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The effect of crop diversification in small fields on the community of natural enemies on the ground: A strip cropping experiment with white cabbage in the Netherlands

Michiel van de Glind

Master of Science in Agroecology

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Student

Michiel van de Glind
920828266020

MSc Agroecology (Norwegian University of Life Sciences)

michiel.vandeglind@wur.nl / michielvdlind@hotmail.nl / michiel.van.de.glind@nmbu.no

Supervisors

Dirk van Apeldoorn (Wageningen University and Research)
Tor Arvid Breland (Norwegian university of Life Sciences)
Luuk Croijmans (Wageningen University and Research)
Merel Hofmeijer (Wageningen University and Research)

Examiner

Felix Bianchi (Wageningen University and Research)

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MSc thesis Michiel van de Glind^{ab,†*}

Supervisors: Dirk van Apeldoorn, Tor Arvid Breland^b, Luuk Croijmans^a, Merel Hofmeijer^a. Examiner: Felix Bianchi^a

[†] MSc student Agroecology. Registration number NMBU: 998945 Registration number WUR: 920828266020. Thesis code: M30-AE & FSE-80430.

^a Wageningen University and Research, Farming System Ecology, PO Box 430, 6700, AK, Wageningen, the Netherlands

^b Norwegian University of Life Sciences

ABSTRACT

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Agricultural development has contributed significantly to the decline in biodiversity over the last century. Crop diversification is promoted as a strategy to mitigate this ongoing catastrophe. This study investigates the effects of strip cropping as a means of crop diversification on the activity density of ground dwelling natural enemies and the composition of ground beetles. Natural enemies were captured by placing pitfall traps in a cabbage crop over the course of three years. Although several studies have shown a positive effect of crop diversification on biodiversity and the abundance of natural enemies, in this study no such effect was detected. A possible explanation might be that the surface designated to each treatment was smaller than 0.28 hectares and located in a diverse landscape (with e.g., an abundance of semi-natural habitat and field edges). Therefore, the treatments were probably already exposed to a diversity and an abundance of ground-dwelling natural enemies, making potential effects of crop diversification on small fields relatively marginal and thus, below the detection limit. Yet, the results could also be an indication that applying crop diversification in larger fields may have a greater impact on ground dwelling natural enemies than in small fields (<0.28 ha).

1. Introduction

In 2017, Hallman et al. startled the world by concluding that between 1989 and 2016, the biomass of flying insects declined by more than 75 percent in protected areas in Germany. These dramatic figures soared concern in the Netherlands and led to an investigation of biodiversity decline. The study conducted by Hallman et al. (2019) used data from two locations in the Netherlands and showed similar trends as in Germany. They estimated that the total biomass of macro-moths and ground beetles declined by 61% and 42% respectively. In individual numbers, macro-moths, beetles, caddisflies and ground beetles declined per year by 3.8, 5.0, 9.2 and 4.3 % respectively. Exact contributions of drivers such as pesticide application, habitat fragmentation and nitrogen load are still unclear, but it is certain that the development of agriculture and the accompanying homogenization of the landscape over the past century played a significant role (see e.g., Habel & Schmidt, 2018; Martin et al., 2019; Sirami et al., 2019). As such, biodiversity conservation strategies solely focusing on preserving high quality habitats in designated 'natural' areas are not sufficient to bring biodiversity loss to a halt (Habel & Schmidt, 2018). Which type of agroecosystem management then, is able to bring the loss of biodiversity to a halt and simultaneously maintain a well-functioning agroecosystem?

The Dutch answer is circular agriculture: a strategy focused on creating farming systems in which nutrients are kept within the system and where external inputs are limited to a minimum (Dutch Ministry of Agriculture, Nature and Food Quality, 2019). The country is well known for their highly technologically advanced agricultural sector and is a large

producer of agricultural products in the world, in terms of value. However, these highly productive systems have revealed their flip side in terms of environmental and ecological degradation. Specific solutions coming from the Dutch Ministry of Agriculture, Nature and Food Quality (2019) range from creating climate neutral greenhouses to the diversification of the agricultural landscape. The latter could lead to a direct increase of biodiversity (Sirami et al., 2019). Additionally, through the potential attraction of more natural enemies, there is also potential to decrease the levels of pests (Letourneau et al., 2011), allowing farmers to use less pesticides. In turn, a decrease in the use of pesticides will lead to an increased level of biodiversity. However, whether a more complex landscape truly attracts more natural enemies and decreases the abundance of pests is still debated (Karp et al., 2018).

Crop diversification, increasing semi-natural habitat and decreasing field sizes are examples that can aid in diversifying the agricultural landscape. Intercropping (a type of crop diversification) has been put forward by Martin-Guay et al. (2018) as a farming system that could start a new green revolution; on average it improves the production of gross energy by 38% and requires 23% less land (Martin-Guay et al., 2018). In relation to pest suppression, farming systems with a high degree of crop diversity compared to systems with a low degree of crop diversity demonstrated to reduce crop damage by 23% while increasing the abundance of natural enemies and the mortality of herbivores by 44% and 54% respectively (Letourneau et al., 2011). Effects of landscape composition on natural enemies, pest abundance and crop damage are still under scrutiny as demonstrated by

*Corresponding author. E-mail address: Michiel.van.de.glind@nmbu.no (M. van de Glind)

Karp et al. (2018), who found that the outcomes of different studies were all highly dependent on the local context of the farming system and on the surrounding landscape. However, no single landscape variable (e.g., forest) could be appointed as having either a negative or positive influence. In contrast, Martin et al. (2019) discovered that pest-control and pollination increased by 1.4 and 1.7 times respectively in landscapes with high edge densities. Likewise, Sirami et al. (2019) investigated the effect of crop heterogeneity on biodiversity in multitrophic levels in a meta-analysis. They found that: 1) increasing crop diversity had a positive effect on species richness when semi natural cover exceeded 11%, and 2) decreasing field size below 6 ha was an effective measure to promote biodiversity. The latter was exuberated in landscapes with less than 8% semi-natural cover. In summary, these different meta-analyses show that the effect of the agricultural landscape on biodiversity, pest-control and crop performance depends on both landscape composition and the type of farming system. Although these meta-studies are of crucial importance to understand these ecological processes, Kremen & Miles (2012) argue that there is a high urgency to investigate specific farming systems that can contribute to pest regulation through crop diversification. They suggest that detailed system specific studies may aid farmers in deciding the best way in which they can utilize crop diversification strategies.

In Wageningen (the Netherlands) experiments are conducted with organic strip cropping. Strip cropping is “the practice of growing two or more species in alternate, multi-row strips wide enough to allow independent cultivation but narrow enough to support ecological interaction” (Ditzler et al., 2020 submitted). Strip cropping is thus a specific type of intercropping and simultaneously a means to increase crop

diversity. One of the key goals of Wageningen’s project is to create a well-functioning agroecosystem, while simultaneously offering a system that can be applied in large scale farming operations. Furthermore, the strip cropping system at Wageningen intends to increase functional biodiversity (e.g. pollinators and natural enemies) through three levers:

- 1) Offering shelter in a nearby habitat in case of field disturbance (if one crop is harvested, natural enemies can find shelter in the strip neighbouring the harvested crop allowing for faster recolonization).
- 2) Providing nectar and pollen.
- 3) Providing alternative prey. (Rowen, 2019; Gontijo, 2019; Gurr et al., 2017; Landis et al., 2000)

The aim of this study was to contribute to the body of knowledge on the relationship between crop diversity and ground dwelling natural enemies. To disentangle the effects of different types of crop diversification, eight treatments were tested in six different crops between 2018 and 2020 (for more details see Section 2.1). This study focused specifically on the presence of ground dwelling natural enemies in white cabbage.

The research questions of this study were:

- 1) What is the effect of different types of crop diversification on the activity density of ground dwelling natural enemies?
- 2) What is the effect of different types of crop diversification on the composition of ground beetle taxa?

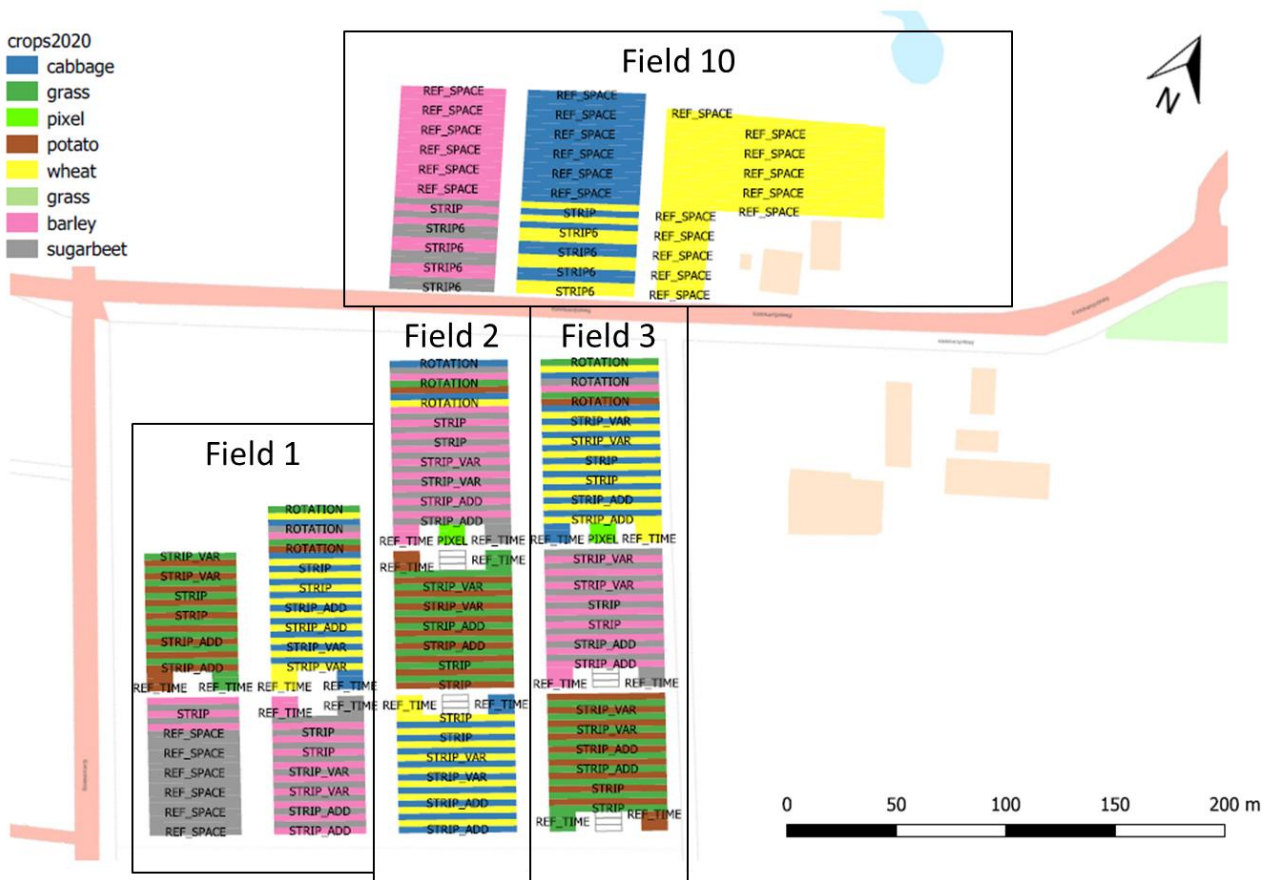


Figure 1, Field map of 2020 at Droevendaal Experimental farm. Field maps of 2018 and 2019 are presented in Appendix J.

2. Materials and methods

2.1 Location and experimental set-up

The experiments were conducted over three years at the Droevendaal Experimental Farm in Wageningen, the Netherlands (51°59'33.06" N, 5°39'43.56" E). The farm was located on a sandy soil and converted to organic in 2003. Land use in the area consisted mainly of grassland for dairy production, but the landscape could still be considered as diverse because: 1) parcels in the area were small (0-5 ha with a few exceptions), 2) the strip cropping fields were adjacent to many other test fields of the university of Wageningen containing a wide variety of crops, and 3) trees, shrubs, edges and ditches were abundant features in the landscape. In all years, a monoculture and three strip treatments with different levels of diversification were tested. All crops in these strip treatments were grown in crop pairs, which meant that cabbage was strip intercropped with wheat in alternating strips (see, e.g., wheat and cabbage in Figure 1, field 1). In addition, in 2018 three treatments were tested based on strip cropping where each crop had a different main crop as a neighbour on each side (e.g. sugar beet and wheat as neighbours of cabbage). These three treatments were merged into the most diversified treatment labelled as "ROTATION" in 2019 and 2020 (see Table 1 & 2).

All STRIP, STRIP_VAR and STRIP_ADD treatments contained at least six strips of which the four innermost strips were measured to avoid measuring edge effects. In total there were three replicates of each strip treatment which were spread out over three fields. These three treatments followed a complete randomized block design. The cabbage in the ROTATION treatment had different crop neighbours depending on the year and field (see Figure 1, and Appendix J). In the field with the larger scale reference treatment, an additional number of strip treatments were placed to be able to account for field effects.

2.2 Pitfall Placement

To measure the activity density of ground-dwelling arthropods, pitfall traps were used. In total 261 pitfall traps were placed over three years. In 2018 pitfall traps were placed by Jansen (2019) and in 2019 and 2020, the placement and collecting of pitfall traps followed a protocol set-up by the research group of the strip-cropping project. In summary, each trap consisted of a plastic cup that was buried with the edge levelled with the ground. Each trap was filled with 3-5 cm of water and two drops of unperfumed detergent and stationed for 5 days. An additional cup with drilled holes was placed under the collection cup for drainage. To avoid the accumulation of dirt, rain or the disruption by large animals, a plastic lid was installed to function as roof. Captured specimens were placed in labelled jars with 70% ethanol (for more details see Appendix A).

In all types of strip treatments, one pitfall trap was placed in each strip (except in the outermost strips of each treatment to avoid effects of the adjacent treatments). In all the ROTATION treatments one pitfall trap was placed in each ROTATION strip of cabbage. In 2018, three traps were placed in REF_SPACE, while in 2019 and 2020 six traps were placed in REF_SPACE. A randomized location for all traps with a distance of at least 10 meters from the outset of each treatment was generated in R.

2.3 Identification of arthropods

Natural enemies were identified to different levels: order (spiders and harvestmen), family (lady beetles and rove beetles), and genus or species (ground beetles). Ground beetles captured in 2018 were identified to species level by Jansen (2019) who used an identification key of Vionita

Table 1: Overview of crops, treatments, sample dates and amount placed pitfall traps in the strip cropping project at Droevendaal over the last three years

	2018	2019	2020
Crops	Cabbage Wheat Potato Grass Leek Grass	Cabbage Wheat Potato Grass Grass (replaced sugar beet due to crop failure) Barley	Cabbage Wheat Potato Grass Pumpkin (replaced sugar beet due to crop failure) Barley
Treatments	Ref_space* Strip Strip Var Strip Add Rot_Mono Rot_Var Rot_add	Ref_Space** Strip Strip Var Strip Add Rotation	Ref_Space** Strip Strip Var Strip Add Rotation
Date of collecting pitfall trap	16 July 30 July 17 September	24 June 23 July 20 August 10 September	2 June 28 June
Total amount of pitfall traps in cabbage	29 x 3 rounds = 87	29 x 4 rounds = 116	29 x 2 rounds = 58

* Only the following crops: potato, cabbage and leek

** Only the following crops: potato, cabbage, wheat and sugar beet

Table 2: Overview of the properties of each treatment

Treatment	Strip - Cropping	Multiple cultivars	Nectar source in adjacent wheat strip (legumes)	In rotation with 5 other crops
Ref_Space	-	-	-	-
Strip	✓	-	-	-
Strip_Var	✓	✓	-	-
Strip_Add	✓	-	✓	-
Rot_Mono	✓	-	-	✓
Rot_Var	✓	✓	-	✓
Rot_Legumes	✓	-	✓	✓
Rotation	✓	✓	✓	✓

(2019) and two handbooks for the identification of ground beetles (Luff and Turner, 2007; Muilwijk et al., 2015). In 2019 and 2020, ground beetles were identified to species or genus level by using Hackston's identification keys (2019) and a key by Muilwijk et al. (2015).

