 | 0.1408 | -20.33 | 1.67 | |
| strip - mintill == 0 | -10.25 | 3.75 | -2.726 | 0.0491 | -20.43 | -0.03 | * |
| tillage - mintill == 0 | -23.48 | 4.70 | -4.992 | <0.001 | -36.26 | -10.69 | *** |
| plant - mulch == 0 | 22.16 | 4.72 | 4.694 | <0.001 | 9.33 | 34.99 | *** |
| strip - mulch == 0 | 21.25 | 4.47 | 4.755 | <0.001 | 9.10 | 33.40 | *** |
| tillage - mulch == 0 | 8.00 | 5.29 | 1.513 | 0.5485 | -6.37 | 22.38 | |
| strip - plant == 0 | -0.90 | 3.54 | -0.255 | 0.999 | -10.53 | 8.72 | |
| tillage - plant == 0 | -14.1523 | 4.53 | -3.119 | 0.015 | -26.48 | -1.82 | * |
| tillage - strip == 0 | -13.2479 | 4.27 | -3.099 | 0.016 | -24.86 | -1.63 | * |

Significance codes: p*<0.001='***', p<0.01='**', p<0.05='*', p<0.1='.'*

From the total count of ground-dwelling natural enemies, 8 natural enemy groups were identified in *strip* treatment, 7 groups were identified in *plant* and *mulching* treatment, and 5 groups were identified in *tillage* and *minimum tillage* treatments (Fig. 15). *Plant* treatment showed to have the highest representation from the total count of ground-dwelling natural enemies. And from the analysis it showed to be significantly different from *tillage* and *mulching* treatments, but not different from *strip* and *minimum tillage.*