2.4 Statistical analysis

All statistical analysis was performed by using R software (version 4.0.1) for Windows (R Development Core Team, 2020). To assess whether the amount of crop diversity affects the activity density of ground dwelling natural enemies, generalized linear mixed-effect models from the 'lme4' package were fit to the data (see Bates et al., 2014). Poisson regressions were fit to the data but showed overdispersion using a test from the "performance" package (Lüdtke, 2020). The model for ladybeetles did not show overdispersion but instead showed unsatisfying residual plots. As such, Negative binomial models were fit to the data. Best models were

selected based on the Akaike Information Criterion (AIC). Overdispersion was checked again and showed a satisfactory ratio of residual deviance on degrees of freedom for natural enemies, ground beetles, rove beetles and spiders. Ladybeetles and harvestmen showed under dispersion in the negative binomial models. Zero-inflation was checked with the DHARMA package (Hartig, 2017), but showed no significant indication of zero-inflation. Most models also showed strong patterns in Pearson residuals over the fitted model (Appendix B), therefore a non-parametric test (Wilcoxon signed paired rank test) was applied to the data as well.

Multivariate analysis was used to visualize and identify differences in the composition of ground beetle taxa. This type of analysis allows for the exploration, interpretation and discrimination of data comprising multiple response variables and is commonly used for the analysis of abundance data (Paliy & Shankar, 2016). The analysis was split in two parts: 1) an analysis of all identified ground beetle taxa in order to explore their composition in each treatment, and 2) an analysis of three groups of ground beetles grouped by their size. Although the size of ground beetles is not the only morphological trait that influences prey pressure (Cole et al., 2002), Rouabah et al. (2014) found that it is an important characteristic and therefore argue that farmers should focus on attracting large carabids. As such, ground beetles were divided into three groups: 1) small (0-9mm), 2) medium (9.1-13 mm) and 3) large (>13.1mm) (Rouabah et al., 2014). Some assumptions about the species composition of each genus of ground beetles were based on data collected in 2018 and 2020 when a greater detail of taxonomic identification was achieved (see Appendix I). The average size of a species or genus was determined by information in Aukema (1990), Hackston (2019), Jelaska & Durbes (2009), Larochele & Larivière (1989), and Magura et al. (2006) (see Appendix I).

In addition to the multivariate analysis, the richness of ground beetle taxa per sample, was fit to a Poisson generalized linear mixed effect model. Model selection was conducted in a similar fashion as with the analysis of activity

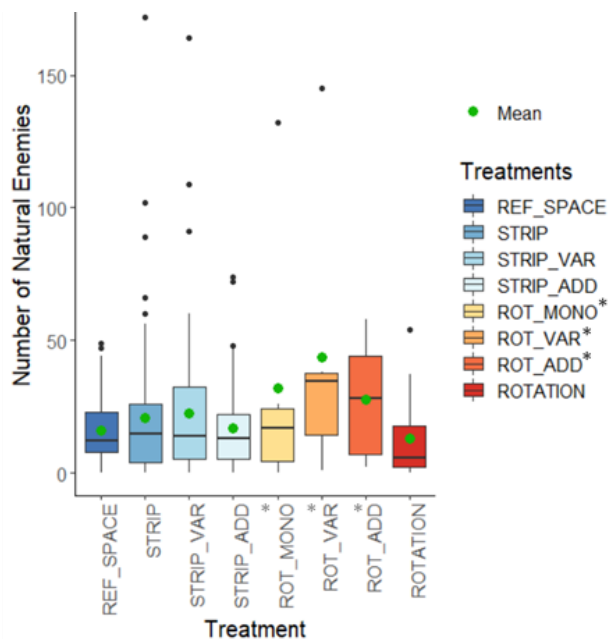


Figure 2, Boxplot of the amount of natural enemies per treatment, differences in mean and median were not significant.

* The ROT_MONO, ROT_VAR and ROT_ADD were only present in 2018 and show a higher activity density mainly as an effect of seasonal influences.

density. However, the Poisson model showed underdispersion. A check for zero-inflation using the DHARMA package, indicated zero-inflation in the model. As such, a zero-inflation factor was added by using the package 'glmmTMB' (Magnusson et al., 2017). In contrast, the results of the glmmTMB showed that the zero-inflation component of the model was non-significant (see Appendix C). As such the output of the original Poisson model was investigated but could not be validated due to strong patterns in the residual plot.

For both multivariate analyses the vegan package was used (Oksanen et al., 2013). To decide whether redundancy analysis (RDA) or canonical correspondence analysis (CCA) should be used, the gradient length was checked by performing detrended correspondence analysis (DCA) (Šmilauer & Lepš, 2014). The results of the DCA indicated that a CCA should be used for the ungrouped ground beetle taxa and an RDA for the analysis in which ground beetles were grouped by size. Prior to both analyses, empty rows were removed and prior to the RDA, a common root transformation was applied to compress large values in the data set (Buttigieg & Ramette, 2014). Function ordistep was used to select the best model for each analysis (see Appendix D and E).

Multicollinearity between predictors was checked in the glmers using the 'car' package (Fox et al., 2012) and in the multivariate analysis using the 'vegan' package.

3. Results

In general, the results did not show an indication that the different degrees of crop diversification had an influence on either the activity density of ground beetles or on the composition of ground beetles.

3.1 Activity density of natural enemies

In total, 5209 individual arthropods, that were considered as natural enemies in this study, were caught in pitfall traps over the course of three years. The caught taxa consisted of 51.8% ground beetles (2700), 23.9% rove beetles (1247), 21.2% spiders (1104), 2.3% harvestmen (119) and 0.8% ladybeetles (39). Initial data exploration showed that the median and mean of natural enemies between the treatments tended to be different. Especially the three rotation treatments that were tested in 2018 tended to show higher activity densities. In contrast, the rotation treatment that was tested in 2019 and 2020 tended to have fewer natural enemies than the other treatments (see Figure 2, and Appendix F & G). However, the fitted generalized linear model showed that these differences were not significant and were mainly caused by random effects (see Table 3 & 4). Models for individual groups of natural enemies could not be validated after inspection of Pearson residuals (Appendix B). Therefore, Wilcoxon paired signed rank tests were applied but these showed no significant differences between treatments (see Table 4, and Appendix H), except for one comparison which had a p-value just under 0.05. The ROTATION treatment had a greater activity density of harvestmen than STRIP_ADD ($p < 0.05$) (see Appendix H).

3.2 Composition of ground beetles

Over three years 21 different taxa of ground beetles were identified (Appendix I), followed by multivariate analysis to visualize differences between communities and a glmer to compare taxonomic richness.

The CCA did not show a significant effect of treatments on the presence of certain ground beetles. Field, year and month were significant predictors and explained 21% of the variation together. However, CCA1 and CCA2 together only explained 12% of the variation. (see Appendix D). A partial CCA revealed that the field effect accounted for 7% of the

Table 3: Output of the negative binomial mixed effect model on natural enemies. The output indicates that there was no significant difference between the treatments. Instead differences were explained by the random effects “field” and “sampling date” (see p values and Marginal R² vs Conditional R²). Furthermore, τ values show that the date of sampling explained most of the variation in the model.

Predictors	Incidence Rate Ratios	CI	Statistic	p
(Intercept)	11.63	5.24 – 25.81	6.04	<0.001
Strip [1]	1.04	0.72 – 1.49	0.20	0.845
Multiple.Varieties [1]	1.03	0.82 – 1.28	0.24	0.811
Legumes [1]	0.88	0.70 – 1.10	-1.16	0.247
Rotation [1]	1.04	0.77 – 1.41	0.24	0.810
Random Effects				
σ^2	0.37			
τ_{00} Sampling.date	1.10			
τ_{00} field	0.10			
ICC	0.76			
N Sampling.date	9			
N field	6			
Observations	261			
Marginal R ² / Conditional R ²	0.002 / 0.764			

Table 4: A table showing the comparison of the activity density of natural enemies between different treatments per sampling round and field by using Wilcoxon paired signed rank tests. For instance, during all nine sampling rounds, pitfall traps were placed in STRIP and STRIP_VAR in field 1, 2 and 3. Therefore 9 x 3 = 27 comparisons could be made. These results show that there was no difference between the treatments.

Treatment 1 (T1)	Treatment 2 (T2)	Amount of comparisons	T1 > T2	T2 > T1	Tie	P Value
STRIP	REF_SPACE	9	4	4	1	0.6241
STRIP	STRIP_VAR	27	13	13	1	0.7994
STRIP	STRIP_ADD	27	14	12	1	0.4767
STRIP_VAR	STRIP_ADD	27	15	11	1	0.5674
STRIP	ROTATION	18	11	7	0	0.5419
STRIP_VAR	ROTATION	18	11	7	0	0.2484
STRIP_ADD	ROTATION	18	11	7	0	0.6471

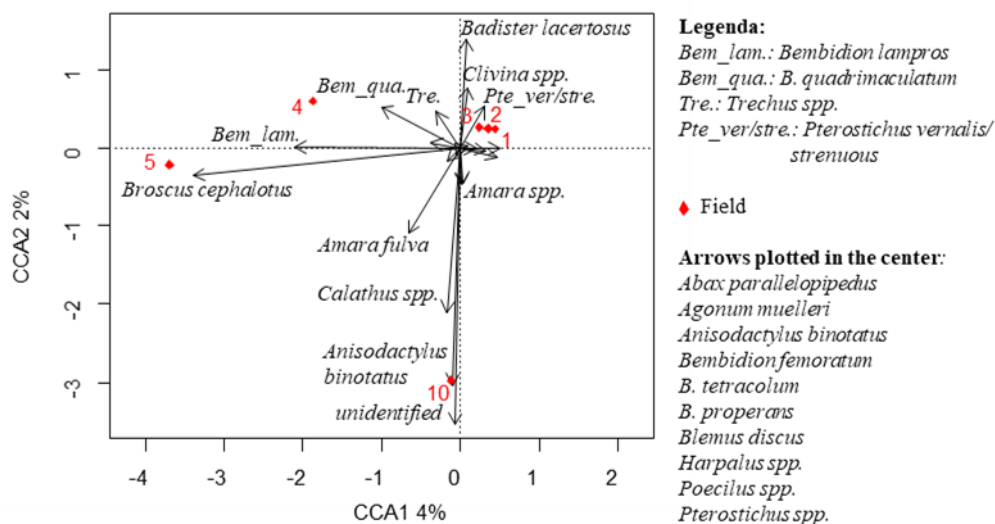


Figure 3, Partial CCA, with field as the main effect and year and month as conditioned effects, scaling = 2. The axis of the CCA indicate that the variation explained by the different fields is very low. Furthermore, most taxa are plotted in the center of the ordination. In summary the fields in this study do not have a noteworthy effect on the composition of ground beetles.

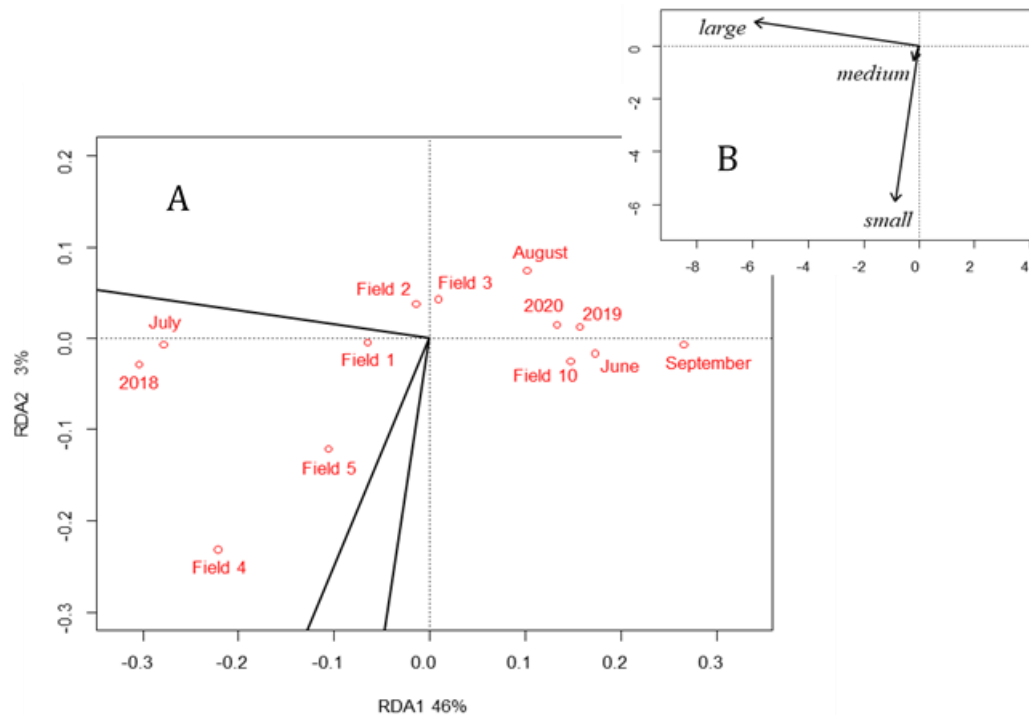


Figure 4, (A): displays the effects of year and month on the presence of three different groups of ground beetles (see B). Although significant in the model, the partial CCA revealed that the field effect was too small (6%) to be considered as a meaningful factor in the presence of either large, medium or smaller sized carabids. In contrast the year and month of sampling together explained 42% of the variation, showing that July and the year 2018 had a positive effect (although marginal due their location on the plot) on the presence of large ground beetles, whereas the year 2020, 2019, and the months June, August and September were slightly negatively correlated with large carabids. These results can partly be explained due to the outliers of one sampling round which took place on the 30th of July in 2018. **(B):** Showing distribution of three groups of ground beetles: 1) Large (>13.1mm), 2) Medium (9.1-13mm) and 3) Small (<9mm), over the ordination axis of an RDA with scaling = 1

CCA2 to 4% and 2% respectively. As such, field effects could only marginally explain the presence of a few taxa that had low counts in general (e.g., *Anisodactylus binotatus* and the unidentified taxa both only occurred once). However, the vast majority of taxa showed no preference for a certain field. Therefore, the community of ground beetles did not show major differences between the fields (see figure 3). The Poisson model, comparing the taxa richness between the treatments, could not be validated due to underdispersion and unsatisfactory Pearson residuals (see Section 2.4 & Appendix C).

The RDA did not show a significant effect of the different treatments on the presence of large, medium or small sized ground beetles. Field, year and month were significant predictors and together explained 48% of the variation. RDA1 and RDA2 together explained 45% and 3% of the variation respectively (see Figure 4, and Appendix E). A partial RDA revealed that the field effect accounted for 6% of the variation (Appendix E), indicating that the date of sampling was the most important predictor (43%). Drawing an imaginary perpendicular line from the centroids onto the arrows revealed some effects of the date of sampling on the presence of large species. The month July, and the year 2018 were correlated with the presence of large ground beetles. In contrast, the years 2019 and 2020, and the months August, September and June were slightly negatively correlated with the presence of large ground beetles compared to July 2018. Although the field effect was indicated as a significant factor in the model, the effect explained such a small amount of the variance that no genuine inference could be made.

4. Discussion

The results indicate that the different treatments had no significant effect on the activity density of natural enemies or the composition of ground beetle taxa. These findings are in stark contrast with findings from Letourneau et al. (2011), who found that the abundance of natural enemies increased by 44% in farming systems with a high crop diversity versus systems with a low crop diversity. Similarly, Shackelford et al. (2013) found a significant positive effect on natural enemies by increasing complexity at both landscape and local scales. In addition, Bertrand et al. (2016) found that an increase in temporal heterogeneity (crop diversity and availability of resources over time) increased the total abundance of ground beetles. However, results with regard to the richness of ground beetles are mixed in literature. Neither Shackelford et al. (2013) or Bertrand et al. (2016) found significant evidence for an increase in the species richness of predatory ground beetles by increased levels of spatial complexity. In contrast, Bertrand et al. (2016) did find that both temporal and spatial heterogeneity affects the assemblages of ground beetles. Furthermore, in a more recent meta-analysis, Sirami et al. (2019) found a positive effect of crop diversification on species richness when semi-natural cover exceeded 11%. The study also concluded that, especially in landscapes with less than 8% semi-natural cover, decreasing the size of fields below six ha is an effective measure to promote biodiversity. The results of this strip cropping experiment in cabbage are somewhat in contrast with the evidence from the meta-analyses mentioned above. There are various factors that potentially led to the result of not finding differences in the presence of natural enemies between the tested treatments, chief among them are: 1) experimental set-up, 2) field conditions, and 3) simplification of ecological processes in the analyses.

4.1 Experimental set-up

The contrasting results of the experiment in Wageningen could perhaps be explained by the use of small areas for each treatment (0.08 - 0.28 ha). In comparison, Bertrand et al.'s (2016) mean crop field size ranged from 1.02 to 3.59 ha, and the smallest field size considered in the study by Sirami et al. (2019) was 0.48 ha. However, 75% of the fields were larger than 1.71 ha and the largest field was 12.71 ha. In contrast, the treatment representing a monoculture in Wageningen was 0.28 ha in 2018 and 2019 (54m x 51m), and 0.23 ha in 2020 (45m x 51m), whereas the strip treatments had a surface of only 0.08 ha (3m x 42m x 6 strips). As the measurements in Wageningen took place on such a small scale, a spillover of natural enemies between the treatments themselves and the surrounding landscape is likely. Therefore, the effects of crop diversification on this scale may be marginal and thus below the detection limit. The importance of testing these treatments on a larger scale is emphasized by the fact that only minor differences were found between fields which were slightly larger than treatment surfaces (0.3 - 0.34 ha, see Figure 1, and Appendix J). In addition, the spatial and temporal heterogeneity in the landscape around test locations should be quantified in order to disentangle the effects of the surrounding landscape and agricultural treatments on the measured richness and abundance of species (Bertrand et al., 2016; Shackelford et al., 2013). This can be done in a similar fashion as Bertrand et al. (2016), who used multispectral satellite images from five years, identified land use using ENVI 4.7 software, and validated the output by using ground surveys and data from local institutions that keep track of agricultural land use. Furthermore, in order to disentangle the effects between treatments and seasonal influences on ground dwelling natural enemies, sampling should take place in the same week each year. This was not the case in the experimental set-up of this study (see Table 1).

Finally, pitfall trapping does not necessarily reflect the abundance of each taxa that is present in the field in an accurate way. Several factors may influence the capture rate of an arthropod, such as the body size of a ground beetle and the temperature during the sampling period (Hancock & Legg, 2012; Esch et al., 2008). Furthermore, Bergeron et al. (2013), showed that if the edge of the pitfall trap is placed 10-15 centimeters below the surface, different communities were captured than when the edge was placed at ground level; Halsall & Wratten (1988) argue that the ability of a ground beetle to maintain their balance once it reaches the edge of a trap, influences capture rates as well; and Ward et al., (2001) found that a greater distance between traps increased the amount of beetle morphospecies that were captured.

4.2 Field conditions

Several conditions in the field may have influenced the results. First, the legumes grew too vigorous in 2019 and outcompeted the main crop, whereas in 2020 the legumes barely established. As such, the legumes did not provide as much nectar and pollen for natural enemies as intended in 2020. Spiders and ladybeetles make use of this food source (Jackson et al., 2001; Lundgren, & Seagraves, 2011) and may therefore show different trends in field conditions with normal growth of legumes. Secondly, the strip next to cabbage in the ROTATION treatment was bare in 2019 for the first part of the season and nearly bare in 2020 as it contained pumpkin that had only just been sown during sampling. This may explain the tendencies towards a lower activity density of natural enemies (except for harvestmen) in rotation than in the other strip treatments, although the difference was not significant.

4.3 Simplification of ecological processes in the analyses

Both the analyses of mixed effect and multivariate models could be improved. First, the mixed effect models mainly overestimated field 4, field 5, and the sampling round in September. Therefore, options to improve model fit could be to remove the year 2018 from the analyses, because it contained 1) outliers in the data, and 2) treatments and fields that were only measured for one year. The DHARMA zero-inflation test was not significant in most cases, but this only meant that zero-inflation was not proven; it does not mean that there was no zero-inflation in some of the models. Especially in the case of lady beetles and harvestmen, of which only 119 and 39 specimens were caught in 261 samples respectively, a zero-inflated model could be reconsidered.

Secondly, the multivariate analyses could be improved by increasing the accuracy of identification up to species level for each genus and by considering other factors than body size when creating functional biodiversity groups. Cole et al. (2002) proposed several factors to be included in their classification method, such as: overwintering capabilities, duration of life cycle, diet, breeding season, and whether species were nocturnal. The weakness of creating functional groups based on size as the only trait is clear when considered that *Harpalus rufipes* (the most frequent captured ground beetle in the experiment, see Appendix I), is not only a predator, but also a generalist that feeds on weed seeds as well (Jørgensen & Toft, 1997). Furthermore, a smaller sized ground beetle, such as *Calathus melanocephalus* may eat 72 eggs from the cabbage root fly (*Delia radicum*) a day, while larger sized beetles such as *Pterostichus melanarius* and *P. Niger* ate none (Finch, 1996). In a next step, linking the abundance of natural enemies, their life traits, the abundance of pests, the degree of crop damage, and the yields, can provide a better understanding of the effects of crop diversification on pest-predator relationships and agroecosystem performance (Shackelford et al., 2013).

Thirdly, the landscape of multivariate analysis is still developing, methods are becoming more rigorous and standards for multivariate analysis are being developed (Paliy & Shankar, 2016). Yet, clear guidelines for when to apply which multivariate analysis and data transformation, covering the whole of the multivariate landscape, are scarce (Legendre & Gallagher, 2001). For instance, the debate on the removal of rare species from the data before analysis remains a highly debated subject and is sometimes applied without clear justification (Poos & Jackson, 2012). From a statistical point of view, rare species may introduce noise into the calculations and removing them may strengthen the statistical analysis (Gauch & Gauch, 1982; McCune & Grace, 2002). Some studies confirmed this argument by showing that common species play the most important role in assessing the difference between assemblages (Graça et al., 2017; Sgarbi et al., 2020). However, in other studies the data provided by rare species was crucial to differentiate species composition between assemblages (Poos & Jackson, 2012 & Leitão et al., 2016). Other counter arguments from a biological perspective have been offered by Poos & Jackson (2012). They argue that species that are the most interesting from a biological perspective may be removed (e.g., endangered species), and assessing the biological community may become more difficult as the total amount of species to assess is reduced. Moreover, the sampled species may not reflect the actual abundance of each species due to sampling protocols (Arscott et al., 2006). Rare species may also be a sign of a habitat that is associated with a reduced rate of anthropogenic stress (Poos & Jackson, 2012) and thus provide valuable information about the effect of landscape management (e.g., agricultural practices) on biodiversity. Habel and Schmidt (2018) countered this argument partially by showing that intermediate species lingering between

specialist and generalist species may, in some cases, be struck even harder by landscape and habitat fragmentation. Due to the lack of scientific consensus, justification, and specific criteria for the removal of rare species in multivariate analysis with respect to ground beetles, it was decided to retain all the species in the multivariate assessment.

Finally, all analyses may be improved by using more complex models that account for: 1) the temporal and spatial heterogeneity of the landscape, and 2) the potential biases created using pitfall traps as discussed in Section 4.1.

Conclusion

One of the ultimate goals of crop diversification is that it should lead to an increase in biodiversity directly, or indirectly through the reduced need for pesticides. However, this study did not identify significant differences in the activity density of ground dwelling natural enemies or the composition of ground beetle taxa between several types of crop diversification, or when comparing these treatments to a slightly larger sized reference treatment. The surface of each treatment was small (0.08 – 0.28 ha) and this was likely to be an important factor in the inability of this experiment to detect a difference between the treatments. Even between the larger sized fields, which contained multiple treatments, the differences were negligible. Yet, the results could also be an indication that further crop diversification in fields smaller than 0.28 ha may only hold marginal increases in abundance and richness of ground dwelling natural enemies. Therefore, the practice of crop diversification might have a greater impact in larger fields if the aim is to attract more ground dwelling natural enemies.

In the future, research focused on the interaction between crop diversity and natural enemies should consider making use of: 1) larger surfaces for each treatment (larger treatment sizes are more likely to reduce the noise introduced by natural enemies from adjacent fields or field edges), 2) a more detailed classification of natural enemies based on their morphology and life traits, 3) linking the presence of specific natural enemies to pest abundance and crop performance, 4) more complex models that account for the spatial and temporal composition of the landscape, and 5) knowledge on the potential biases created by the use of pitfall trapping and adjust the models accordingly.

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Appendix A: Protocol hPro_029: Placing and processing pitfall samples to assess arthropod abundance and diversity

Written by: Lenora Ditzler, based on protocol by Yayang Vionita (diagrams made by Yayang)

Written on: November 23 2018 (by Yayang)

Last updated: April 04 2020 (by Luuk Croijmans)

Updaters note: Part 2 "Collecting pitfalls" was updated and performed as described below from 2019 onwards. The old protocol is at the bottom of this document.

Goal: This protocol describes the methods for: 1) placing pitfall traps in the field, 2) collecting pitfall trap samples, 3) cleaning pitfall trap samples, 4) weighing samples in pitfall traps, and 5) storing pitfall trap samples for further analysis. *How to identify arthropods in pitfall samples is explained in Pro_048.*

Materials needed:

1. Placing pitfalls:

- Plastic cups without holes in the bottom (same number as total pitfall locations)
- Plastic cups with holes in the bottom (same number as total pitfall locations)
- Black plastic pitfall lids + wire anchors (same number as total pitfall locations)
- Long white sticks
- Label flags (same as for blue stakes)
- Auger (Dutch: grondboor)
- Measuring tape
- Clean fresh water
- Neutral dish soap (no dye or perfumes)

2. Collecting pitfalls:

- 200 ML jars (w/ blue lids)
- Plastic bags (40 cm x 60 cm)
- Sample labels
- Waterproof marker
- Squeeze bottle filled with clean fresh water for flushing

samples

3. Cleaning and storing pitfall samples:

- Clean water
- 70% Ethanol in squeeze bottle
- Sieve
- Large petri dishes (100 mm x 15 mm)
- Paintbrush
- Tweezers
- Plastic sample bottles (40 ml or 50 ml)
- Sample labels (x2 – sticker for outside of bottle, hand-written label on paper w pencil for inside bottle)
- Plastic storage tray

4. Weighing pitfall samples:

- Paper towels
- Glass beaker (200 ml)
- Sieve (Aluminum sieve 330 mm stainless mesh 330 mm x 0.4 mm)

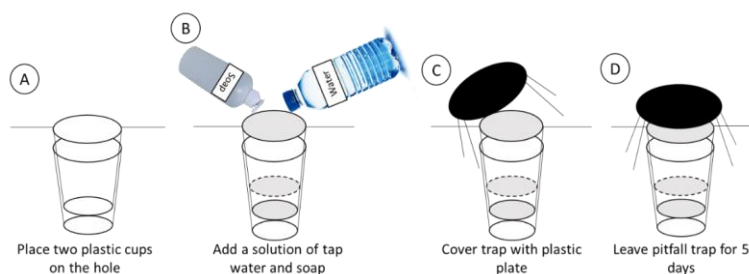
- Scale (Sartorius Genius series) with a level of precision 0.0000 gram
- Tweezers
- 70% ethanol in squeeze bottle
- Data sheet (digital or paper)

Time estimation: Placing pitfalls with a team of four (two to find and measure the location, two to dig holes and place pitfalls) takes about 5-10 minutes per pitfall. Picking up pitfall samples is markedly quicker. Processing and weighing samples in the lab also takes around 3 minutes per sample with a team of two.

Methods:

1) Placing pitfall traps in the field

- Measure the x coordinate where you want to place the trap using the measuring tape. Make sure to always measure from the “left” side if you were to look at the maps.
- Place a white stick with flag at the location. Make sure to place the label flag relatively far down to the ground, to avoid it to fall off due to management practices.
- Dig a hole approximately the size of the plastic cup.
- Place a cup with holes in the bottom in the hole and fill in soil around it so there is no gap between the walls of the hole and the cup.
- Remove any soil that falls into the cup. Then place a second cup into the first cup. The second cup should **not** have holes in the bottom. *Make sure the rim of the cup as level with the surface of the soil as possible.*
- Pour approximately 3 cm water into the cup and add a dash of soap (just a couple drops is enough)
- Place a black pitfall lid over the cup and push down the anchors so the lid is close to the ground, leaving about 1cm space between the soil surface and the black lid – enough space for bugs to crawl in but not enough space for a mouse ;)
- Leave the pitfall trap in the field for 5 days before collecting the sample



2) Collecting pitfall samples from the field

Day before:

- Ask the farmer if the pitfall traps (or at least the sticks) can remain in the ground.
- Put labels on 200 ML jars and sort them in the bags.