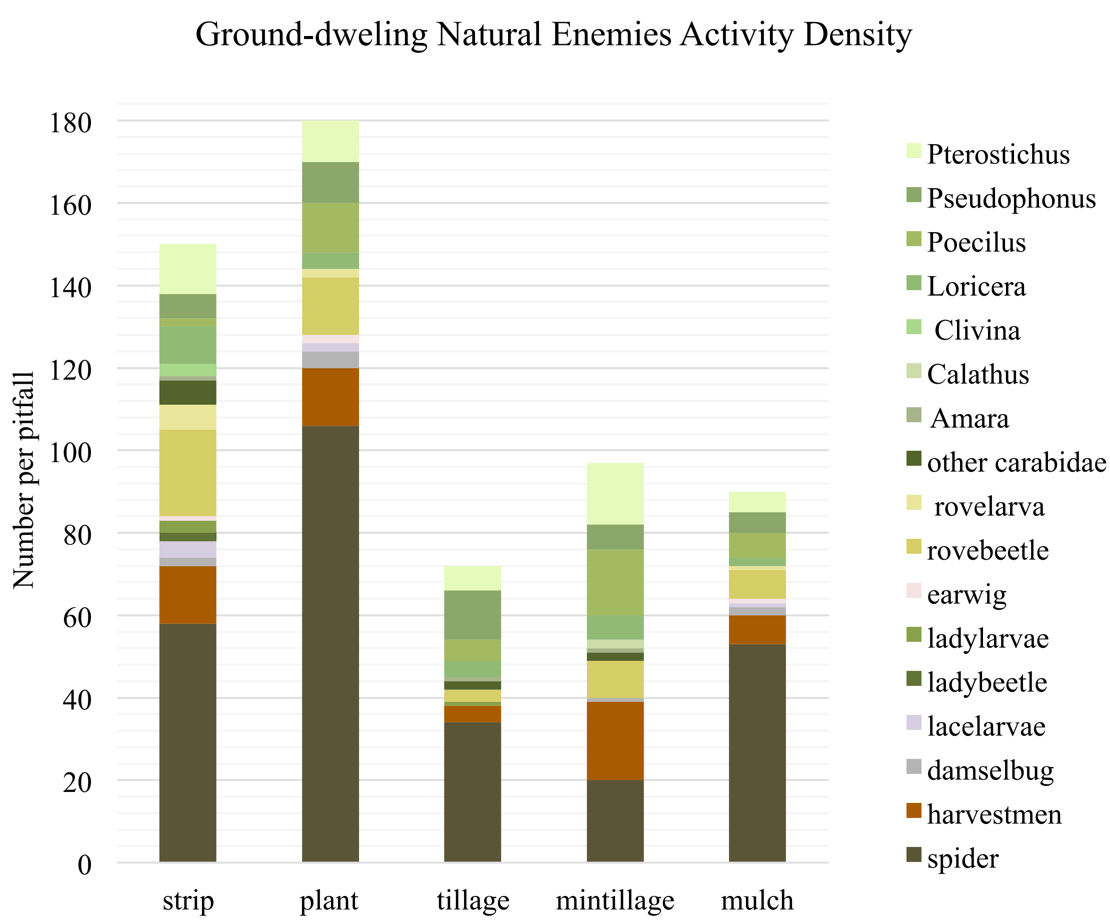


*Figure 15: Ground-dwelling natural enemies activity density (# / pitfall) of five treatments in potato, during the sampling season (Julian days). In total 8 groups of ground-dwelling natural enemies were identified from the whole pitfall trapping.*

# DISCUSSION

The aim of this research was to evaluate the effect of crop diversification (monocropping versus intercropping) and soil management (tillage, manuring and mulching) on population dynamics of aphid pests and natural enemies on different treatments of wheat and potato in a complex cropping system in the Netherlands. According to the hypothesis of this research it was expected that more diverse and complex agricultural systems offer a higher potential for more abundant beneficial arthropods and therefore have a better potential for pest suppression.

For this study two types of cropping diversity were tested. For wheat, three scales of cropping diversity were investigated 1) wheat monocropping at large fields, 2) wheat monocropping at strip scale and 3) by intercropping of wheat and faba bean under a strip cropping system. For potato, cropping diversity was determined by 2 scales of genetic diversity. 1) one cultivar vs 2) variety mixture cropping and 4 different scales of soil disturbance regimes of tillage, manuring and mulching under a strip cropping system.

The results of this research will be discussed as follow. First, the limitations of the approach will be explained. Next, an overview of the results obtained of the effect of cropping diversity and soil disturbance over aphid pests and natural enemies is presented (Table 16). Followed by a comparison of the results with other studies, and finally the conclusions of the research.

## Limitations of the approach

### *Aphids and parasitoids densities vary across years*

Different studies of aphids' populations show there is a clear fluctuation on aphids' population dynamics from year to year. There are years where parasitism is low and the aphid infestation only lasts for a short period of time as for the next year it can be completely the opposite (Sigsgaard, 2002; Leslie et *al.,* 2009). For this study, this `see-saw effect´ is quite noticeable when comparing the results of wheat to the study of last year from Shuang (2015) where the mean aphid density values were about the double. Having this type of continuity of yearly results could give a more clear answer on why the aphid population densities remain low on this research.

### *Biotic and abiotic mortality factors*

Biotic and abiotic factors, such as wind, rain, temperature, drought and light may have potentially influenced the densities of aphid pests and natural enemies of this research. Some authors have shown that biotic and abiotic factors can affect pest outbreaks, parasitoids emergence and other natural enemies presence, which can lead to disproportionately variations on population densities and therefore significantly affect the communities' diversity and evenness (Sigsgaard, 2002). More in detail, a research form Berthe et al. (2015) demonstrated that beetle diversity could be overall reduced by high temperatures. *Pseudoophonus rufipes, Pterostychus melanarius, Calathus fuscipes* and *Harpalus affinis* populations are prone to increase under warmer conditions. Also, an increase in temperature can result in a decrease in parasitoid populations, which can explain why large aphid populations are typically observed during warm seasons (Moiroux, Boivin and Brodeur, 2015). So even when all the sampling sites in this research were under the same weather conditions, the effect of the weather over either aphids populations or natural enemies populations could have influenced their proportions and the relation between them.