At the day of collecting

- Locate the pitfall trap you’re collecting and remove the black lid
- Gently swirl the contents of the pitfall trap and pour into the corresponding 200 ML jar
 - Watch out. If any mice are in the pitfall trap, please first gently remove these without removing any of the insects. Do NOT put mice in the 200 ML jars.
 - Write with a waterproof marker on the jar how many mice were in the trap.
- Store samples in refrigerator and transfer insects to alcohol as soon as possible
- When you can leave the traps in the field:

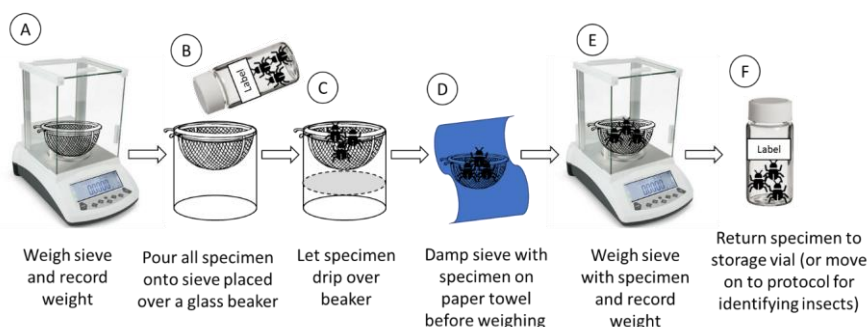
- i. Remove cup without holes.
 - ii. Leave cup with holes.
 - iii. Push black lid closely to the bottom, try to leave no space.
- g. When you can only leave the white sticks:
- i. Remove the entire trap.
 - ii. Leave the white stick
- h. When you can't leave anything, well... take everything.

3) Cleaning and storing pitfall samples

- a. Label a clean plastic vial with the sample ID using a sticker on the outside and a hand-written label inside (pencil on a slip of paper) and fill with 70% ethanol (plenty to submerge all insects)
- b. Pour the contents of a 250 ML jar through a sieve and flush the sieve to clean the insects.
- c. Keep the sieve upside down above a petri dish and flush to get its contents in the petri dish.
- d. Using a brush and tweezers, separate samples from excess soil. If the sample is very muddy, transfer specimens to a second petri dish filled with clean water.
- e. Transfer cleaned contents of the pitfall trap (only arthropods, no earthworms or slugs) into the prepared vial. If it is a very large sample, make sure all specimens are submerged in ethanol and use a second vial if necessary.

4) Weighing pitfall samples (see also instructional movie, [Pro_29 pitfall sample weighing.wmv](#))

- a. Weigh the sieve you will use for filtering specimens and record weight on a datasheet
- b. Pour specimen into the sieve placed over a glass beaker to collect the ethanol
- c. Let the specimen drip dry for a minute or so (until dripping stops)
- d. Damp the sieve on a paper towel to remove excess ethanol
- e. Weigh the specimen-filled sieve and record weight on datasheet
- f. Return the specimen and paper label to the storage vial. Discard the ethanol collected in the beaker and re-fill the sample vial with fresh ethanol.



Old protocol sections

Collecting pitfall traps before 2019

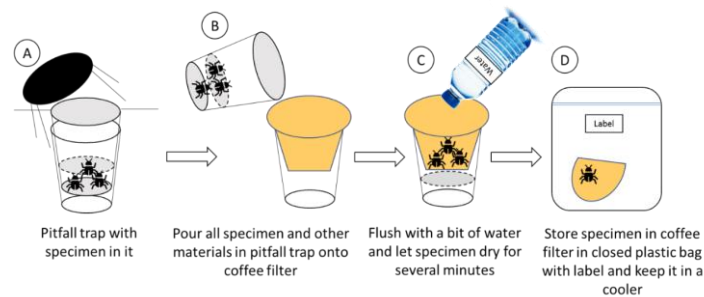
Materials needed

Collecting pitfalls:

- Coffee filters (size no. 4)
- Empty plastic cup (no holes)
- Plastic bags (40 cm x 60 cm)
- Sample labels
- Squeeze bottle filled with clean fresh water for flushing samples

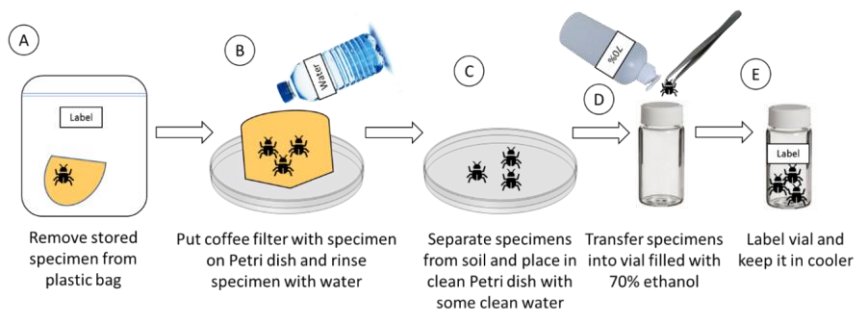
Methods

- Day before, ask the farmer if the pitfall traps (or at least the sticks) can remain in the ground.
- Locate the pitfall trap you're collecting and remove the black lid
- Gently pour the contents of the pitfall trap into a coffee filter positioned over an empty cup
- Flush the coffee filter with a bit of clean water if there is a lot of mud and then let the specimen dry for a couple minutes
- Place the coffee filter (filled with specimens) in a clean plastic storage bag and label with the pitfall date and ID, seal, and store in a cooler while in the field and then transfer to the refrigerator room



Cleaning and storing pitfall samples

- Label a clean plastic vial with the sample ID using a sticker on the outside and a hand-written label inside (pencil on a slip of paper) and fill with 70% ethanol (till 1cm below rim)
- Remove the coffee filter with the specimen from the storage bag
- Open the coffee filter over a petri dish and flush contents off filter and into the petri dish with clean water. If the sample is very muddy, use a fine sieve to filter the sample.
- Using a brush and tweezers, separate samples from excess soil. If the sample is very muddy, transfer specimens to a second petri dish filled with clean water.
- Transfer cleaned contents of the pitfall trap (only arthropods, no earthworms or slugs) into the prepared vial. If it is a very large sample, make sure all specimens are submerged in ethanol and use a second vial if necessary.



Appendix B1, Generalized linear mixed effect models and analysis of variance: Natural enemies

Selected model: `glmer.nb(Total ~ Strip + Multiple.Varities + Legumes + Rotation + (1 | Sampling.date) + (1 | field, data = mydata)`

Table 5: Output of the glmer model over natural enemies

<i>Predictors</i>	<i>Incidence Rate Ratios</i>	Total		
		<i>CI</i>	<i>Statistic</i>	<i>p</i>
(Intercept)	11.63	5.24 – 25.81	6.04	<0.001
Strip [1]	1.04	0.72 – 1.49	0.20	0.845
Multiple.Varities [1]	1.03	0.82 – 1.28	0.24	0.811
Legumes [1]	0.88	0.70 – 1.10	-1.16	0.247
Rotation [1]	1.04	0.77 – 1.41	0.24	0.810
Random Effects				
σ^2	0.37			
τ_{00} Sampling.date	1.10			
τ_{00} field	0.10			
ICC	0.76			
N Sampling.date	9			
N field	6			
Observations	261			
Marginal R ² / Conditional R ²	0.002 / 0.764			

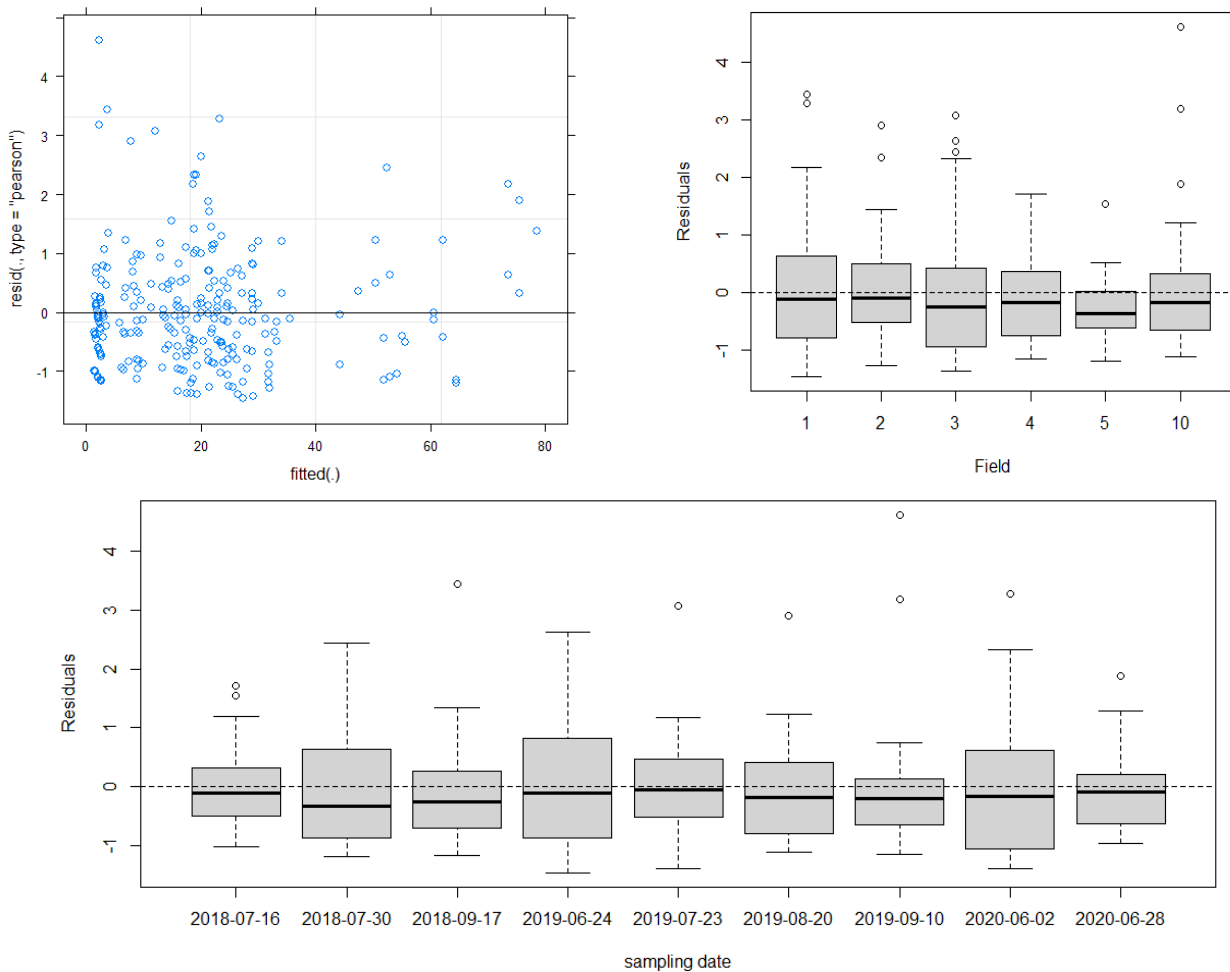


Figure 5, Top left: Pearson residuals vs the model look reasonably centered around zero, top right: Pearson residuals of the random field effect, most fields are predicted well with the exception of field 3 and 5, bottom: Pearson residuals over sampling date, the model overestimates a few sampling dates. In general residuals look reasonable, but one must be careful with the interpretation due to some overestimations by the model, especially in field 5.

Appendix B2, Generalized linear mixed effect model and analysis of variance: Ground beetles

Selected model: `glmer.nb(Ground.Beetles ~ Strip + Multiple.Varities + Legumes + Rotation + (1 | Sampling.date) + (1 | field), data = mydata)`

Table 6: Output of the glmer model over ground beetles

Ground Beetles				
Predictors	Incidence Rate Ratios	CI	Statistic	p
(Intercept)	4.17	1.65 – 10.54	3.02	0.002
Strip [1]	1.04	0.67 – 1.60	0.18	0.860
Multiple.Varities [1]	0.97	0.75 – 1.26	-0.20	0.839
Legumes [1]	0.79	0.60 – 1.03	-1.77	0.078
Rotation [1]	1.04	0.73 – 1.48	0.22	0.825
Random Effects				
σ^2	0.50			
τ_{00} Sampling.date	1.46			
τ_{00} field	0.14			
ICC	0.76			
N Sampling.date	9			
N field	6			
Observations	261			
Marginal R ² / Conditional R ²	0.005 / 0.763			

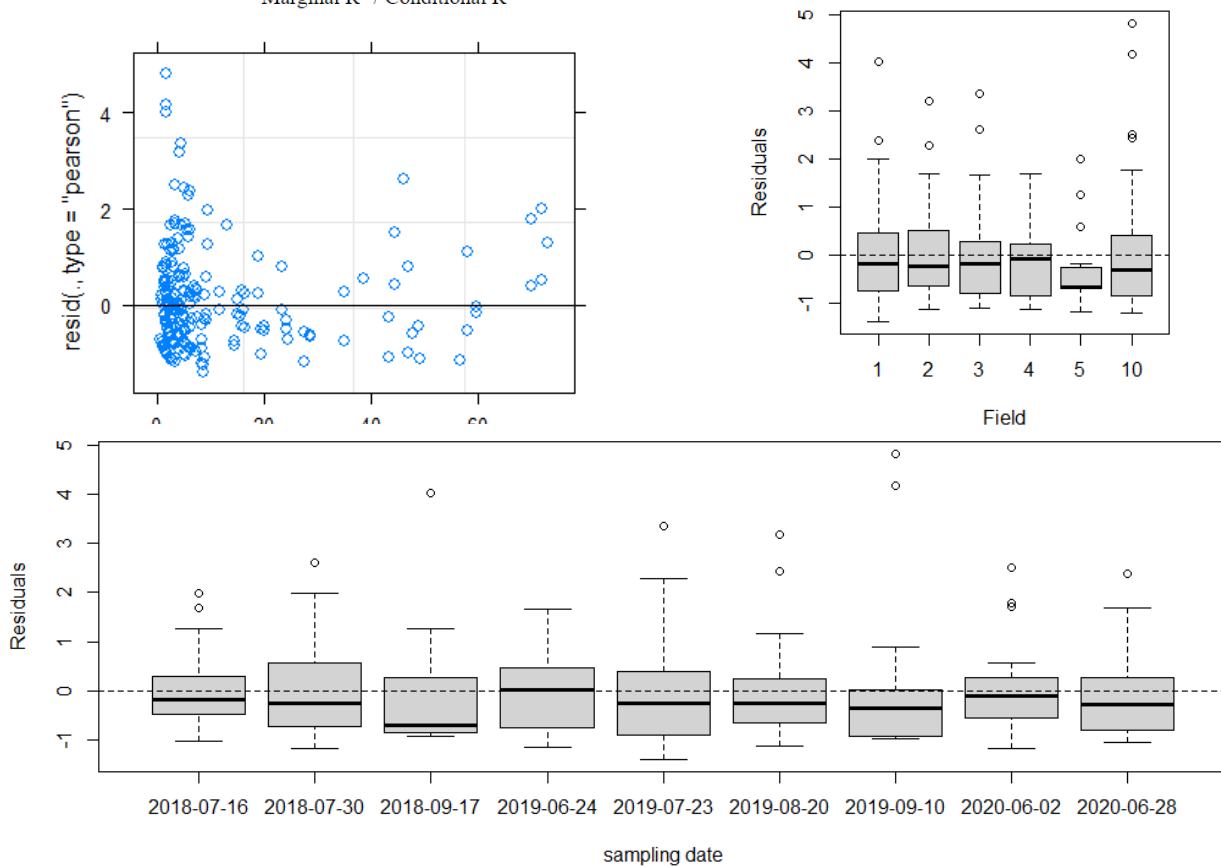


Figure 6, Top left: Pearson residuals vs the model look reasonably centered around zero with some outliers around lower values, top right: Pearson residuals of the random field effect, fields are not predicted very well with the exception of field 4, bottom: Pearson residuals over sampling date, the model overestimates a few sampling dates. The model is overestimating too many effects and can therefore not be validated.

Appendix B3, Generalized linear mixed effect model and analysis of variance: Rove beetles

Selected model: `glmer.nb(Rove_beetle ~ Strip + Multiple.Varities + Legumes + Rotation + (1 | Sampling.date), data = mydata)`

Table 7: Output of the glmer model over rove beetles

Rove beetle				
<i>Predictors</i>	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
(Intercept)	2.14	0.60 – 7.61	1.17	0.242
Strip [1]	0.81	0.51 – 1.26	-0.94	0.348
Multiple.Varities [1]	1.07	0.73 – 1.57	0.33	0.741
Legumes [1]	0.82	0.56 – 1.20	-1.04	0.298
Rotation [1]	1.46	0.89 – 2.41	1.49	0.136
Random Effects				
σ^2	0.90			
τ_{00} Sampling.date	3.35			
ICC	0.79			
N Sampling.date	9			
Observations	261			
Marginal R ² / Conditional R ²	0.007 / 0.789			

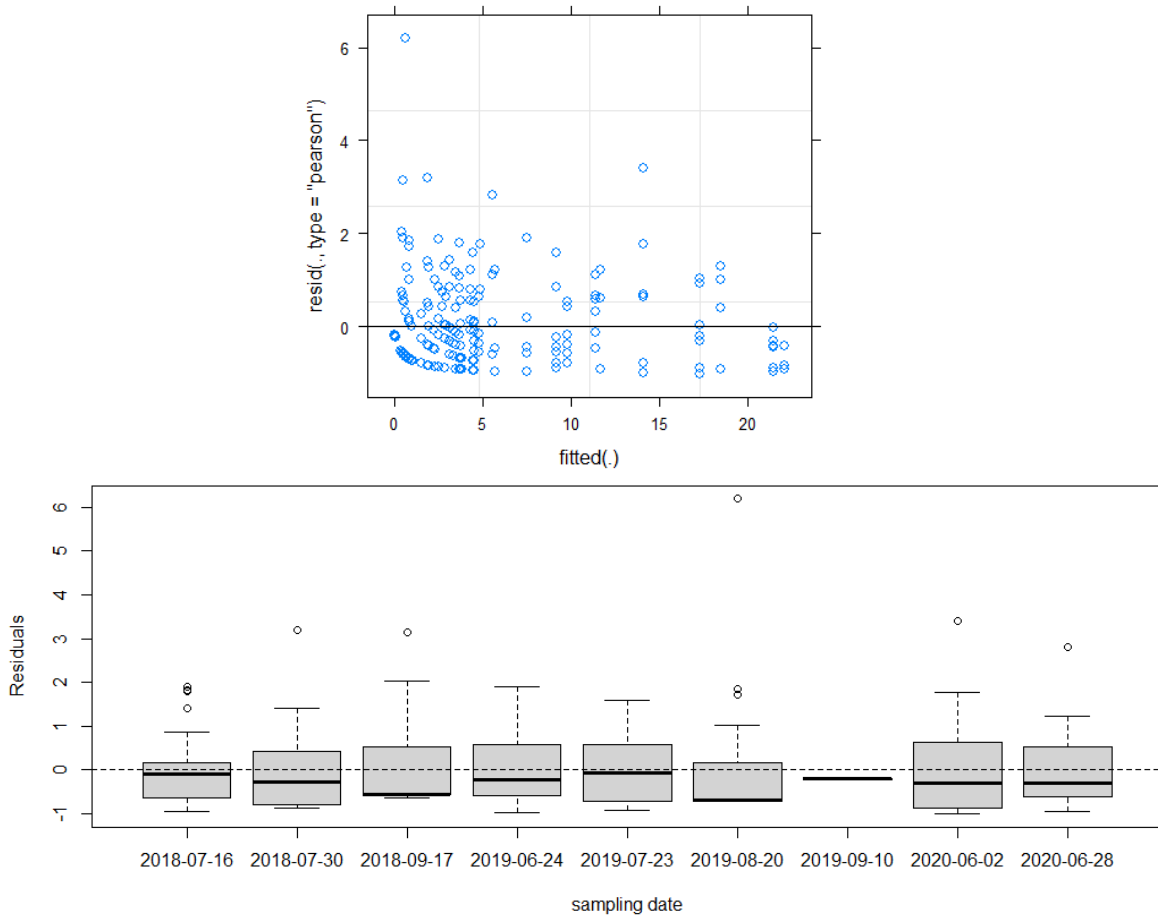


Figure 7, top: Pearson residuals over fitted model, residuals show some patterns at lower values but are still primarily centered around zero, bottom: Pearson residuals over random effect sampling date, the model overestimates several sampling dates. The model cannot be validated due to many overestimations by the model and some patterns in the overall residual plot.

Appendix B4, Generalized linear mixed effect models and analysis of variance: Spiders

Selected model: `Glmer.nb Spider ~ Strip + Multiple.Varities + Legumes + Rotation + (1 | Sampling.date) + (1 | field), data = mydata)`

Table 8: Output of the glmer model over spiders

<i>Predictors</i>	Spider			
	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
(Intercept)	2.75	1.33 – 5.67	2.74	0.006
Strip [1]	1.03	0.68 – 1.56	0.13	0.894
Multiple.Varities [1]	1.05	0.79 – 1.40	0.35	0.725
Legumes [1]	0.88	0.66 – 1.18	-0.85	0.397
Rotation [1]	0.83	0.56 – 1.24	-0.91	0.362
Random Effects				
σ^2	0.58			
τ_{00} Sampling.date	0.81			
τ_{00} field	0.06			
ICC	0.60			
N Sampling.date	9			
N field	6			
Observations	261			
Marginal R ² / Conditional R ²	0.006 / 0.604			

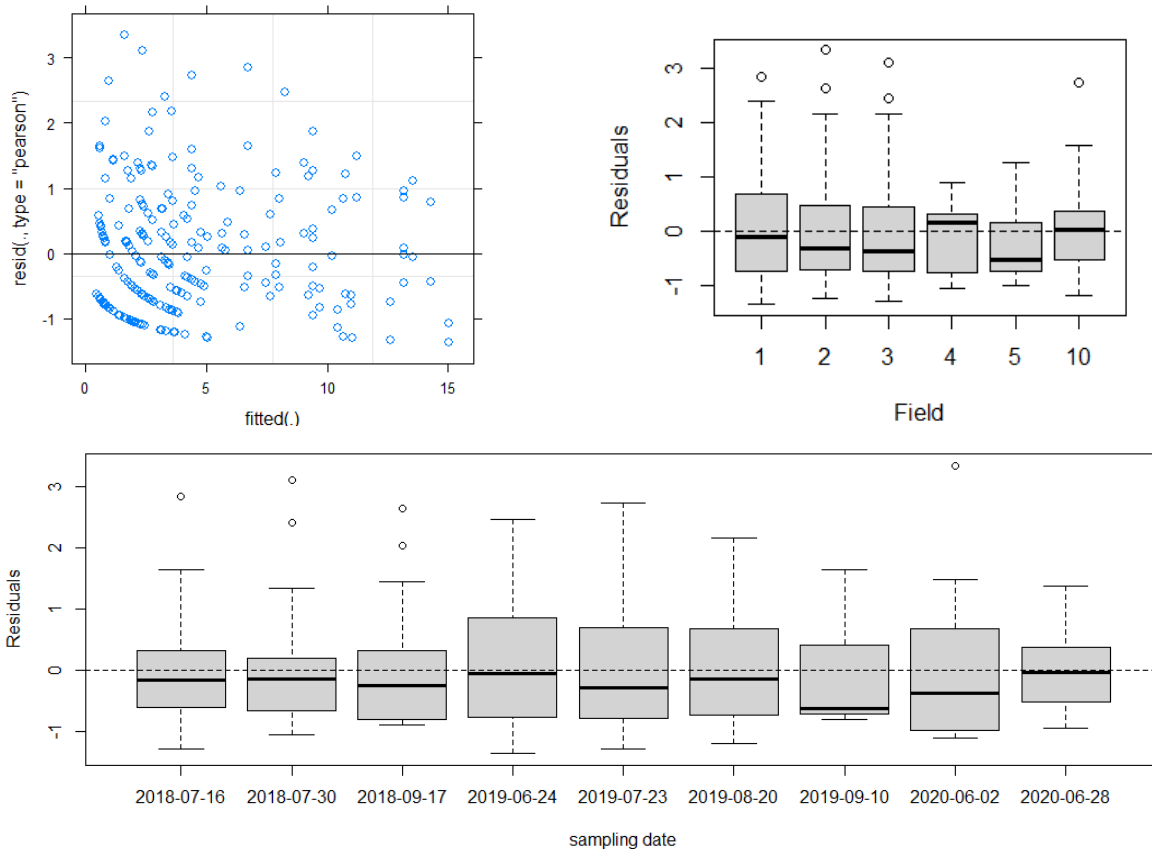


Figure 8, top left: Pearson residuals over fitted model, residuals show strong patterns at lower values but are still primarily centered around zero, top right: Pearson residuals over random effect field, model overestimates field 2,3 and 5 and underestimates field 4, bottom: Pearson residuals over random effect sampling date, the model overestimates several sampling dates. The model cannot be validated due to many overestimations by the model and strong patterns in the overall residual plot.

Appendix B5, Generalized linear mixed effect models and analysis of variance: Harvestmen

Selected model: `glmer.nb(Harvestmen ~ Strip + Multiple.Varities + Legumes + Rotation + (1 | Sampling.date), data = mydata)`

Table 9: Output of the glmer model over harvestmen

Harvestmen				
Predictors	Incidence Rate Ratios	CI	Statistic	p
(Intercept)	0.13	0.05 – 0.31	-4.55	<0.001
Strip [1]	2.88	1.20 – 6.91	2.37	0.018
Multiple.Varities [1]	1.14	0.69 – 1.91	0.51	0.609
Legumes [1]	0.83	0.48 – 1.43	-0.67	0.501
Rotation [1]	2.29	1.20 – 4.38	2.51	0.012
Random Effects				
σ^2	1.45			
τ_{00} Sampling.date	0.28			
ICC	0.16			
N Sampling.date	9			
Observations	261			
Marginal R ² / Conditional R ²	0.142 / 0.279			

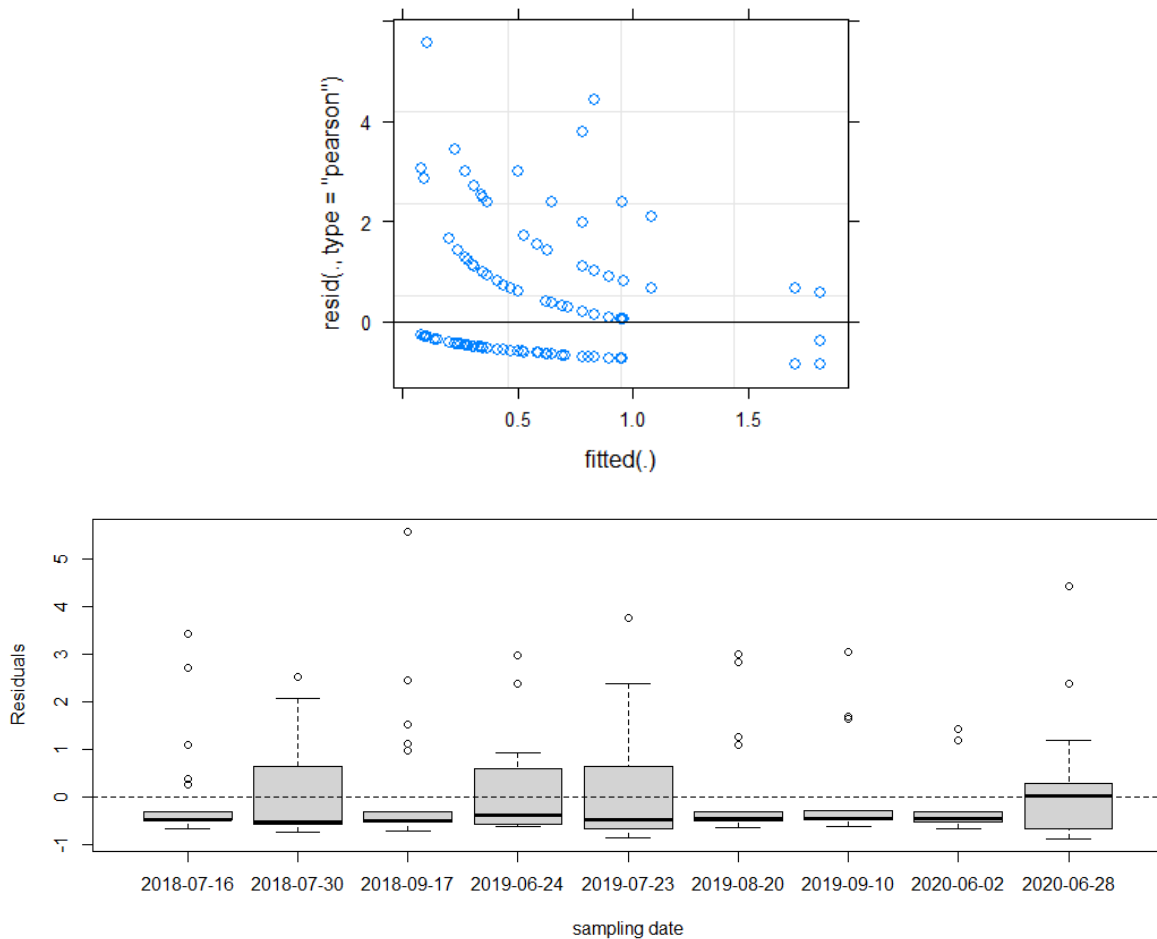


Figure 9, top: Pearson residuals over fitted model, residuals show strong patterns at lower values, bottom: Pearson residuals over random effect sampling date, the model overestimates several sampling dates. The model cannot be validated due to marginal and conditional R2 values, many overestimations by the model and the strong patterns in the overall residual plot.