### *´Group specific´ effects of agricultural management practices: tillage, manuring and mulching*

Different studies agree that agricultural soil management practices can have a positive effect on the habitat and diversity of predators. Yet, these beneficial effects are more likely to occur in specific groups of predators and parasitoids that have a direct interaction with the soil (Thomson and Hoffman, 2007; Roger-Estrade, 2010; Gill et *al.,*, 2011). Although the data of this research allow evaluating the effect of agricultural soil management practices (e.g. tillage, manuring and mulching) on diversity of natural enemies and their effectiveness on biocontrol, there was insufficient time to conduct this analysis. However, it is suggested to the coordinator of this project to take into account the data for future analysis.

## Overview of the results

*Table 16 Overview of the results obtained of the effect of cropping diversity and soil disturbance over aphids pests and natural enemies of wheat and potato.*

**Table 16. Overview of the results obtained of the effect of cropping diversity and soil disturbance over aphids pests and natural enemies of wheat and potato.**

*Wheat*

**Aphid density**

- Aphid density was significantly higher at *plant* level than at *strip* level.
- *Strip* level was significantly lower than *field-edge* level.

**Parasitized aphids**

- The estimate of parasitized aphids tended to decrease in time.
- No significant differences between treatments.

**Plant-dwelling natural enemies density**

- *Plant* treatment showed to have the higher representation of plant-dwelling natural enemies.
- No significant differences between treatments.

**Ground-dwelling natural enemies density**

- *Plant* treatment showed the highest total number of plant-dwelling natural enemies.
- No significant differences between treatments.

*Potato*

**Aphid density**

- *Plant* and *strip* aphid densities tend to decrease in comparison with *tillage, minimum tillage* and *mulching* aphid densities.
- Low significant difference between *plant* and *strip* treatments.
- *Plant* aphid densities tend to be lower than *strip* aphid densities.

**Parasitized aphids**

- *Plant* treatment was significantly different from treatments *tillage, minimum tillage* and *mulching*.
- *Strip* treatment significant different from *tillage* and *mulching* treatments.
- *Strip* was barely significantly different from the treatment *minimum tillage*.
- No significant differences between *plant* and *strip* treatments.

**Plant-dwelling natural enemies density**

- *Plant* treatment showed to have the higher representation of natural enemy groups.
- *Strip* treatment presented the highest total number of plant-dwelling natural enemies.
- No significant differences between any of the treatments

**Ground-dwelling natural enemies density**

- *Plant* treatment showed to have the highest representation from the total count of ground-dwelling natural enemies
- *Plant* and *strip* treatment were significantly different from *mulching* and *tillage* treatments.
- *Plant* was not significantly different from *strip* and *minimum tillage.*
- *Minimum tillage* was significantly different from *strip* and *mulching* treatments.

## Comparison of results with other studies

## Cropping diversity on aphid and natural enemies density

### *Cropping diversity on aphids density*

Aphid densities in wheat and potato stayed at low levels in all cropping diversity treatments during the whole sampling season. For both crops the mean density values along the sampling season remain below the economic thresholds, for wheat is set at around 3 to 8 aphids per tiller depending on crop stage and for potato around 20 aphids per 100 leafs (Larsson, 2005; UC, 2014). This was not expected but there are studies that confirm that fluctuations on aphid densities and population dynamics are common between seasons and years. Even in agricultural systems with high diversity (e.g. strip cropping) the number of aphids can vary depending on the year (Saguez et *al.,* 2013, Plécas et *al.,* 2014). These fluctuations on aphid densities are referred by some authors as 'see-saw effect', and refer to alternating high and low densities of some aphids' populations between years. This effect can be a consequence of different factors like: weather conditions between years, natural enemies or access to sufficient resources (Leslie et *al.,* 2009).