Appendix B6, Generalized linear mixed effect model and analysis of variance: Ladybeetle

Selected model: `glmer.nb(Ladybeetle ~ Strip + Multiple.Varities + Legumes + Rotation + (1 | Sampling.date), data = mydata)`

Table 10: Output of the glmer model over ladybeetles

<i>Predictors</i>	Ladybeetle			
	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>Statistic</i>	<i>p</i>
(Intercept)	0.11	0.03 – 0.38	-3.44	0.001
Strip [1]	0.53	0.22 – 1.31	-1.37	0.172
Multiple.Varities [1]	0.60	0.20 – 1.81	-0.91	0.362
Legumes [1]	1.55	0.64 – 3.76	0.96	0.335
Rotation [1]	0.53	0.09 – 3.01	-0.72	0.471
Random Effects				
σ^2	2.84			
τ_{00} Sampling.date	1.86			
ICC	0.40			
N Sampling.date	9			
Observations	261			
Marginal R ² / Conditional R ²	0.049 / 0.425			

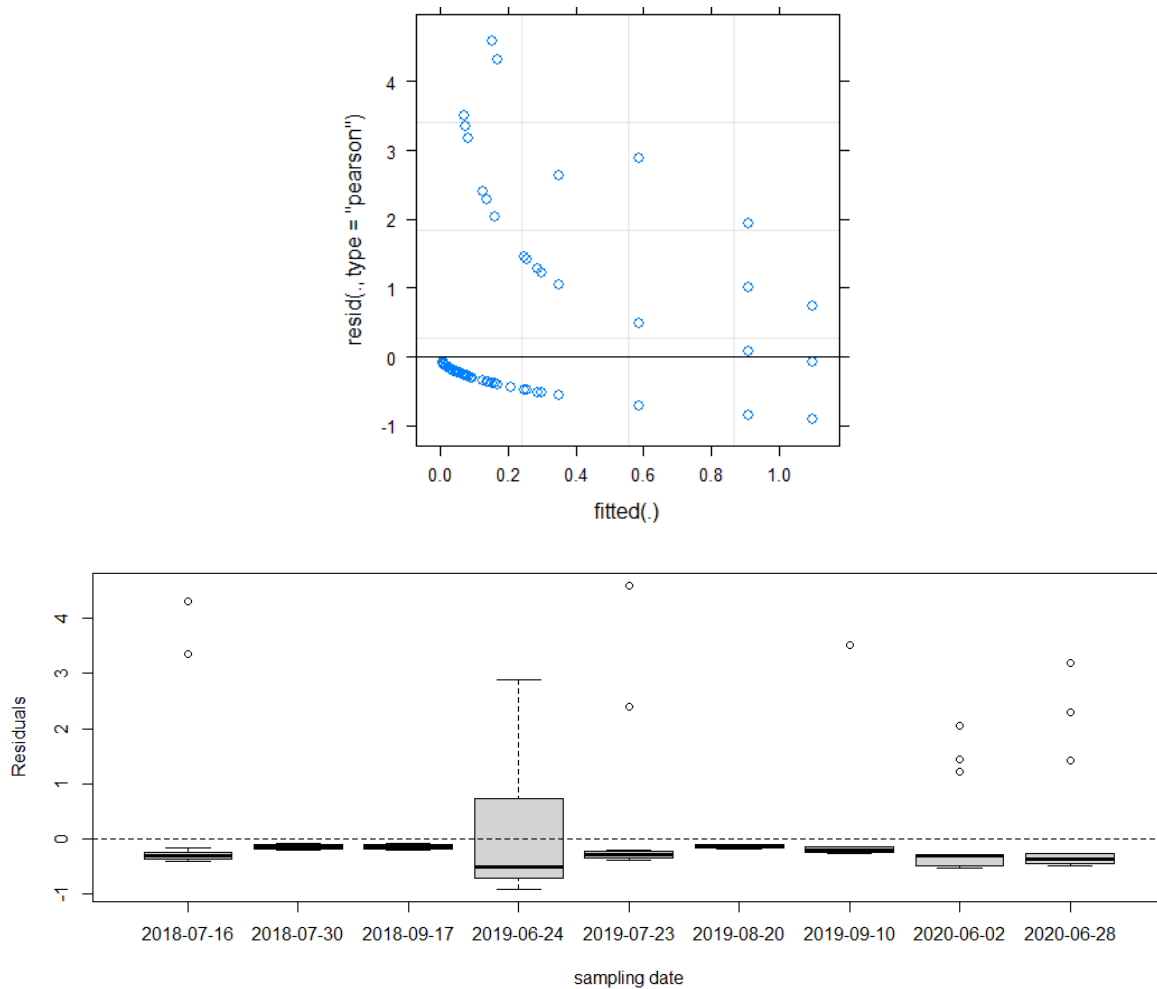


Figure 10, top: Pearson residuals over fitted model, residuals show strong patterns at lower values, bottom: Pearson residuals over random effect sampling date, the model overestimates for all sampling dates. The model cannot be validated due to marginal and conditional R2 values, many overestimations by the model and the strong patterns in the overall residual plot.

Appendix C, glmer over species richness

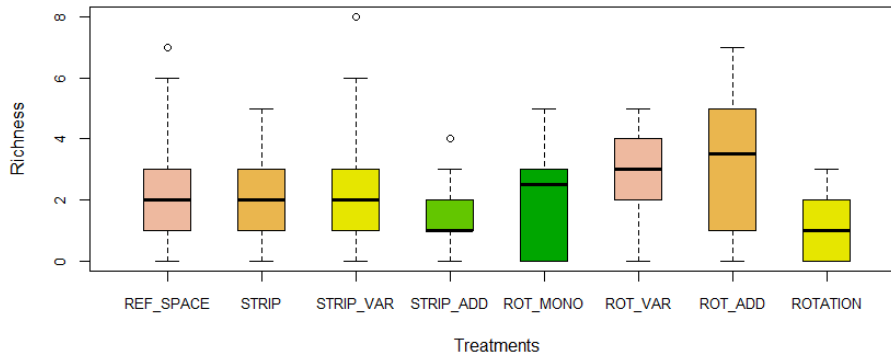


Figure 11, Boxplots of ground beetle taxa richness per treatment

Table 11: Output of the Poisson glmer over richness without zero-inflation

<i>Predictors</i>	<i>indice 2\$Richness</i>		
	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>p</i>
(Intercept)	1.66	1.02 – 2.67	0.040
Strip	0.92	0.66 – 1.26	0.591
Multiple.Varieties	1.04	0.83 – 1.31	0.723
Legumes	0.84	0.66 – 1.06	0.144
Rotation	0.95	0.70 – 1.30	0.764
Random Effects			
σ^2	0.52		
τ_{00} Month:Year	0.25		
τ_{00} field	0.02		
τ_{00} Year	0.00		
ICC	0.35		
N Month	4		
N Year	3		
N field	6		
Observations	261		
Marginal R ² / Conditional R ²	0.014 / 0.356		

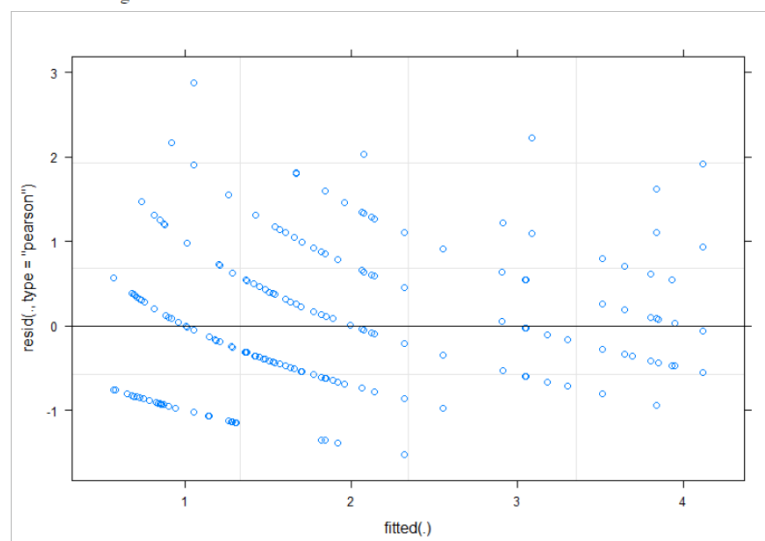


Figure 12, Residual plot of the Poisson glmer without zero-inflation, formula that was used: `glmer(indice2$Richness ~ Strip + Multiple.Varieties + Legumes + Rotation + (1 | Year/Month) + (1 | field),data=mydata, family = poisson(link = \"log\")`

Table 12: Output of the Poisson glmer with zero-inflation over richness.
The p value of the second intercept indicates that zero inflation is not significant

<i>Predictors</i>	indice 2SRichness		
	<i>Incidence Rate Ratios</i>	<i>CI</i>	<i>p</i>
(Intercept)	1.66	1.02 – 2.68	0.040
Strip	0.92	0.66 – 1.27	0.592
Multiple.Varities	1.04	0.83 – 1.31	0.724
Legumes	0.84	0.66 – 1.06	0.146
Rotation	0.95	0.70 – 1.30	0.765
(Intercept)	0.00	0.00 – Inf	0.996
Random Effects			
σ^2	0.62		
τ_{00} Month:Year	0.25		
τ_{00} Year	0.00		
τ_{00} field	0.02		
N_{Month}	4		
N_{Year}	3		
N_{field}	6		
Observations	261		
Marginal R^2 / Conditional R^2	0.017 / NA		

Appendix D, CCA

R output showing that a CCA should be used for the analysis and that treatments are not a significant predictor

```
> decorana(species) ## Gradient length first 2 axes: 3.5 and 4.11
```

```
Call:
decorana(veg = species)

Detrended correspondence analysis with 26 segments.
Rescaling of axes with 4 iterations.

Eigenvalues      DCA1  DCA2  DCA3  DCA4
Decorana values  0.5440 0.4137 0.3932 0.2731
Axis lengths     3.5551 4.1146 3.9613 3.4151
```

Axis length to long for RDA, try Hellinger transformation:

```
> decorana(Species_hel) ##Gradient length still too long for RDA, switch to CCA
```

```
Call:
decorana(veg = Species_hel)

Detrended correspondence analysis with 26 segments.
Rescaling of axes with 4 iterations.

Eigenvalues      DCA1  DCA2  DCA3  DCA4
Decorana values  0.7624 0.5456 0.3694 0.4370
Axis lengths     3.6160 4.3631 3.0608 2.2256
```

Hellinger transformation does not decrease axis length, therefore use CCA. Use Ordstep to select the best model.

Model selection using ordistep showed that Treatments had no significant effect.

```
> ordistep(my.cca)
```

```
Start: species ~ field + Year + Month + Strip + Multiple.Varities + Legumes + Rotation
```

	Df	AIC	F	Pr(>F)
- Legumes	1	888.84	0.3883	0.960
- Multiple.Varities	1	888.93	0.4650	0.915
- Rotation	1	889.03	0.5647	0.670
- Strip	1	890.02	1.4923	0.135
- Year	2	892.41	2.8228	0.005 **
- field	5	896.01	3.0095	0.005 **
- Month	3	905.03	6.7066	0.005 **

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Step: species ~ field + Year + Month + Strip + Multiple.Varities + Rotation
```

	Df	AIC	F	Pr(>F)
- Multiple.Varities	1	887.54	0.6538	0.765
- Rotation	1	887.50	0.6166	0.675
- Strip	1	888.44	1.4972	0.125
- Year	2	890.86	2.8570	0.005 **
- field	5	894.59	3.0567	0.005 **
- Month	3	903.40	6.7235	0.005 **

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Step: species ~ field + Year + Month + Strip + Rotation
```

	Df	AIC	F	Pr(>F)
- Rotation	1	886.17	0.5875	0.625
- Strip	1	887.13	1.4998	0.130
- Year	2	889.53	2.8560	0.005 **
- field	5	893.21	3.0569	0.005 **
- Month	3	902.03	6.7356	0.005 **

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
Step: species ~ field + Year + Month + Strip
```

	Df	AIC	F	Pr(>F)
- Strip	1	885.75	1.5037	0.140

```

M. van de Glind
- Year 2 888.14 2.8630 0.005 **
- field 5 892.65 3.2370 0.005 **
- Month 3 900.64 6.7608 0.005 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Step: species ~ field + Year + Month

      Df    AIC      F Pr(>F)
- Year  2 887.62 2.8249 0.005 **
- field 5 895.49 3.9242 0.005 **
- Month 3 900.11 6.7538 0.005 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Call: cca(formula = species ~ field + Year + Month, data = fixed)

Total      Inertia Proportion Rank
Constrained  5.2198      1.0000    10
Unconstrained 4.1350      0.7922    20
Inertia is scaled Chi-square

Eigenvalues for constrained axes:
CCA1  CCA2  CCA3  CCA4  CCA5  CCA6  CCA7  CCA8  CCA9  CCA10
0.4326 0.2193 0.1925 0.0929 0.0533 0.0439 0.0219 0.0178 0.0063 0.0043

Eigenvalues for unconstrained axes:
CA1  CA2  CA3  CA4  CA5  CA6  CA7  CA8
0.5331 0.4225 0.3874 0.3759 0.3173 0.2899 0.2453 0.2200
(Showing 8 of 20 unconstrained eigenvalues)

```

Checking collinearity

```
> vif.cca(my.cca2) ## VIF should be 10 or ideally below 5, most are below 5, June = 6
```

field2	field3	field4	field5
1.293974	1.261611	1.102454	1.087005
field10	Year2019	Year2020	
1.543723	2.046158	5.050642	
MonthJuly	MonthJune	MonthSeptember	
5.302528	5.994072	1.617108	

No collinearity detected as the level of VIF is below 10 (Oksanen et al., 2019, see <https://cran.r-project.org/web/packages/vegan/vegan.pdf>)

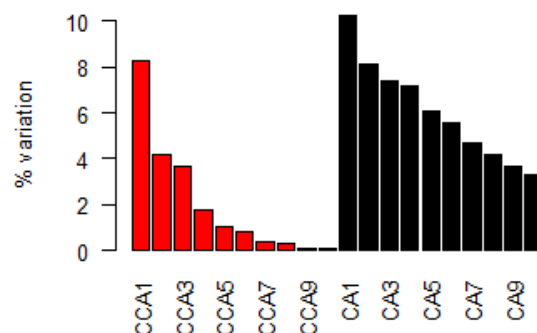


Figure 13, Barplot showing explained variation per ordination axis

Appendix E, RDA

Model selection using ordistep showed that treatments were not significant in the RDA.

```
> a <- ordistep(my.rda) #remove Treatments
```

Start: sqrt(species) ~ field + Treatments + Year + Month

	Df	AIC	F	Pr(>F)
- Treatments	7	262.68	1.0068	0.420
- field	5	279.20	3.8574	0.005 **
- Year	2	308.10	21.7837	0.005 **
- Month	3	327.65	22.9457	0.005 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Step: sqrt(species) ~ field + Year + Month

	Df	AIC	F	Pr(>F)
- field	5	275.97	4.6687	0.005 **
- Year	2	305.69	24.8953	0.005 **
- Month	3	320.91	23.6200	0.005 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
> vif.cca(my.rda2)
```

	field2	field3	field4	field5	field10	Year2019	Year20
20	1.508810	1.490710	1.169969	1.265647	1.599369	2.450434	4.2497
58	MonthJuly 3.476354	MonthJune 3.952400	MonthSeptember 2.056613				

No collinearity detected as the level of VIF is below 10 (Oksanen et al., 2019, see <https://cran.r-project.org/web/packages/vegan/vegan.pdf>)

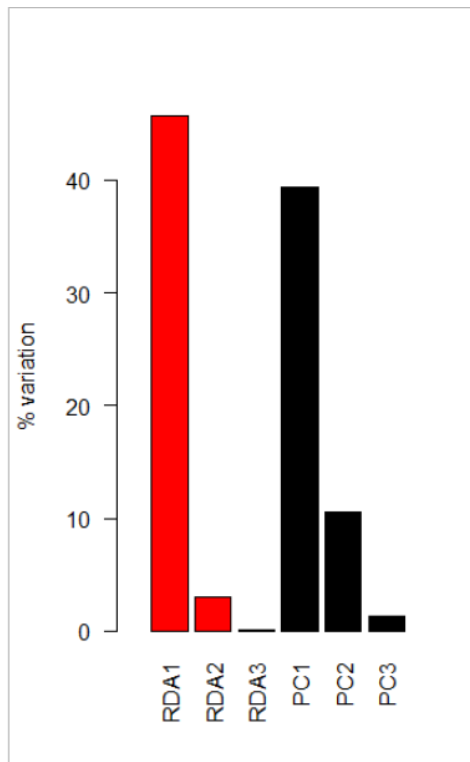


Figure 14, variation explained per axis

Appendix F, Data exploration through boxplots showing means and medians of natural enemies caught in pitfall traps of each treatment over three years

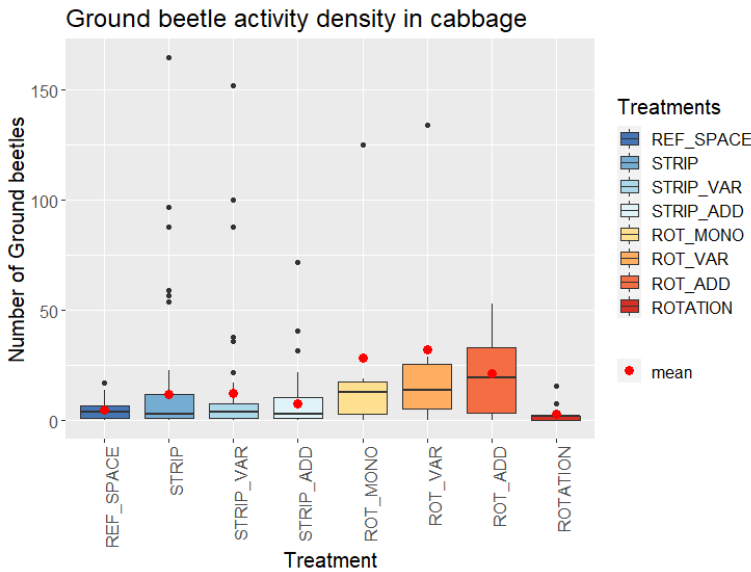


Figure 15, Boxplot of the amount of ground beetles per treatment, differences in mean and median were not significant.

* The ROT_MONO, ROT_VAR and ROT_ADD were only present in 2018 and show a higher activity density mainly as an effect of seasonal influences.

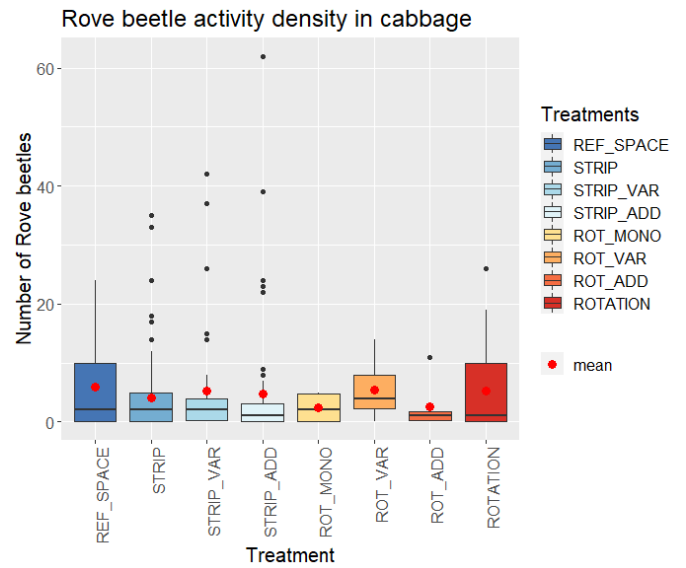


Figure 16, Boxplot of the amount of rove beetles per treatment, differences in mean and median were not significant.

* The ROT_MONO, ROT_VAR and ROT_ADD were only present in 2018.

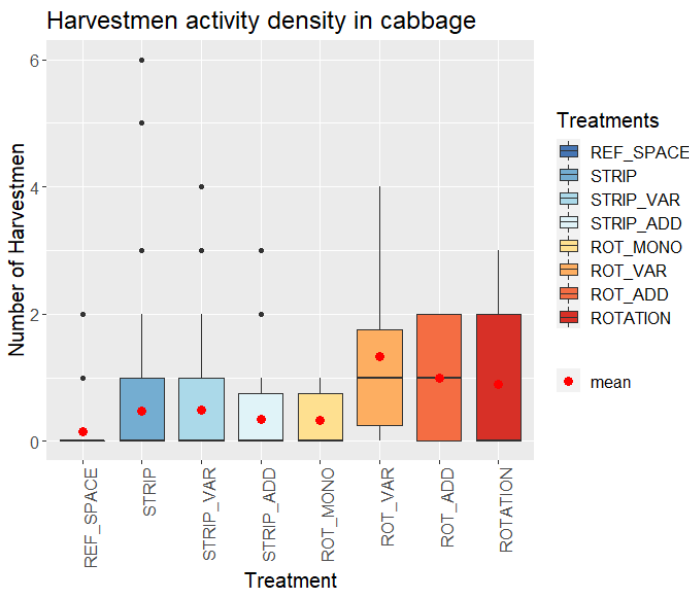


Figure 17, Boxplot of the amount of harvestmen per treatment, differences in mean and median were not significant.

* The ROT_MONO, ROT_VAR and ROT_ADD were only present in 2018.

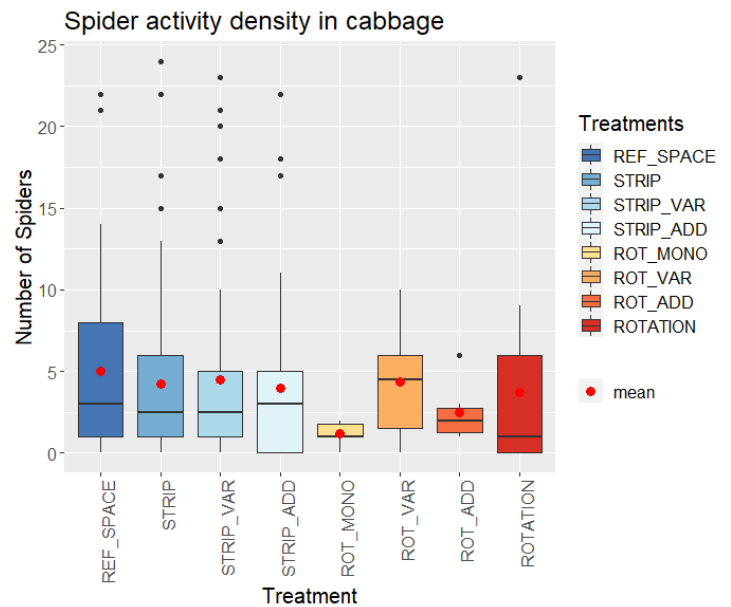


Figure 18, Boxplot of the amount of spiders per treatment, differences in mean and median were not significant.

* The ROT_MONO, ROT_VAR and ROT_ADD were only present in 2018.

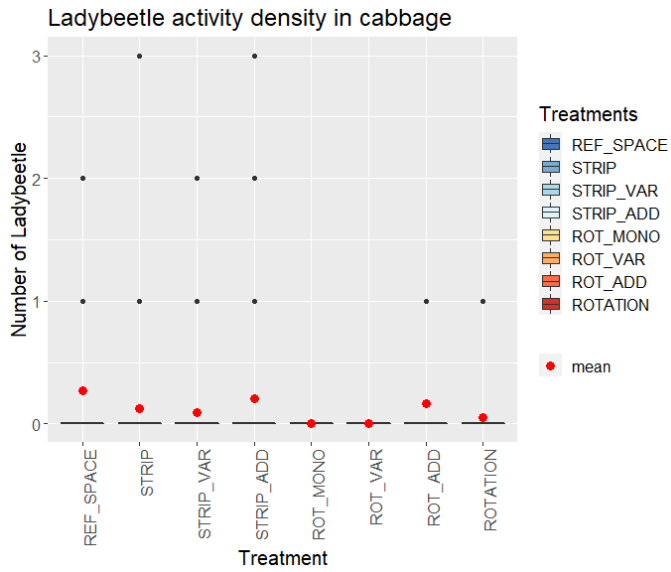


Figure 19, Boxplot of the amount of ladybeetles per treatment, differences in mean and median were not significant.

* The ROT_MONO, ROT_VAR and ROT_ADD were only present in 2018.

Appendix G: Mean of captured specimens per treatment split by sampling date and field:

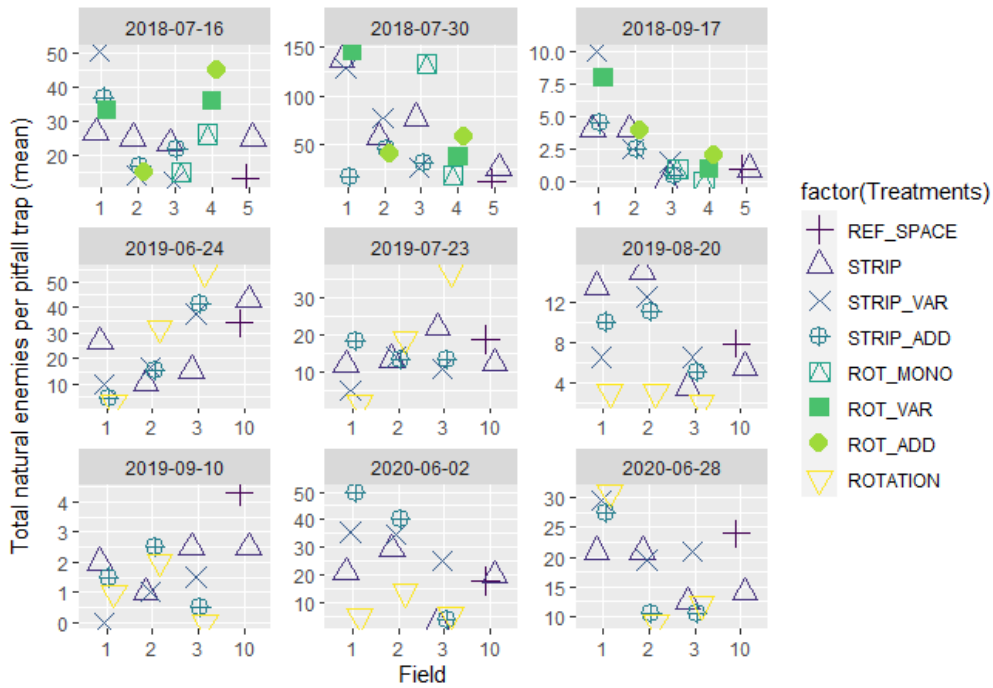


Figure 20, Plots showing the mean of captured natural enemies per pitfall trap split by sampling date and field

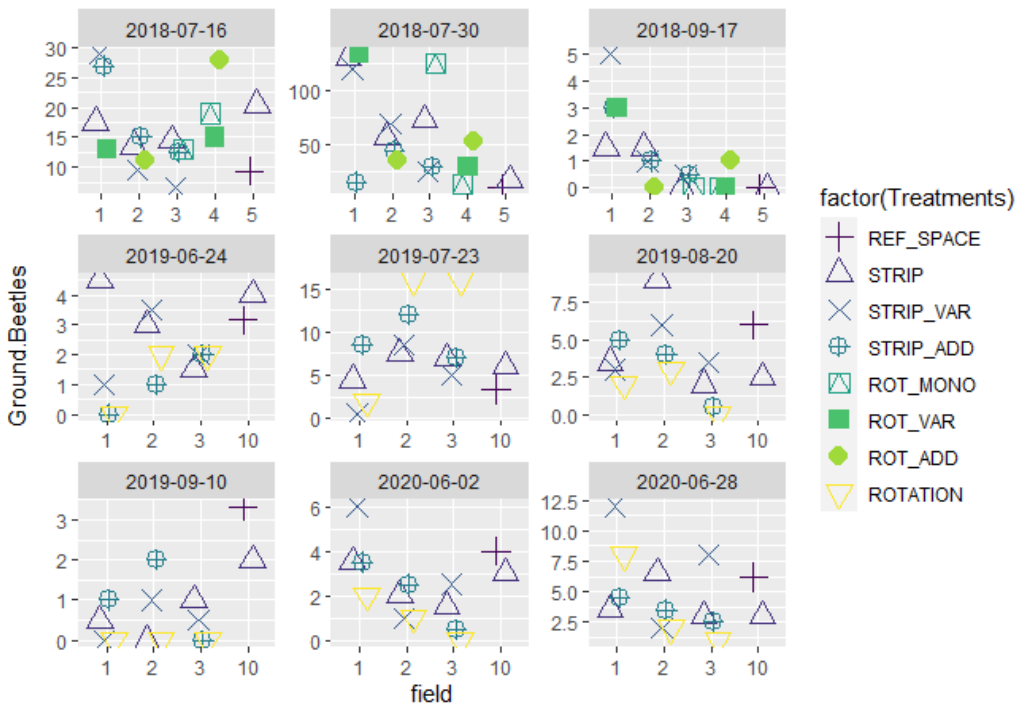


Figure 21, Plots showing the mean of captured ground beetles per pitfall trap split by sampling date and field

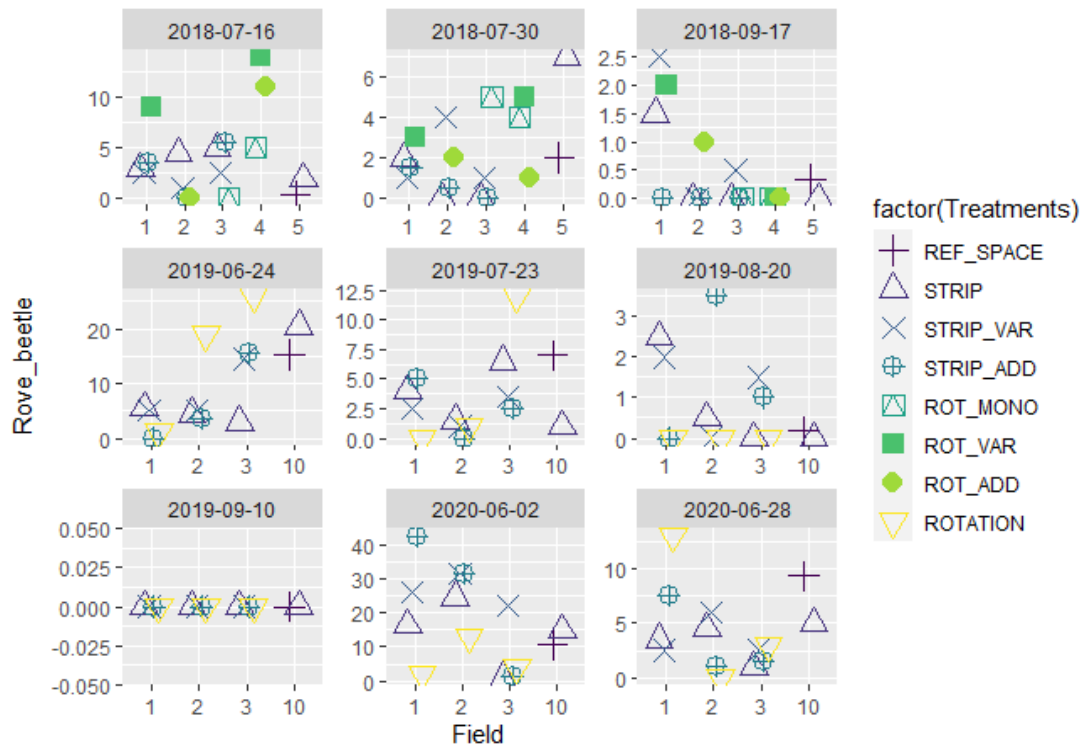


Figure 22, Plots showing the mean of captured rove beetles per pitfall trap split by sampling date and field