When comparing aphid densities between cropping diversity treatments, for wheat *plant* was significantly higher aphid density than *strip*, while *strip* was significantly lower aphid density than *field-edge*. *Plant* treatment presented the highest aphid densities while *strip* treatment presented the lowest aphid density. Different studies of agricultural landscape complexity explain that more complex agricultural systems have an enhancing effect over aphids' population growth but it is also expected to have an increase in aphid suppression. More diverse systems offer more alternative resources and non-crop habitats, which may benefit the reproduction and colonization of pests and natural enemies (Plécas et *al.,* 2014, Martin et *al.,* 2015). These studies support the results of high aphid densities in the most diverse treatment (strip cropping), but it differs from the results for parasitized aphids. Even when no significant differences were determined, higher aphid densities were found on *plant* treatment when compared with *field-edge* and *field-middle* treatments, and higher densities were found on *field-edge* in comparison with *field-middle*. Here *field-edge* is considered to have a 'higher diversity' as it is closer to the edge when comparing with *field-middle*. These two examples agree with the tendency of higher aphid densities in the more diverse treatments and it could be said that aphids are benefitting more from the additional resources from the surroundings. In this case, one would expect that densities of parasitized aphids would have higher in the most diverse treatments but this was only true when doing individual comparisons between *plant* treatment and *strip* and when comparing *field-edge* with *field-middle*. In the overall result *field* resulted to have the higher parasitized aphid values, even when no significant differences where determined. It can be considered that parasitoids populations and effective colonization were affected by other factors like the high aphid densities at the faba plants diluting parasitation on the intercropped wheat or predation/protection of ants attracted by aphids excreted honey dew.

For aphid densities in potato the effect of cropping diversity is more difficult to determine, as the dates of sampling were more to the end of the growing season. That is the time when aphid populations are already decreasing. Nevertheless, from the overall results significant differences between some treatments were found. For aphid densities, *plant* appears to be significantly lower than *strip*, and both of them were significantly lower from *tillage, minimum tillage* and *mulching* treatments. And for parasitoid aphid densities, *strip* treatment appear to be significantly lower from *tillage* and *mulching,* and *plant* treatment appear to be significantly lower from *tillage, minimum tillage* and *mulching.* The statistical significant differences showed that the highest aphid densities were found in *strip* treatment and the lowest in *mulching* treatment. Which match with the parasitoid aphid densities that were the lowest in *strip* treatment and the highest in *mulching* treatment. This can be supported by studies where mulching shows to have a negative impact over arthropod pests and a positive impact over predator populations (Johnson, Hough-Goldstein and Vangessel, 2004, Gill, McSorley and Branham, 2011). When comparing aphid densities under *tillage* and *minimum tillage* treatment they show to have a positive effect when compared with *strip* treatment but a negative effect when compared with *plant* and *mulching* treatment. Tillage is an agricultural practice that is known for making a great disturbance in all soil biointeractions. Even when tillage may not have a direct impact over the aphid populations it does have a direct negative effect over ground-dwelling predators (Thomson and Hoffman, 2007; Roger-Estrade, 2010).

### *Cropping diversity on natural enemies*

Natural enemies densities varied for both crops between the plant-dwelling and ground-dwelling samplings. Overall the highest densities of natural enemies were ground-dwelling. This could be related more to the characteristics of the samplings.

When comparing natural enemies densities of wheat, plant-dwelling and ground-dwelling, no significant differences were found between any of the treatments. However, the higher densities where found in *plant* treatment while the lowest where found in *field* treatment. Which was the expected result according to the hypothesis of this research that support that 'more diverse and complex agricultural systems offer higher potential for more diverse beneficial arthropods' (Weibull, Östman and Granqvist, 2003; Burgio, 2015).

For potato plant-dwelling natural enemies no significant differences between any of the treatments were found. *Strip* followed by *plant* treatments presented to have the highest densities, while *tillage, minimum tillage* and *mulching* remain below half of the maximum total count. Tillage, minimum tillage and mulching are agricultural practices directly related to soil, so it is expected that the higher impact will be to soil organisms (Roger-Estrade, 2010; Gill, McSorley and Branham, 2011). This can be the reason why plant-dwelling natural enemies will not be directly affected by the treatments *tillage*, *minimum tillage* and *mulching.* However, for the other two treatments (*strip* and *plant*) the overall results were opposite as expected, as *strip* presented the highest densities of plant-dwelling natural enemies. But when looking at the individual sampling dates it can be identified that it is not the same case in all of them. This may be explained by the effect that biotic and abiotic factors have on the emergence of parasitoids and other natural enemies (Sigsgaard, 2002). Some authors have argued that, specifically for beat samplings, the effectiveness may be affected by the fluctuations of weather conditions (such as temperature, rain and wind). However, if this was the case for this research it would have been expected to have a clear Treatment-Julian day interaction in the results for beat samplings (Wade et *al*., 2006). Still, even when the hypothesis is rejected, for future research it is suggested to take into account these considerations, as well as the increase of sampling efforts.

Ground-dwelling natural enemies on potato do presented significant differences between some of the treatments. *Plant* and *strip* treatments ground-dwelling natural enemies were significantly higher when compared with *mulching* and *tillage* treatments. And *minimum tillage* treatment ground-dwelling natural enemies were significantly higher when compared with *strip* and *mulching treatment.* As mentioned before *tillage, minimum tillage* and *mulching* are agricultural practices that due to their characteristics have a greater impact on soil biointeractions and diversity (Roger-Estrade, 2010; Gill, McSorley and Branham, 2011).

Different studies have shown that mulching is an agricultural practice that can have a positive effect over predators and parasitoids abundance. But there are certain groups of predators, which tend to be more positively affected by mulching (e.g. Carabidae beetles and spiders). While some studies show that parasitoids wasps remain unaffected (Johnson, Hough-Goldstein and Vangessel, 2004; Thomson and Hoffman, 2007; Gill, McSorley and Branham, 2011). However, the results of this research are not in line with other studies, as the overall densities of ground-dwelling natural enemies of *mulching* treatment remain lower when compared to *plant* and *strip* treatment. As mentioned before some ground-dwelling natural enemies populations are affected by climate conditions (e.g. temperature) and it has been shown that mulch has an effect over soil temperature and humidity creating a microclimate (Johnson et *al.,* 2004). It could be argued that the microclimate created by mulch was not the much favourable for the ground-dwelling natural enemies during this research.

Decreasing in tillage practices, showed to have a general positive effect over the increase in abundance and diversity of natural enemies. Tillage has negative effects over some natural enemies. For example, predators are very sensitive to tillage, because it directly affects the soil food web (Thomson and Hoffman, 2007; Lalonde et *al.*, 2011). Which agrees with the results of this research as *tillage* treatment presented the lowest densities of ground-dwelling natural enemies in comparison to the rest of the treatments. Which may have an indirect negative effect over the control of aphid populations, as mentioned before when discussing about parasitized aphid densities (Thomson and Hoffman, 2007; Roger-Estrade et *al.*, 2010).

## CONCLUSIONS

From this research it can be concluded that there is a tendency to have more controlled aphid pests populations and more abundant natural enemies populations in agroecosystems with higher diversification. However, the abundance of both aphid pests and natural enemies appear to be influenced also by the type of agricultural management practices.

Aphid pest's populations in wheat resulted to be higher in the most diverse treatments; this could mean that aphids are benefitting more from the additional resources from the surroundings. However, parasitized aphid densities were not correlated with the aphid densities. The less diverse treatments presented higher densities of parasitized aphids. In this case the aphid densities in the other crops of the strip cropping system may have influenced the effective colonization of parasitoids populations in wheat. Aphids pest's populations in potato appear to be lower in the most diverse treatments and parasitized aphids appear to be higher in the most diverse treatments, which indicates that soil management practices that enhance soil biodiversity such as mulching have a negative impact aphids pests and a positive impact over aphid parasitoids.

From plant-dwelling and ground-dwelling natural enemies the results presented a tendency of higher abundance in more diverse treatments for both wheat and potato that can lead to a more stable biocontrol of aphid pests. But when it comes to define the degree of diversity in the agroecosystem taking into account soil management practices is important in order to boost natural enemies that are directly or indirectly related to soil composition and soil biointeractions. From potato ground-dwelling natural enemies results it was concluded that mulching not only have an impact over the soil biointeractions but it has an effect over soil temperature and humidity creating a microclimate which can have either positive or negative effects over natural enemies abundance.

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## ANNEXES

### Annex i. Crop species and varieties composition of the strip cropping system in spring 2015

**Table 16. Crop species and varieties composition of the strip cropping system in spring 2015**

| Strip | Crop | Sowing date | Species and varieties per type of treatment | |
|---|---|---|---|---|
| | | | No-mix treatment (*strip* scale) | Mix- treatment (*plant* scale) |
| 1 | Grass-clover | May 2014 | *Lolium multiflorum* (Sultano), Trifolium pratense (Lucrum) | *Lolium multiflorum* (Sultano), *Lolium perenne* (brand: Country Balance), Trifolium pratense (Lucrum), Trifolium repens (Alice and Riesling) |
| 2 | Wheat | April 15, 2015 | *Triticum aestivum* (Lennox) | *Triticum aestivum* (Lennox), *Vicia faba* (Nile) |
| 3 | Maize | Failure | *Zea mays* (Ronaldinio) | *Zea mays* (Roadrunner) |
| 4 | Oilseed rape | April 14, 2015 | *Brassica napus* | *Brassica napus, L. Trifolium* |
| 5 | Potato | April 23, 2015 | *Solanum tuberosum* (Toluca) | *Solanum tuberosum* (Toluca, Tiamo, Anabelle, Ditta) |
| 6 | Grass-clover | May 2014 | *Lolium multiflorum* (Sultano), Trifolium pratense (Lucrum) | *Lolium multiflorum* (Sultano), *Lolium perenne* (Country Balance), Trifolium pratense (Lucrum), Trifolium repens (Alice and Riesling) |
| 7 | Flower mix | June 2014 | *Fagopyrum esculentum, Achilleamille folium, Anethum graveolens, Cichoriumin tybus, Foeniculum vulgare, Pastinaca sativa, Angelica sylvestris, Anthriscus sylvestris, Papaver rhoeas, Chrysanthemum segetum, Ammimajus, Centaureacyanus* | |
| 8 | Potato (experimental) | | *Solanum tuberosum* (Toluca)<br>Treated with differents types of mulching | |

## Annex ii: Layout of the experimental design of the strip cropping system

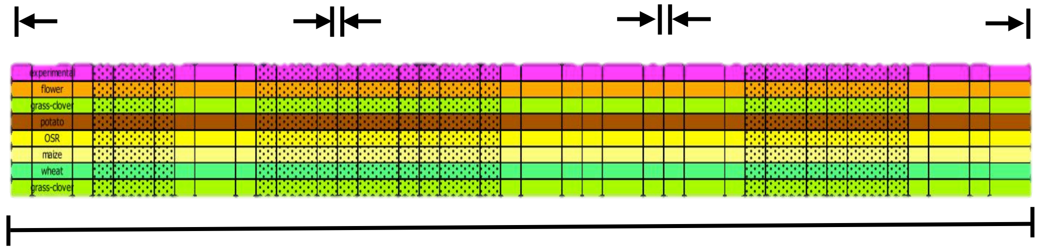


*Figure 16 Layout of the experimental design of the strip cropping system and the distribution of blocks.*

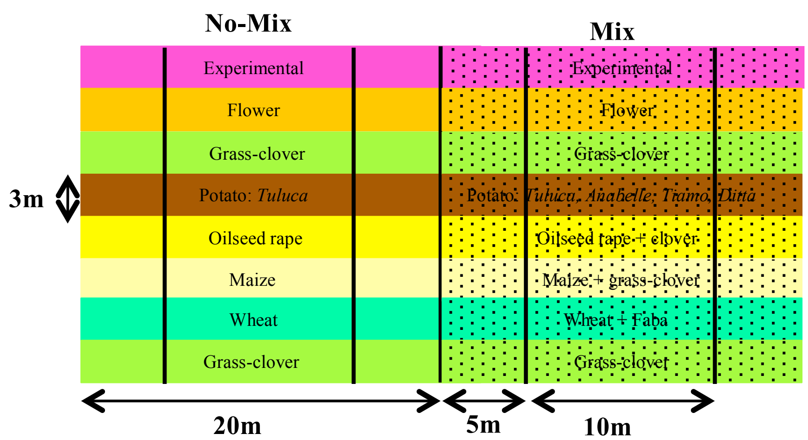


*Figure 17. Graphical representation of the dimensions of the plots, experimental area and buffer zone of the strip cropping system design. Each plot was 3 meters wide and 20 meters large, with a buffer area of 5 meters at the start and end of each plot. Leaving a total experimental area of 3 meters x 10 meters.*