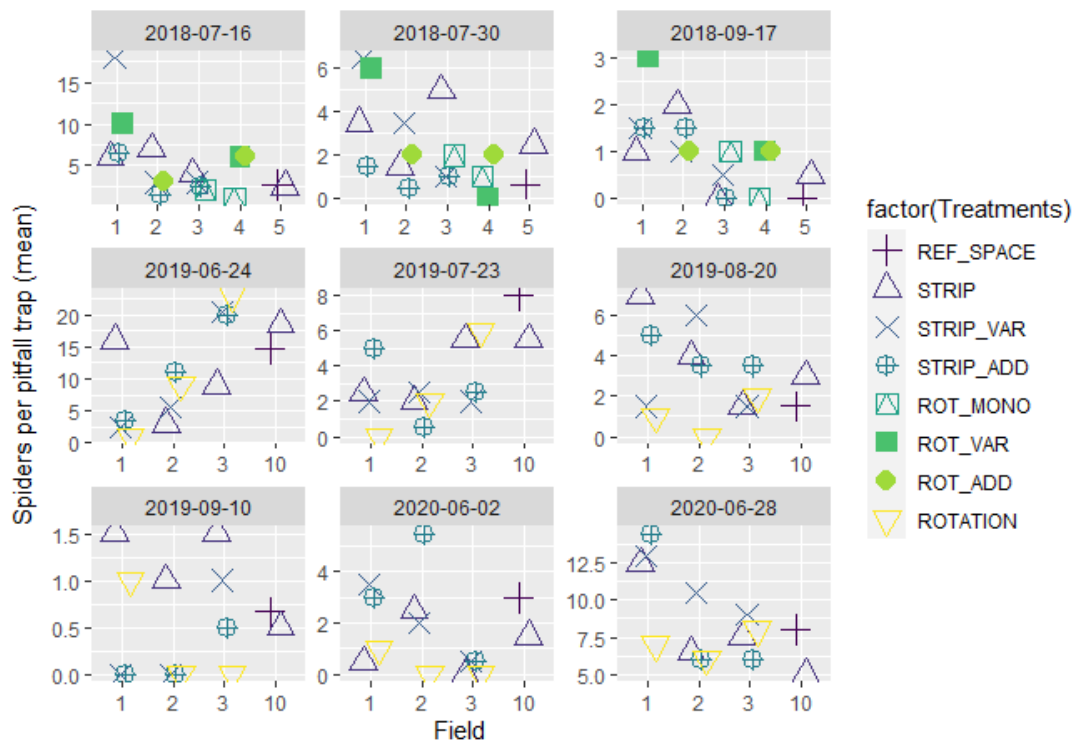


Figure 23, Plots showing the mean of captured spiders per pitfall trap split by sampling date and field

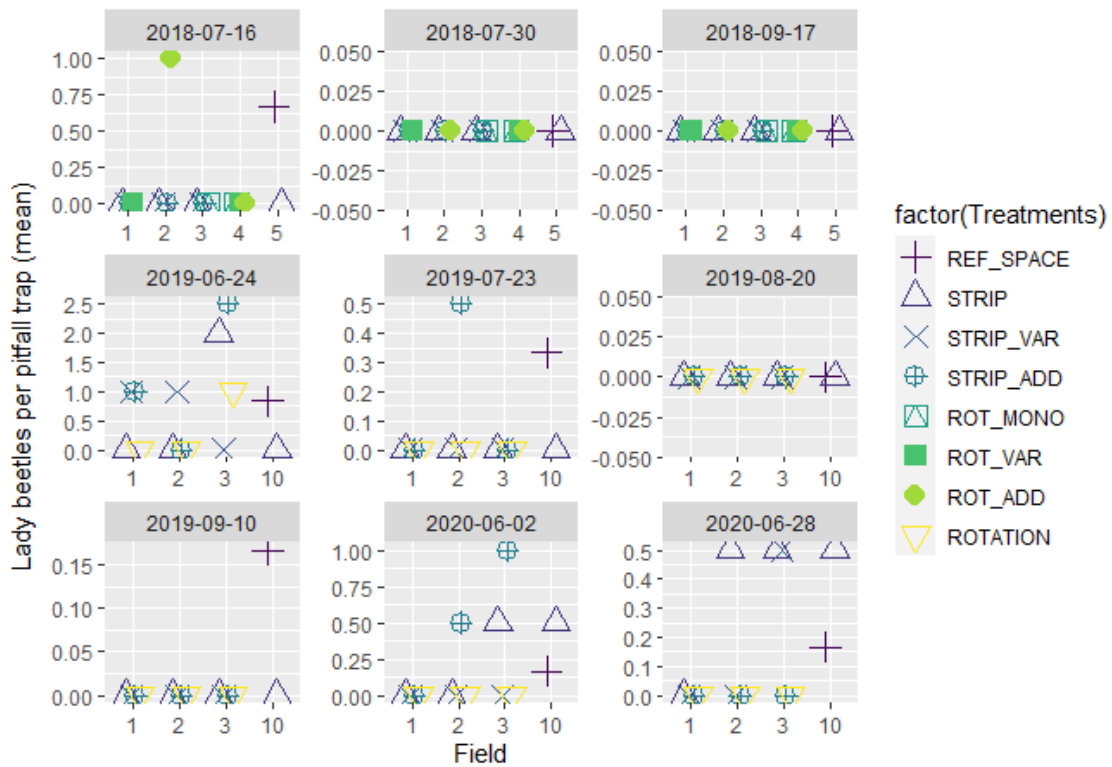


Figure 24, Plots showing the mean of captured ladybeetles per pitfall trap split by sampling date and field

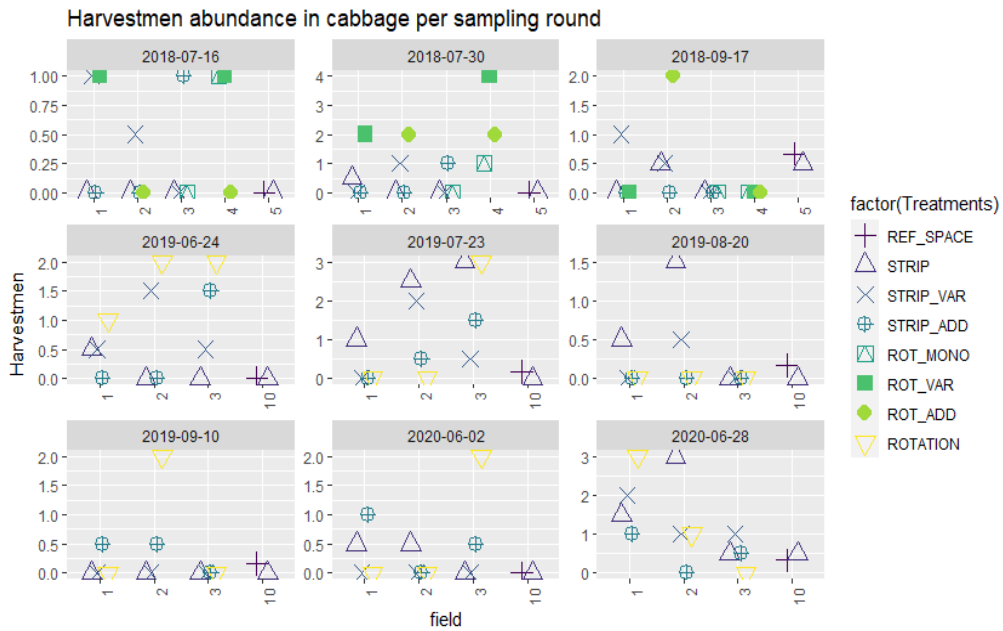


Figure 25, Plots showing the mean of captured harvestmen per pitfall trap split by sampling date and field

Appendix H, Pairwise comparisons using Wilcoxon signed rank test**Table 13:** Wilcoxon paired signed rank test of Ladybeetles

Treatment 1 (T1)	Treatment 2 (T2)	Amount of comparisons	T1 > T2	T2 > T1	Tie	P Value
STRIP	REF_SPACE	9	2	4	3	0.5282
STRIP	STRIP_VAR	27	3	2	22	1
STRIP	STRIP_ADD	27	2	5	20	0.2402
STRIP_VAR	STRIP_ADD	27	2	4	21	0.4568
STRIP	ROTATION	18	4	0	14	0.08897
STRIP_VAR	ROTATION	18	3	1	14	0.5708
STRIP_ADD	ROTATION	18	5	0	13	0.05676

Table 14: Wilcoxon paired signed rank test of Ground beetles

Treatment 1 (T1)	Treatment 2 (T2)	Amount of comparisons	T1 > T2	T2 > T1	Tie	P Value
STRIP	REF_SPACE	9	4	4	1	0.8336
STRIP	STRIP_VAR	27	14	13	0	0.8004
STRIP	STRIP_ADD	27	13	12	2	0.4031
STRIP_VAR	STRIP_ADD	27	13	11	3	0.5669
STRIP	ROTATION	18	13	4	1	0.1764
STRIP_VAR	ROTATION	18	11	3	4	0.2202
STRIP_ADD	ROTATION	18	11	4	3	0.3772

Table 15: Wilcoxon paired signed rank test of natural enemies

Treatment 1 (T1)	Treatment 2 (T2)	Amount of comparisons	T1 > T2	T2 > T1	Tie	P Value
STRIP	REF_SPACE	9	4	4	1	0.6241
STRIP	STRIP_VAR	27	13	13	1	0.7994
STRIP	STRIP_ADD	27	14	12	1	0.4767
STRIP_VAR	STRIP_ADD	27	15	11	1	0.5674
STRIP	ROTATION	18	11	7	0	0.5419
STRIP_VAR	ROTATION	18	11	7	0	0.2484
STRIP_ADD	ROTATION	18	11	7	0	0.6471

Table 16: Wilcoxon paired signed rank test of Rove beetles

Treatment 1 (T1)	Treatment 2 (T2)	Amount of comparisons	T1 > T2	T2 > T1	Tie	P Value
STRIP	REF_SPACE	9	4	4	1	0.8336
STRIP	STRIP_VAR	27	11	12	4	0.299
STRIP	STRIP_ADD	27	9	12	6	0.8343
STRIP_VAR	STRIP_ADD	27	14	8	5	0.415
STRIP	ROTATION	18	8	6	4	0.9749
STRIP_VAR	ROTATION	18	8	5	5	0.4846
STRIP_ADD	ROTATION	18	6	8	4	0.753

Table 17: Wilcoxon paired signed rank test of Harvestmen

Treatment 1 (T1)	Treatment 2 (T2)	Amount of comparisons	T1 > T2	T2 > T1	Tie	P Value
STRIP	REF_SPACE	9	1	4	4	0.5827
STRIP	STRIP_VAR	27	9	8	10	0.8092
STRIP	STRIP_ADD	27	11	7	9	0.2858
STRIP_VAR	STRIP_ADD	27	12	8	7	0.3697
STRIP	ROTATION	18	8	6	4	0.9242
STRIP_VAR	ROTATION	18	3	7	8	0.2
STRIP_ADD	ROTATION	18	4	8	6	0.04819*

Signif. codes: '**' 0.05

Table 18: Wilcoxon paired signed rank test of Spiders

Treatment 1 (T1)	Treatment 2 (T2)	Amount of comparisons	T1 > T2	T2 > T1	Tie	P Value
STRIP	REF_SPACE	9	4	5	0	1
STRIP	STRIP_VAR	27	12	14	1	0.8185
STRIP	STRIP_ADD	27	16	10	1	0.4918
STRIP_VAR	STRIP_ADD	27	13	9	5	0.5573
STRIP	ROTATION	18	10	6	2	0.1933
STRIP_VAR	ROTATION	18	12	5	1	0.1688
STRIP_ADD	ROTATION	18	11	5	2	0.1029

Appendix I, Ground beetles caught by taxa and classification by size (next page)**Table 19:** Overview of captured ground beetle taxa

<i>Harpalus spp.</i>	1890
<i>Pterostichus spp.</i>	395
<i>Bembidion quadrimaculatum</i>	98
<i>Bembidion properans</i>	50
<i>Bembidion tetracolum</i>	44
<i>Clivina spp.</i>	42
<i>Calathus spp.</i>	38
<i>Bembidion lampros</i>	29
<i>Bembidion femoratum</i>	28
<i>Amara spp.</i>	24
<i>Broscus cephalotus</i>	19
<i>Poecilus spp.</i>	12
<i>Amara fulva</i>	10
<i>Trechus spp.</i>	7
<i>Abax parallelopedus</i>	4
<i>Pterostichus vernalis/strenuus</i>	3
<i>Agonum muelleri</i>	1
<i>Anisodactylus binotatus</i>	1
<i>Badister lacertosus</i>	1
<i>Blemus discus</i>	1
<i>Unidentified</i>	1

Table 20: Overview of classification of ground beetles by size

Large (> 13.1 mm)	Medium (9.1-13 mm)	Small (<9 mm)	Source
<i>Pterostichus spp.</i> *			Hackston (2019)
<i>Brosicus cephalotes</i>			Hackston (2019) Larochelle & Larivière (1989)
<i>Abax parallelopedus</i>			Jelaska & Durbes (2009)
<i>Harpalus spp.</i> **			Jelaska & Durbes (2009); Hackston (2019)
	<i>Amara fulva spp.</i>		Hackston (2019)
	<i>Poecilus spp.</i>		Hackston (2019)
	<i>Anisodactylus binotatus</i>		Hackston (2019)
		<i>Amara spp.</i> ***	Hackston (2019)
		<i>Bembidion femoratum</i>	Hackston (2019)
		<i>B. properans</i>	Hackston (2019)
		<i>B. tetracolum</i>	Hackston (2019)
		<i>B. lampros</i>	Jelaska & Durbes (2009); Hackston (2019)
		<i>B. quadrimaculatum</i>	Hackston (2019)
		<i>Blemus discus</i>	Hackston (2019)
		<i>Calathus spp.</i> ****	Aukema (1990)
		<i>Clivina spp.</i>	Hackston (2019)
		<i>Trechus spp.</i>	Hackston (2019)
		<i>Pterostichus vernalis/strenuus</i>	Jelaska & Durbes (2009); Hackston (2019); Magura et al. (2006)
		<i>Agonum muelleri</i>	Hackston (2019)
		<i>Badister lacertosus</i>	Hackston (2019); Magura et al. (2006)

* All species of large *Pterostichus* were *P. melenarius* and *P. niger* in 2018 and 2020, both species are > 13.1 mm. Smaller species (*P. vernalis/strenuus* are grouped in the small group.

** Majority of the caught *Harpalus spp.* ground beetles are *Harpalus rufipes*, 1722 in 2018 (99.71% of *H. spp.* caught) and 42 (71.19% of *H. spp.* caught) in 2020. Over all three years 1890 *H. spp.* were caught in total. So at least 93% are *H. rufipes* with an average size > 13.1mm

***All *Amara* species (7 individuals) caught in 2018 other than *Amara fulva* had an average size of < 9mm

**** Ground beetles from the genus *Calathus* were not identified to species level, the source describes three species in the Netherlands <9mm, however other species may be larger

References

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Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitenskapelige universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway