# Evaluating the Impact of Cropping Systems on the Taxonomic and Functional Composition of Arthropod Communities: Recommended Pitfall Traps and Sampling Strategies

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## Abstract

1. Ground dwelling arthropods are affected by agricultural practices, and analyses of their responses to different crop management are required. The sampling efficiency of pitfall traps has been widely studied in natural ecosystems. In arable agroecosystems, arthropod communities are more simplified than in natural ones and sampling techniques need to be adjusted to these specific communities. In particular, the ability to distinguish between simplified communities and the sampling effort required have been little investigated. We evaluated the suitability of pitfall traps for characterizing the effects of arable cropping systems on the taxonomic and functional composition of spider and carabid communities.

2. In a field experiment comparing three cropping systems, we compared the effects of two pitfall trap diameters, of type of preserving fluid used in pitfall traps and of sampling effort on six metrics describing communities: activity-density, richness and community weighted mean (CWM) of body size, each one for carabid and spiders.

3. Trap size affected the observed composition of carabid and spider communities, with large traps yielding a higher relative proportion of spiders, and a higher species richness and CWM body size for both taxa. The type of preserving fluid had no marked effect on any of the metrics considered.

In the case of our experiment conducted in arable crops, simulations with various sampling efforts showed that only very different communities could be significantly distinguished with less than ten traps per field or less than 30 field-year replicates.

The relationship between the arthropod community differences and the minimum sampling effort required to detect it was similar for activity-density and species or genus richness

metrics. Fewer traps were required to find differences between cropping systems for CWM body size than for other metrics.

For the three arable cropping systems studied here, carabid activity-density, carabid CWM body size and spider genus richness were the variables better distinguishing between cropping systems with the smallest sampling effort.

4. The minimum sampling effort required for community comparisons under different arable cropping systems was smaller for functional composition than for activity-density in case of spiders and richness in case of carabids. The trap design, arthropod community metrics and crops selected were the principal levers for optimizing the trade-off between sampling effort and the ability to detect arthropod community responses to cropping systems.

## Keywords

activity-density, arthropod community, cropping system, CWM body size, ground beetle, pitfall trap, sampling effort, species richness, spider

## 1. Introduction

Agriculture is facing numerous challenges, including biodiversity loss, climate change and dependence on synthetic inputs. These challenges are driving the design and assessment of innovative sustainable cropping systems targeting all these issues together (Foley et al. 2011). Sustainability will require a fostering of biotic interactions to strengthen the ecosystem services on which agriculture depends (Gaba et al. 2018). In particular, agricultural techniques that enhance the biodiversity of soil organisms, such as ground dwelling arthropods, not only promote conservation biological control (Thérond et al. 2017), but also support higher trophic levels, such as vertebrates.

Ground dwelling arthropods have important roles in the dynamics of soil functioning and associated ecosystem services. They are often used as biological indicators of the effects of anthropogenic activities because they respond to them with community changes (Gallé et al. 2019). In agroecosystems, carabids and spiders are important predators for the regulation of phytophagous insect and weed populations (Kromp 1999; Marc et al. 1999). In terms of biological control, their impact depends on their density, the species richness and functional composition of their communities (Jonsson et al. 2017; Rusch et al. 2015). Assessing arthropods communities under different cropping systems, involving lower levels of chemical inputs and less physical disturbance of the soil, can help telling if the cropping system preserves the soil biodiversity, its functioning and the associated services. The most commonly utilized metrics to assess carabid and spider assemblages are activity-density (as a measure of abundance), taxonomic richness (as a measure of taxonomic diversity) and measures of functional composition, such as community weighted means of traits (Eyre et al. 2012; Martin et al. 2019).

Pitfall traps are widely used to sample ground dwelling arthropod assemblages (Woodcock 2005). The design of pitfall traps varies considerably between studies, and a number of features, including trap diameter and the type of preserving fluid used within them, have been shown to

have a significant impact on capture rates (Brown and Matthews 2016). Several methodological studies have been performed (Corti et al. 2013; Lange et al. 2011; Work et al. 2002) in natural or semi-natural habitats, such as forests, grasslands and riverbeds. However, in these ecosystems, vegetation structure is more complex than in arable fields, leading to more diversified arthropod communities. It is thus unclear to which extent the results of these methodological studies can be generalized to characterise the simplified communities of disturbed ecosystems, such as agroecosystems.

First, trap diameter varies considerably between studies (Brown and Matthews 2016). Large-diameter pitfall traps collect more individuals of all taxa than smaller traps, but they also collect more individual spiders (Lycosidae), more spider species and fewer small carabid individuals than expected when catch rates are corrected for trap circumference (Lange et al. 2011; Work et al. 2002). Indeed, capture rates depend on the mobility and behaviour of the organisms. In arable fields, in which the architecture of the surface vegetation is less complex than in natural habitats (little or no litter on the soil surface, bare soil between rows), ground-dwelling arthropods are likely to be more mobile, potentially reducing the effect of trap diameter on capture rates. Small and large pitfall traps could, therefore, provide more similar results for carabid and spider communities than in more structurally complex habitats.

Second, many types of preserving fluids are available, and the pros and cons of each have been hotly debated (Woodcock 2005). Ethylene glycol and formalin are the most widely used (Brown and Matthews 2016), as they are highly effective for their capture efficiency and to conserve dead individuals, but both are toxic. Nontoxic fluids should be preferred, where possible, to prevent intoxication following accidental ingestion (e.g. by humans or wild mammals). Salt water and vinegar are other possible alternatives. Both have been evaluated for preservation efficacy, which is lower than that of ethylene glycol or formalin, but their capture efficiency has rarely been assessed. Capture efficiency relates to the fact that preserving fluids

may be attractant or repulsive and that trapped arthropods could escape from the trap depending on the density and the surface tension of the fluid. It has been suggested that acetic acid (vinegar) may act as an attractant (McCravy and Willand 2007; Scheller 1984) and that the low density of salt water may enhance the escape ability of arthropods. The effect of this on capture efficiency remains however unclear (Koivula et al. 2003).

Third, the number of traps is a key point in the sampling strategy. The use of 20 to more than 70 traps has been recommended for the determination of carabid species richness at a given site (Woodcock 2005). This sampling effort is often impracticable when it has to be replicated over a network of multiple sites to account for landscape effects for instance, in terms working time to settle and harvest the traps and to identify the captured arthropods.

Determining absolute values of richness or abundance is not necessary when the goal is to compare arthropod communities under different cropping systems. Looking at relative values or performing comparisons between might require a tailored sampling effort. Arable fields are generally characterized by frequent intense disturbances, and by the presence of little abundant arthropods with low diversity (Geiger et al. 2010; Postma-Blaauw et al. 2010), in which carabids and spiders have a highly heterogeneous spatial distribution (Holland et al. 1999). A large sampling effort is therefore likely to be required for accurate comparisons of activity-density and richness values between fields managed with different cropping systems. This can be problematic in terms to feasibility. Studies investigating the functioning of these communities, such as predation on phytophagous insects, also require a characterization of the functional composition of the arthropod community in terms of diet, body size… (Rudolf 2012; Rusch et al. 2015). Such metrics of the functional composition of arthropod communities have never been evaluated in methodological studies comparing pitfall trap designs. Community weighted means (CWM) of trait values are generally determined by the identity and proportion of the dominant species, with rare species making only a minor contribution. A smaller

sampling effort should therefore be required for the estimation of this metric than for species richness. To summarize, we miss data on the level of sampling effort to reach our objective because it depends on (1) the fact that we want to compare arthropod communities, rather than estimating absolute metrics, (2) the low abundant arthropod communities with low diversity and (3) the different metrics chosen, especially those related to community functioning, which may require different sampling efforts.

In this study, we aimed to determine how sampling should be conducted in order to characterize differences among communities under different arable cropping systems. This included comparing pitfall traps characteristics, the associated preservative fluid and the sampling effort. A two-year large field sampling was carried out in a cropping system experiment, in which cropping systems differed in terms of soil tillage, pesticide use and the types of crops and intercrops grown. We first compared the effects of two pitfall trap diameters and two different preserving fluids (salt water and vinegar) on community composition and on six response variables, i.e. activity-density (number of individuals per trap), species or genus richness and CWM of body size for both carabids and spiders. This functional metric was chosen because it is highly sensitive to community composition and because body size is a major trait affecting trophic interactions (Ball et al. 2015). We then used field data for three cropping systems for a simulation study in which we varied the number of pitfall traps per field and the number of field replicates. We calculated the minimum sampling effort required to differentiate between the effects of contrasting cropping systems on the three metrics of carabid and spider communities described above.

## 2. Materials and methods

*2.1. Experimental design*

This experiment was performed at a long-term experimental site at Grignon, France (N 48.84, E 1.95), where cropping systems with innovative objectives and techniques combinations have been implemented since 2008 in adjacent 63 × 65 m fields (for more information, see Colnenne-David and Doré 2015; Colnenne-David et al. 2017). This site has a deep homogeneous loamy clay soil.

We studied three cropping systems within this existing experimental design. The first was a productive high-environmental performance cropping system (PHEP), designed to meet high targets for both production and environmental criteria. Pesticide applications were allowed but the treatment frequency index and ploughing frequency were halved in comparison with conventional cropping systems. The amount of mineral nitrogen fertilizer was reduced from 190 to 56 $kg \cdot ha^{-1} \cdot year^{-1}$ on average in comparison with conventional cropping systems in the surrounding. This system was used as a "reference" here, although its environmental objectives were higher than those of the conventional cropping systems used in the vicinity. The other systems were designed to meet additional environmental targets. The second cropping system was a no-pesticide cropping system (No-Pest), in which no pesticide use was tolerated, even pesticides authorized in organic agriculture. However, the application of inorganic chemical fertilizers was permitted in this system (average over years: 27 $kg \cdot ha^{-1} \cdot year^{-1}$) and ploughing occurred three times over the six-year rotation. The third cropping system was a low-greenhouse gas emission cropping system (L-GHG), designed to reduce greenhouse gas emissions by 50% relative to the PHEP system, by increasing carbon sequestration in the soil, maintaining a low level of nitrogen use (average over years: 52 $kg \cdot ha^{-1} \cdot year^{-1}$) and decreasing $N_2O$ emissions. These objectives were achieved through the maintenance of a continuous soil cover and the elimination of soil tillage. The targeted level of pesticide used was the same as in the PHEP system.

All three cropping systems were required to meet the same environmental goals, including reductions of pesticide use and losses, inorganic nitrogen use and losses, through crop management practices, such as the sowing of catch crops, and diversification of the crop rotation (Colnenne-David and Doré 2015; Colnenne-David et al. 2017). Each cropping system was replicated three times, but the replicates did not always contain the same crop simultaneously (they are however cultivated with the same crop sequence).

*2.2. Arthropod sampling with different kinds of pitfall traps*

In 2014 and 2015, we sampled ground-dwelling arthropods (1) in a winter cereal field (generally wheat, but barley in one case) for each of the three cropping systems, and (2) in a winter oilseed rape field for the PHEP and L-GHG cropping systems. This resulted in the sampling of five “cropping system × crop” combinations each year.

Arthropods were sampled with pitfall traps placed in April, May and June, corresponding to 800, 1100 and 1500 degree-days, calculated each year as the daily sum of positive mean air temperatures from January 1 onwards.

The pitfall traps consisted of transparent plastic containers, filled with a preserving fluid and inserted into the soil such that their rim was flushed with the soil surface. They were protected from the rain by inverted opaque plastic flower-pot saucers (14 cm in diameter) supported about 2 cm above the soil surface with two nails (Fig. 1 A).

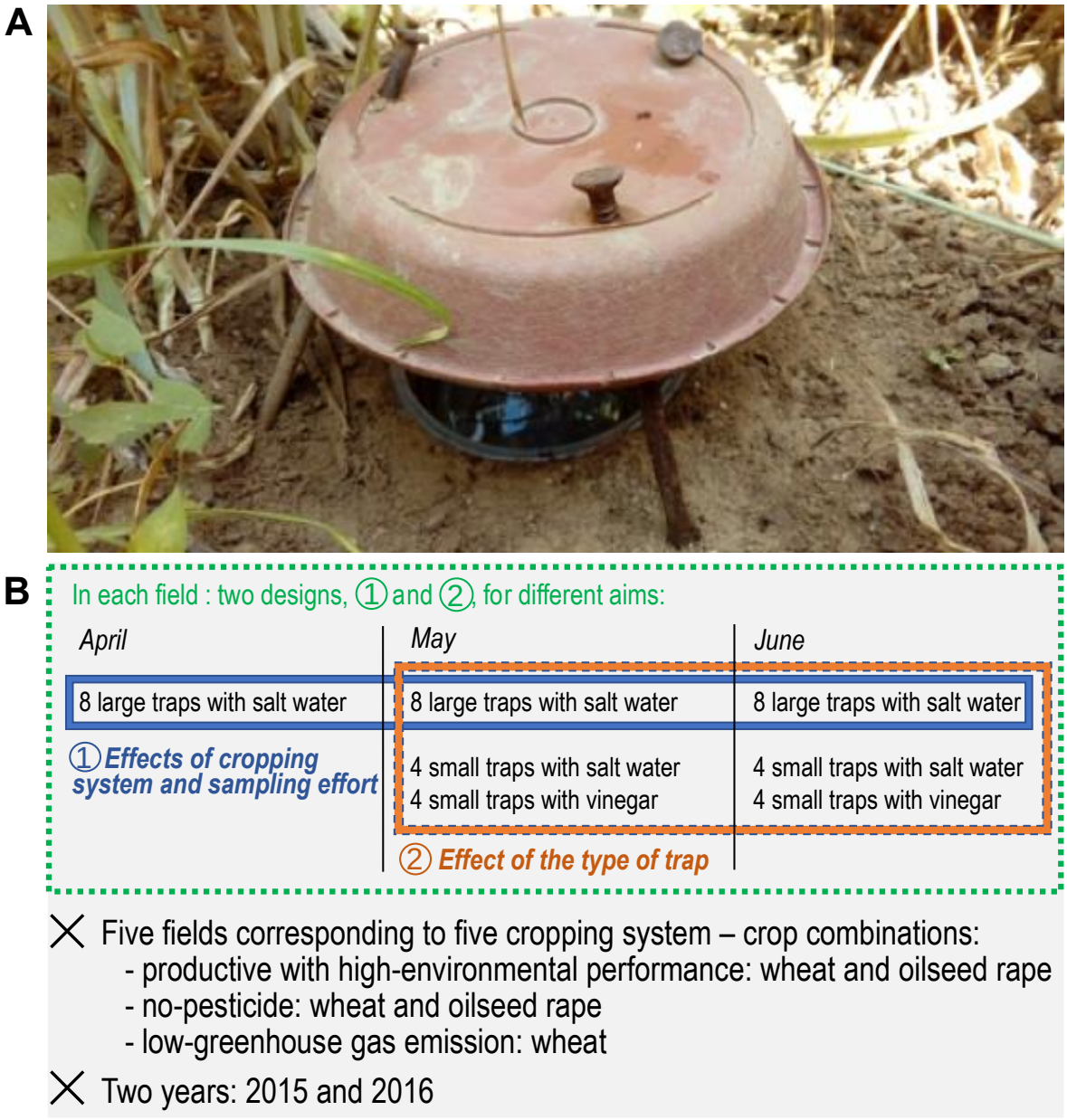


Figure 1. A: Pitfall trap in a cereal field; B: Schematic representation of the sampling design. Data were pooled over months prior to analysis.

In order to satisfy the different objectives, to keep a minimal distance of 15 m between each sampling station (location of each individual trap) so that they remain independent and to avoid depopulating the fields with a too high number of traps, we combined an incomplete factorial design with two sampling designs and different trap numbers at three periods in spring (Fig. 1

B). In each field (one field for each crop × cropping system combination), we placed three types of pitfall trap: (1) eight large traps (9.5 cm diameter, 7 cm high) filled with water, salt (50 g/l) and unscented detergent to break surface tension (20 ml/l); (2) four small traps (5.5 cm diameter, 7 cm high) filled with the water, salt and detergent solution; (3) four small traps (5.5 cm in diameter, 7.5 cm high) filled with alcohol vinegar. Pitfall traps were filled to two-thirds of their volume with the preserving fluid. In April, only the eight large traps with the salt solution were placed in the field, whereas all three types of trap were placed in the field in May and June (Fig. 1 B). We chose here to compare the effect of type of preserving fluid on the months where the arthropod activity-density was the highest.

The sampling stations were 12 m away from the edge of the field. In total, we placed each year 8 traps × 5 “cropping system-crop” combinations in April and 16 traps × 5 “cropping system-crop” combinations per sampling date in May and June, resulting in 80 traps per year (Fig. 1 B). Traps were left open for one week and the arthropods trapped were then preserved in ethanol (70%) for later identification.

### *2.3. Effect of trap size and type of preserving fluid on the trapping of carabid and spider communities*

Carabids were identified to species level, except for *Amara spp*., which were determined to genus level because of identification uncertainties (Roger et al. 2012), and adult spiders, which were identified to genus level (Roberts 1993; Roberts 2014). The amount of individuals in each trap reflects both their density and their activity, and was therefore called “activity-density”. Since the amount of carabids per trap at each date was low and because we did not aim at analysing the temporal dynamics of arthropods, we pooled , for each trap, the data from the two sampling dates (May and June) in the same year.

Statistical analyses were performed with R software, version 3.1.3 (R Development Core Team, 2015). We first analyzed the effect of trap size and type of preserving fluid on the activity-density and species (carabid) or genus (spider) richness values obtained. Rarefied richness (number of species or genera) was determined at five individuals for both carabids and spiders with the *vegan* package (Oksanen 2013). This small number of individuals was a compromise between the need to achieve rarefaction by levelling down community size across all traps without excluding too many traps for which the number of individuals would be below this threshold. In the case of carabids, this led us to remove 38 data points (with less than five individuals) evenly distributed over the three types of traps (10 for large traps with salt water, 14 for small traps with salt water and 14 for small traps with vinegar).

CWM body sizes were calculated as the mean body length weighted by the relative activity-density of each species (carabids) or genus (spiders) at community level using the *FD* package. Body length values originated from the BETSI database (Pey et al. 2014).

In spiders, species level trait values could not be used since they were identified at genus level only. To evaluate how spider body size varies between and within generra, we used a nested analysis of variance coupled with a variance partitioning method. This was carried out by first by fitting a linear mixed model (*nlme* package, Pinheiro et al. 2012) for spider body size (log-transformed). Genus and species nested within genus were entered as sequential random effects. We then calculated the variance components associated with genus and species level ('varcomp' function with ape package, Paradis et al. 2004). The largest proportion of spider body size variation was found at between-genus level (74.4 %) while the remaining variance (25.6 %) occurred at within-genus level. This is consistent with phylogenetically studies showing that spider body size is a conserved trait (Entling et al. 2010; Gonçalves-Souza et al. 2014). Assuming that there is not a wide variation in body size within genus, we therefore

averaged body size values for all the species available in the database at genus level before calculating CWM of spider body size.

We investigated the effects of trap type (three types of trap) on the six response variables (activity-density, richness and CWM body size for carabids and spiders). We used generalized linear mixed models (*lme4* package) with a Poisson error distribution for activity-density and richness or linear mixed models (*lme4* package) for the rarefied richness, CWM body size and when a log- or square root-transformation was necessary to reduce the over-dispersion. Cropping system, crop type and their interaction were introduced as fixed-effect covariates. A random field×year effect was introduced to account for plot and between-year variability. A random station effect, nested within the field×year effect, was used to take into account the location of the pitfall trap within the field. The distribution of residuals was checked with the *DHARMa* package (Hartig 2019). The effect of the type of trap was assessed with an ANOVA. In case of a significant effect, post-hoc Tukey tests were performed for multiple comparisons (multcomp package, Hothorn et al. 2008).

Beyond the abovementioned variables, we deepened our analysis to check if the types of trap led to differences in the recorded community composition. Such differences were identified by a constrained analysis of principal coordinates (CAP), with Bray-Curtis distance used as a measurement of dissimilarity (*vegan* package, Oksanen 2013). Ordination was constrained by the type of trap. Rare species, accounting for fewer than five individuals over the whole dataset, were removed. A $\log_{10}(x+1)$-transformation was performed on activity-density data, to reduce the influence of very abundant species. A permutational analysis of variance (PERMANOVA; Anderson 2001) was then used to test the difference in assemblages of species or genera between trap types.

### *2.4. Effect of sampling effort on the ability to distinguish carabid and spider communities between simulated cropping systems*

#### *2.4.1. Overview of the approach*

We investigated the sampling effort required to distinguish carabid and spider communities between contrasting cropping systems by performing a simulation study in which we varied simultaneously (1) the numbers of pitfall traps and field-year replicates and (2) the difference of a community metrics between two compared cropping systems. We simulated a large range of metrics of arthropod communities taking into account the uncontrolled variability caused by field-year and station effects. For each level of difference between two metrics, we then computed the minimal sampling effort that was required to find a significant difference between them.

#### *2.4.2. Estimation of the cropping system, crop type, field-year and station effects*

Using the field data, we first developed statistical models relating the activity-density, richness and CWM body size of carabids and spiders to the kind of cropping system. As trap type affected several of these metrics, we retained only the data for large pitfall traps filled with salt solution for which we had the largest dataset (eight traps per field and per sampling date). For each trap, we pooled the data for the three sampling dates (April, May and June) in each year. Effects of cropping system, crop type and their interaction were evaluated using the same statistical approach as described in section 2.3 using generalized linear mixed models or linear mixed models, including a random field-year effect and a random station effect. The effects of crop, cropping system and their interaction were assessed with an ANOVA using type III sum of squares. In case of a significant effect, post-hoc Tukey tests were performed for multiple comparisons (*multcomp* package).

We then used the statistical models obtained to predict three community metrics: activity-density, richness and CWM body size, for two taxonomic groups (carabids and spiders), and two crops (cereals and oilseed rape), giving 12 response variables.

*2.4.3. Simulation of a range of trap numbers crossed with a range of cropping system effects*

We first investigated the effect of the number of traps, by generating a dataset for each response variable for a number of pitfall traps ranging from 2 to 40 per field-year, for one or three field-year replicates. This sampling effort encompasses and extends beyond the most common practices in ecological and agronomic studies (e.g. Engel et al. 2017; Eyre et al. 2016; Woodcock 2005). The station effect associated with each trap and the field-year effect were randomly drawn from normal distributions with a variance corresponding to the random effects estimated in the descriptive statistical analyses (section 2.4.2).

Comparisons of cropping system effects were carried out for pairs of systems. The PHEP system was always used as the reference, and the positive or negative effects on arthropod communities relative to this reference were determined for each of the innovative cropping system. We simulated a range of cropping systems via their effect on activity-density, richness and CWM body size values. We varied these metrics from almost -100% to +150% relative to the reference cropping system. The range of positive effects was consistent with our observations and with the literature (Djoudi et al. 2018; Henneron et al. 2015; Rusch et al. 2014), while negative effects were tested for exploration purposes.

*2.4.4. Simulation of a range of field-year replicates for three crop×cropping system combinations*

We then adapted this simulation plan for analysis of the effect of the number of field-year replicates. Since cropping system experiments generally provide two to four field replicates (e.g. Hossard et al. 2014; Meyer et al. 2019), we simulated here one, three and six field-year replicates. To focus on the effect of number of field-year replicates, only the three observed crop×cropping system combinations were studied here (L-GHG and No-Pest in cereal, L-GHG in oilseed rape), which were compared to the reference PHEP cropping system. In this case, the number of traps per field-year ranged from 2 to 500.

*2.4.5. Calculation of the minimum sampling effort to detect a difference between the two cropping systems*

In each tested situation (i.e. each combination of cropping system effect in a given crop, of sampling effort, for each response variable and each taxonomic group), we checked if the response variable (metrics of arthropod community) was significantly different ($P < 0.05$) between the simulated and the reference cropping system. Data for each situation were generated 1000 times to perform as many comparisons. We recorded the number of cases in which there was a significant difference between the two cropping systems with the metrics in question. Significant differences were assessed using generalized linear mixed models for the activity-density and richness variables, with a Poisson error distribution and a log-link function, and a linear mixed model for CWM body size. These models included random effects for trap location and field-year. This resulted in a total of 29,232,000 comparisons.

We then determined the minimum number of traps and field-year replicates required to obtain a significant difference between the community associated with the reference system and the community associated with the simulated cropping system in 95% of simulations.

## 3. Results

In total, 2885 individual carabids and 9525 individual spiders were collected over the five crop-cropping system combinations, two experimental years and three sampling dates per year.

### *3.1. Effect of trap size and type of preserving fluid on the trapped carabid and spider communities*

Activity-density was much lower in small traps (5.5 cm diameter) than in large traps (9.5 cm diameter, Fig. 2, Table S1). Average activity-densities were halved for carabids (mean of 15.6 vs. 8.1 individuals in large vs. small traps, respectively) and divided by three for spiders (86.3 vs. 28.5 individuals, respectively, on average). In addition, activity-density for spiders was slightly lower in traps filled with vinegar than in traps filled with salt water.

For salt-water traps, carabid and spider richness values were higher for large than for small traps (4.1 vs. 2.8 carabid species, 9.3 vs. 5.9 spider genera for large and small traps, respectively). Within small traps, richness was not affected by type of preserving fluid. The differences were smaller, but remained significant, when richness was rarefied to account for the differences in the individuals caught between different types of traps (Fig. 2, Table S1).

CWM body size was lower in small salt-water traps than in large salt-water traps, for both carabids and spiders. CWM body size in small vinegar-filled traps was not significantly different from that for the other two types of trap (Fig. 2, Table S1).

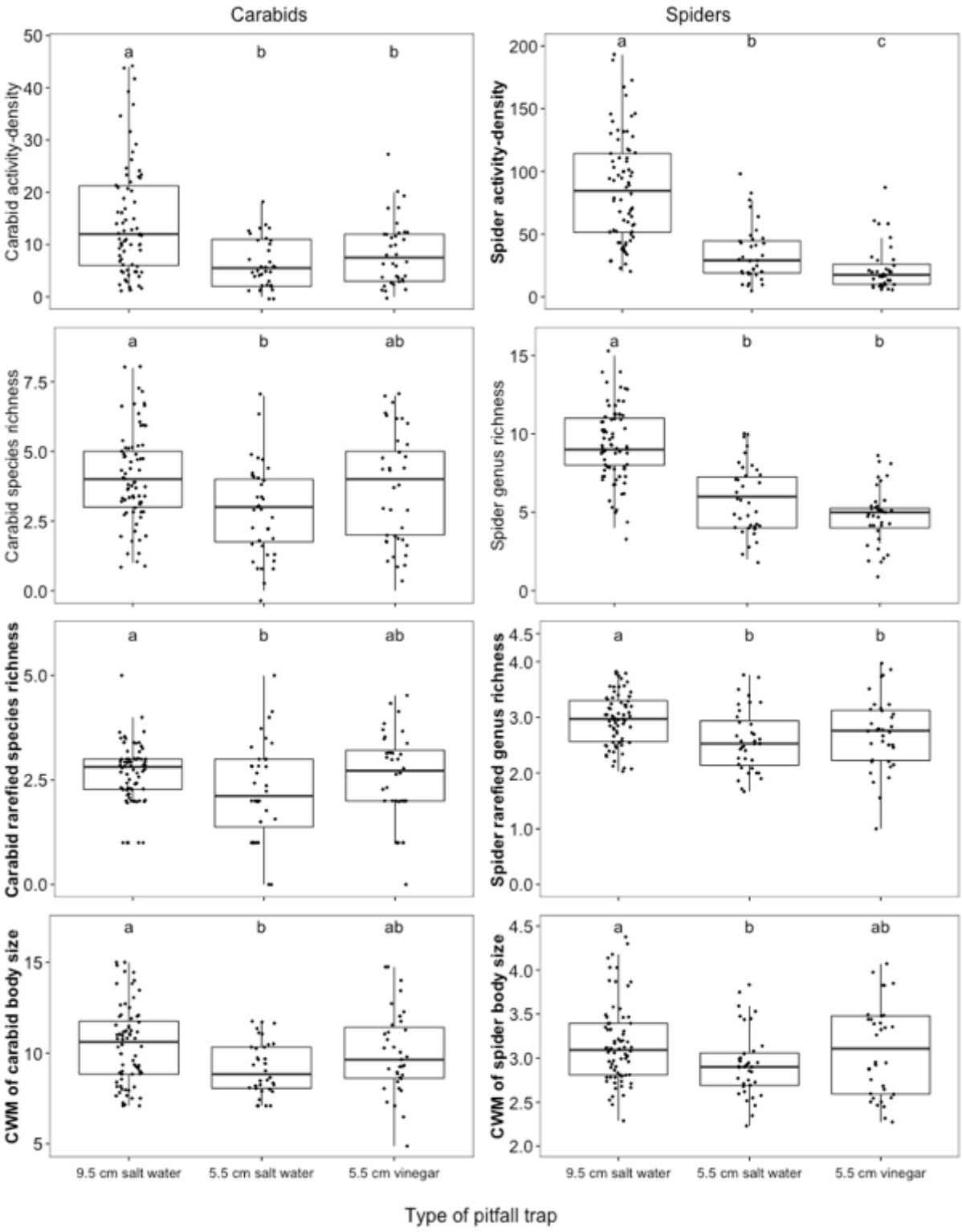


Figure 2. Effect of the three types of pitfall trap on the activity-density, richness, rarefied richness and CWM body size values obtained for carabids and spiders. The letters above the boxplots indicate significant differences between trap types in post-hoc Tukey tests ($P < 0.05$).

The biplot derived from the constrained analysis of principal coordinates explained 12.2% of the total variance (Fig. 3). The PERMANOVA results indicated a significant effect of the type of trap on the results obtained for carabid and spider communities ($P < 10^{-4}$). Constrained axis 1 distinguished between small and large traps. Large traps were associated with a higher activity-density for all taxa, especially for several spider genera, such as *Oedothorax*, *Tenuiphantes*, *Erigone* and *Diplocephalus* (Fig. 3). Variation along constrained axis 2 reflected differences between the types of preserving fluid. Traps filled with vinegar were characterized by a higher activity-density for several carabid species (*Pterostichus melanarius*, *Notiophilus biguttatus*, *Pseudoophonus rufipes*, *Poecilus cupreus*) and for spiders of genus *Tenuiphantes*, and a lower activity-density for *Anchomenus dorsalis* and *Oedothorax*.

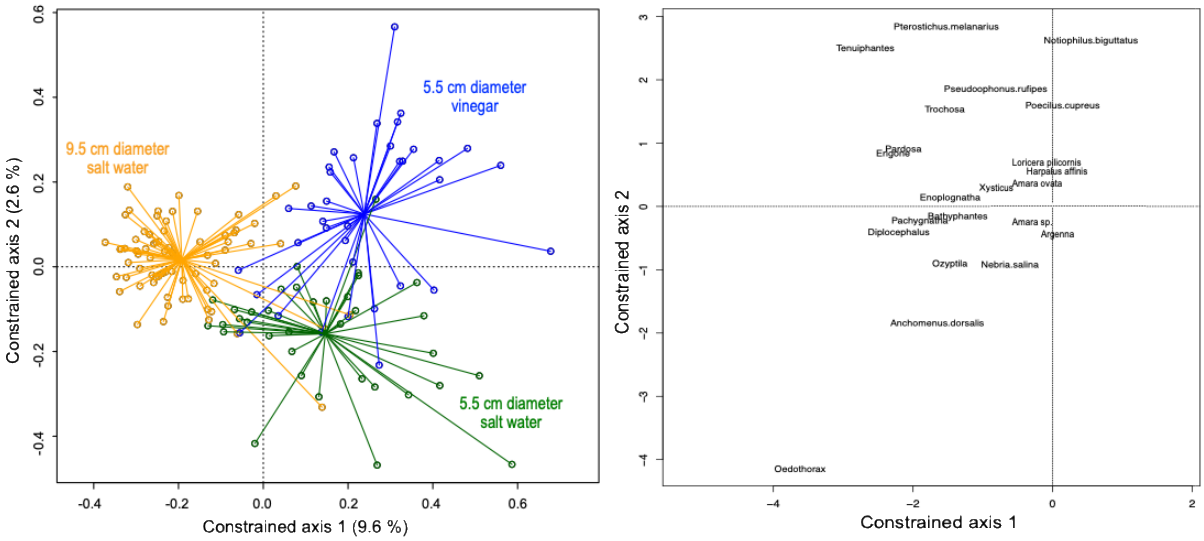


Figure 3. Biplot representation of the constrained principal coordinate analysis based on Bray-Curtis dissimilarities for the activity-densities of carabid species and spider genera in the three types of pitfall trap. Traps (individuals, left) were described by the activity-density of the species (variables, right) they contained. The species and genus which are not well discriminated by the analysis are not shown for readability.

*3.2. Differences in the results obtained for carabid and spiders communities between cropping systems*

The carabid and spider communities caught in the traps were generally affected by the nature of the crop or by a crop×cropping system interaction (Table 1). Activity-densities did not differ significantly between the different cropping systems for a given crop, with the exception of a tendency towards higher numbers of carabids and spiders being caught in the L-GHG system in oilseed rape crops (Fig. S1). Carabid species richness was higher in oilseed rape than in cereal crops, but was not significantly different between cropping systems for any given crop. For spiders, genus richness was highest in the L-GHG system. The CWM body size of carabids was greater in oilseed rape than in cereal crops, with no effect of cropping system. For spiders, CWM body size was greater in the L-GHG and No-Pest cropping systems than in the PHEP system, for both crops studied. Among all the variability due to crop and cropping system, the statistical models summarized in Table 1 provided estimates of the random effects of field-year and sampling station.

Averaged over the six statistical models (Table 1), marginal coefficients of determination, accounting for fixed crop and cropping system effects, equalled 43.5%. The conditional coefficients, accounting also for field-year and station random effects, equalled 68.2%. This goodness of fit was appraised to be acceptable in order to use the models for simulating a wider sampling effort with different values of activity-density, richness and CWM and to represent the uncontrolled field-year and station effects, so as to generate a variability as can be observed in the fields.

Table 1. Effects of the crop and cropping system on carabid and spider activity-densities, richness and CWM body size. These results were obtained with data from for large pitfall traps filled with salt water only, summed over the three sampling dates ($n$ = 72 observations for each model).

| Response variable | Explanatory variable | P-value | Factor levels | Fixed effects: estimate ± SE<br>Random effects: SD |
|---|---|---|---|---|
| **Carabid activity-density** | Intercept (ref = wheat PHEP) | | | 2.81 ± 0.27 |
| (glmer, Poisson log-link function) | Cropping system | 0.057 | No-Pest | -0.19 ± 0.38 |
| | | | L-GHG | -0.090 ± 0.39 |
| | Crop | 0.58 | Oilseed rape | -1.16 ± 0.31 |
| | Crop × cropping system | **$4.98 \cdot 10^{-3}$** | L-GHG×oilseed rape | -1.73 ± 0.62 |
| | Field-year | | | 0.36 |
| | 1\|Station:Field-year | | | 0.32 |
| Marginal coefficient of determination = 0.47 | | | | |
| Conditional coefficient of determination = 0.90 | | | | |
| **Carabid species richness** | Intercept (ref = wheat PHEP) | | | 2.16 ± 0.11 |
| (lmer, square-root transformation) | Cropping system | | No-Pest | 0.05 ± 0.13 |
| | | **$1.66 \cdot 10^{-3}$** | L-GHG | -0.40 ± 0.13 |
| | Crop | 0.29 | Oilseed rape | 0.17 ± 0.17 |
| | Crop × cropping system | **$2.97 \cdot 10^{-3}$** | L-GHG×oilseed rape | 0.64 ± 0.22 |
| | Field-year | | | 0.142 |
| | 1\|Station:Field-year | | | 0.090 |
| Marginal coefficient of determination = 0.40 | | | | |
| Conditional coefficient of determination = 0.63 | | | | |
| **Carabid CWM of body size** | Intercept (ref = wheat PHEP) | | | 10.73 ± 0.88 |
| (lmer) | Cropping system | 0.48 | No-Pest | -0.24 ± 1.23 |
| | | | L-GHG | 0.82 ± 1.23 |
| | Crop | **$1.00 \cdot 10^{-2}$** | Oilseed rape | -2.75 ± 1.52 |
| | Crop × cropping system | 0.81 | L-GHG×oilseed rape | 0.47 ± 1.96 |
| | Field-year | | | 0.63 |
| | 1\|Station:Field-year | | | 0.33 |
| Marginal coefficient of determination = 0.38 | | | | |
| Conditional coefficient of determination = 0.67 | | | | |
| **Spider activity-density** | Intercept (ref = wheat PHEP) | | | 4.86 |
| (glmer, Poisson log-link function) | Cropping system | 0.92 | No-Pest | -0.09 ± 0.25 |
| | | | L-GHG | -0.09 ± 0.25 |
| | Crop | $5.10 \cdot 10^{-2}$ | Oilseed rape | -1.16 ± 0.31 |
| | Crop × cropping system | **$1.52 \cdot 10^{-3}$** | L-GHG×oilseed rape | 0.78 ± 0.40 |
| | Field-year | | | 0.23 |
| | 1\|Station:Field-year | | | 0.27 |
| Marginal coefficient of determination = 0.28 | | | | |
| Conditional coefficient of determination = 0.57 | | | | |
| **Spider genus richness** | Intercept (ref = wheat PHEP) | | | 2.35 ± 0.06 |
| (lmer, log transformation) | Cropping system | **$4.59 \cdot 10^{-2}$** | No-Pest | -0.11 ± 0.08 |
| | | | L-GHG | 0.09 ± 0.06 |
| | Crop | **$< 10^{-4}$** | Oilseed rape | -0.52 ± 0.10 |
| | Crop × cropping system | **$2.65 \cdot 10^{-4}$** | L-GHG×oilseed rape | 0.47 ±0.13 |
| | Field-year | | | 0.05 |
| | 1\|Station:Field-year | | | 0.02 |
| Marginal coefficient of determination = 0.47 | | | | |
| Conditional coefficient of determination = 0.61 | | | | |
| **Spider CWM of body size** | Intercept (ref = wheat PHEP) | | | 1.15 ± 0.04 |
| (lmer, log transformation) | Cropping system | **$6.19 \cdot 10^{-4}$** | No-Pest | 0.19 ± 0.06 |
| | | | L-GHG | 0.25 ± 0.02 |
| | Crop | **$1.03 \cdot 10^{-3}$** | 3Oilseed rape | -0.04 ± 0.07 |
| | Crop × cropping system | $5.26 \cdot 10^{-2}$ | L-GHG×oilseed rape | -0.19 ± 0.09 |
| | Field-year | | | 0.07 |
| | 1\|Station:Field-year | | | 0.05 |
| Marginal coefficient of determination = 0.61 | | | | |
| Conditional coefficient of determination = 0.71 | | | | |

*3.3 Effect of sampling effort on the ability of activity-density, richness and CWM body size estimates to distinguish between contrasting simulated communities*

The relationships between the arthropod metrics, when the differences were expressed in proportions, and the minimum sampling effort required to detect these differences were approximately the same for all three metrics (Fig. 4). This minimum sampling effort decreased when the difference, either positive or negative, between the two metrics increased.

A variation of carabid or spider activity-density of less than 30% (i.e. 5 carabid or about 20 spider individuals) between a simulated and the reference PHEP system could not be demonstrated by a significant difference if there were fewer than 40 traps per field (Figs. 4, S2). With three field-year replicates, five to ten traps were required to detect a variation of 50% in activity-density, corresponding to a difference of about 10 carabids or 30 to 50 individual spiders.

A minimum of three to five traps per field for carabids and five to 15 traps per field for spiders was required to differentiate between two cropping systems differing by five species or genera. The sampling effort increased to more than 20 traps per field for the detection of a difference in richness below two species or two genera.

For CWM body size, the sampling effort required decreased sharply, from 30 to less than five traps, for a difference between two simulated cropping systems of 1 to 2 mm in carabids and 0.3 to 0.7 mm in spiders (Fig. 3).

For all variables, the minimum number of traps per field required to differentiate between cropping systems decreased by a factor of three when the number of field-years increased from one to three.

We then represented the observed effects of the studied cropping systems on these sampling effort curves (see vertical dashed lines on Figs. 4, S2). In general, the observed effects of the

No-Pest cropping system relative to the PHEP system were so small that more than 30 or 40 traps per field, with three field-year replicates, would have been required to detect a significant difference for the three metrics (Fig. 3). In the L-GHG system, particularly for oilseed rape, the differences in activity-densities and in CWM body size were slightly larger and easier to demonstrate.

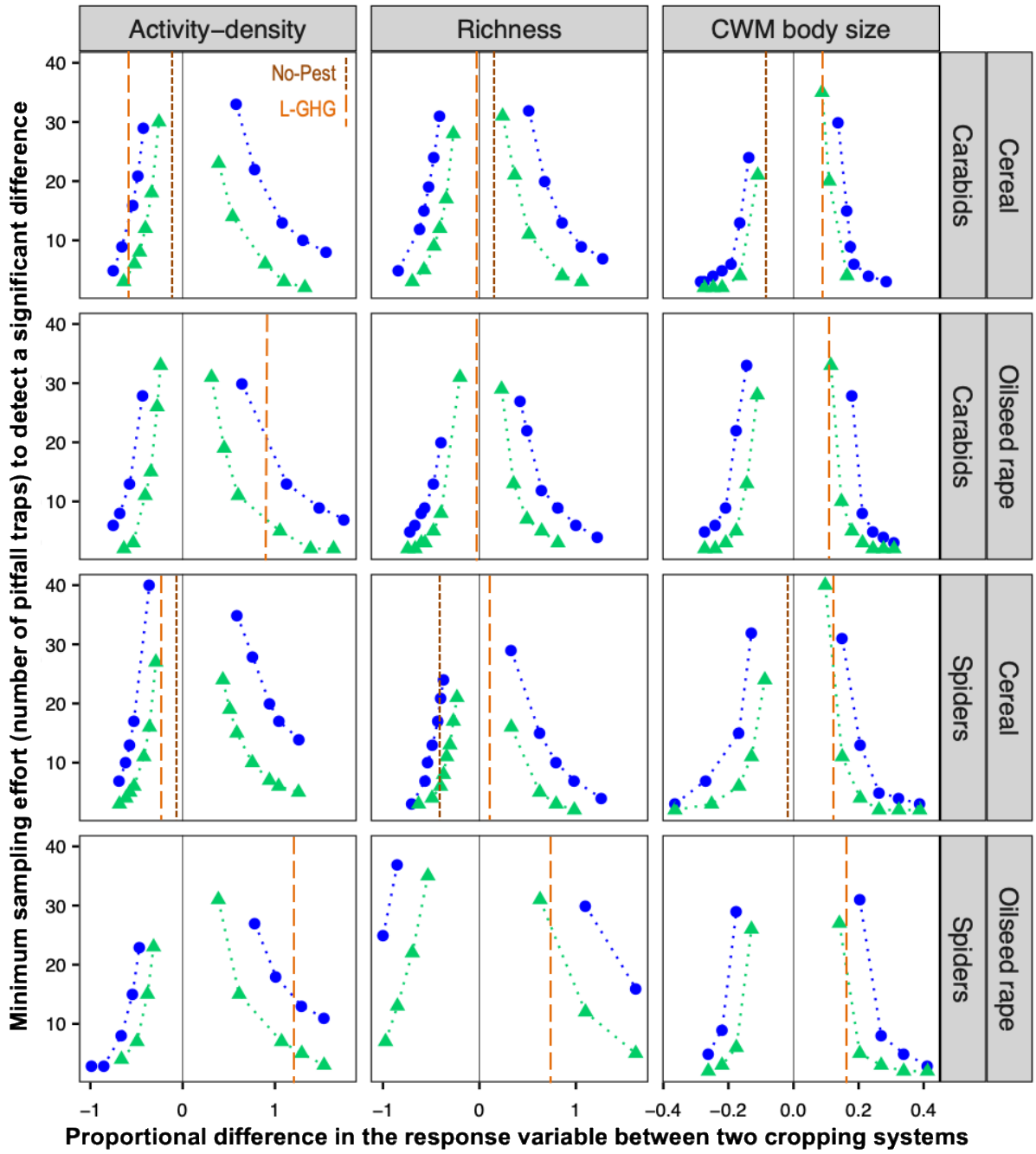


Figure 4. Minimum sampling effort (number of traps) required to differentiate two cropping systems as a function of the simulated proportional difference in the response variables between the two systems, the productive one being taken as a reference (vertical full black line). The vertical dashed lines indicate the observed differences in the cropping system experiment (section 3.2). The response variables were carabid and spider activity-density, richness (number of carabid species or spider genera) and CWM body size (mm). Simulations were carried out

with one (blue circles) or three (green triangles) field-year replicates. The vertical dashed lines indicate the observed differences in the response variable in the cropping system experiment (section 3.2).

### *3.4. Effect of the number of field-year replicates on the ability to distinguish between contrasting simulated communities*

For the observed effects of cropping system, we analyzed the impact of the number of field-year replicates on the minimum number of traps per field required to detect a significant difference between an innovative cropping system and the PHEP cropping system (Table 2). In general, the minimum sampling effort decreased by a factor of three when the number of field-year replicates doubled (from three to six). The decrease in sampling effort was largest for CWM body size (e.g. a decrease from 33 to 6 traps to distinguish between the L-GHG and reference systems in cereals) and for all variables in oilseed rape crops. In cereals, doubling the number of field-year replicates only slightly decreased the minimum sampling effort required to detect differences in carabid richness, spider activity-density and CWM spider body size. For these variables, a very large number of traps (more than 200) would be required to distinguish between the communities present in two cropping systems.

Table 2. Minimum number of pitfall traps per field required to distinguish between an innovative cropping system (L-GHG or No-Pest) and the reference PHEP system in the same crop (a cereal or oilseed rape), for three or six field-year replicates. See the materials and methods for the cropping system abbreviations.

| | | | Cropping system | | |
|---|---|---|---|---|---|
| | | | Cereal | | Oilseed rape |
| **Replicates** | **Organism** | **Variable** | **L-GHG** | **No-Pest** | **L-GHG** |
| **With three field-year replicates** | | | | | |
| | Carabids | Activity-density | 2 | 53 | 6 |
| | | Richness | 291 | 74 | 435 |
| | | CWM body size | 33 | 82 | 36 |
| | Spiders | Activity-density | 510 | > 700 | 14 |
| | | Richness | 520 | 23 | 8 |
| | | CWM body size | 252 | 648 | 17 |
| **With six field-year replicates** | | | | | |
| | Carabids | Activity-density | 1 | 30 | 2 |
| | | Richness | 257 | 36 | 172 |
| | | CWM body size | 6 | 17 | 7 |
| | Spiders | Activity-density | 354 | > 700 | 2 |
| | | Richness | 245 | 12 | 3 |
| | | CWM body size | 195 | 550 | 5 |

## 4. Discussion

We evaluated the extent to which pitfall traps, which are widely used in natural and semi-natural ecosystems, were appropriate for studies characterizing the effects of arable cropping systems on the composition and structure of spider and carabid communities. We first compared the effects of pitfall trap size and type of preserving fluid on carabid and spider communities. These features are the main sources of variation in pitfall trap design, while other characteristics were fixed here (trap and rain guard colour, no funnel), to which our results are subject. We used activity-density and richness as community descriptors, together with functional composition, assessed as the CWM body size, an aspect never before addressed in such methodological studies.

As expected, activity-density increased with trap size. Increasing the trap diameter by a factor of 1.7 (from 5.5 to 9.5 cm) resulted in a similar increase in the number of carabids caught, but a tripling of the numbers of spiders caught, consistent with previous observations (Lange et al. 2011; Work et al. 2002). The effect of trap size has already been shown to differ between the species caught, as a function of their mobility (often related to their size), but we expected these differences in community composition to be smaller due to the simplified structural architecture in cropped fields, in which ground dwelling arthropods may be very mobile (not much litter on the soil surface and bare soil between rows). However, the community composition results were affected by trap size. The constrained analysis of principal coordinates indicated that genera from the family Linyphiidae were overrepresented in the large traps. This increase in the number of catches, which was not proportional to trap diameter, can be explained by large differences in behaviour between carabids and wandering spiders on the one hand, and Linyphiidae spiders on the other, when they encounter the rim of the trap. Linyphiidae spiders

can enter and explore the trap without necessarily falling into the fluid (Topping 1993).

Large traps also captured larger numbers of carabid species and spider genera, even after the differences in trapped individuals were taken into account by a rarefaction procedure. This may be because the large traps are better able to catch species with behaviours enabling them to escape from traps. This has already been reported for Linyphiidae spiders (Topping 1993), but this result was unexpected for carabid richness, which was unaffected by trap size in previous studies (e.g. Lange et al. 2011). In terms of body mass, small arthropod species tend to be underrepresented in pitfall traps because of their lower levels of activity, and the response to sampling effort is greatest for these species (Engel et al. 2017). The use of larger traps should increase the sampling effort and the ability to catch small species, which would result in a higher richness within the sampled communities. However, this hypothesis is not supported by the comparison of CWM body size between small and large traps. Indeed, CWM body size increased with trap size, for both carabids and spiders, perhaps because large species were better able to escape from small traps.

For a fixed trap size, the type of preserving fluid had no effect on the variables considered, except for spider activity-density, for which vinegar-filled traps had a slightly lower trapping efficiency, for which we currently have no clear explanation. The constrained analysis of principal coordinates suggested differences in community composition between types of preserving fluids, but this factor explained a negligible proportion of variance and had no significant effect on community taxonomic richness or functional composition.

These methodological results illustrate how the picture of community composition and structure given by pitfall traps can be affected by the activity and behaviour of the arthropods studied. These limitations have already been reported for activity-density and richness, but we provide the first demonstration here that functional characterization (here CWM body size, one

of the most frequently used functional markers) is also affected. The use of functional indicators, such as the CWM of traits, to predict the role of arthropods in ecosystem functioning, in terms of predation for example, may therefore be risky. The correction of these sampling biases by body mass, as recommended by Engel et al. (2017), is required to obtain a reliable view of community taxonomic and functional composition and structure.

In wheat crops, cropping system had little effect on the carabid and spider communities captured, except that spider CWM body size was markedly lower in the reference PHEP system. In oilseed rape, greater differences between cropping systems were observed for the communities, with higher activity-densities, richness and CWM body size in the L-GHG system (50% decrease in greenhouse gas emissions, no tillage). The observed differences in activity-densities and richness were not specific to our case study and are similar to those already reported in other long-term cropping system experiments comparing conventional, organic and "conservation agriculture" cropping systems (Djoudi et al. 2018; Henneron et al. 2015; Rusch et al. 2014). However, several of these differences were not significant due to high levels of variability across field-year replicates and trap types. Our sampling protocol was not designed to compare cropping systems, but to capture the multiple sources of variability between traps. These random effects indeed accounted for a significant part of the explained variance in the statistical models. Moreover, the reference cropping system PHEP was already a more environmentally friendly cropping system than typical conventional cropping systems.

We then developed a simulation procedure to investigate the effect of the numbers of traps and field-year replicates on the ability to detect cropping system effects on carabid and spider communities, based on three metrics: activity-density, richness and CWM body size. The dataset generated took into account the important variability caused by the trap location and field-year effects. This simulation approach also made it possible to analyse the effect of

sampling effort when comparing more marked cropping system effects and to compare pairs of arthropod communities with metrics theoretically differing by -100% to +150%.

Only very different communities could be significantly distinguished with a reasonable number of traps and field-year replicates. Indeed, a sampling design with ten pitfall traps per field and three field-year replicates was able to detect a 100% increase in carabid or spider activity-density between two cropping systems, but not smaller increases. Five traps were sufficient to distinguish between communities differing by 20 or 30% in CWM body size (for three and one field-year replicates, respectively), but the required sampling effort increased sharply for differences below this threshold.

For the three cropping systems studied, a very high number of traps per field (often more than 30, larger that in our design) would have been required to find significant differences in the metrics studied between the reference system and the two innovative cropping systems (L-GHG or No-Pest, i.e. no-pesticide), due to the large spatial and temporal within-field heterogeneity of the arthropods sampled in pitfall traps. This variability was often greater than the differences generated by the cropping systems, even though the data used originated from an analytical experiment performed at a single site with homogeneous soil and landscape characteristics.

The relationships between the difference in arthropod metrics between cropping systems and the minimum sampling effort required to detect this difference were similar for activity-density and richness, contrary to the findings of Perner (2003), who reported that the required sample size for estimating richness was smaller than that for abundance parameters. A smaller number of traps was required to find differences in CWM body size between cropping systems. This finding highlights the suitability of this metric for analysing the response of arthropod communities to different management techniques. The relationships were also similar between the two taxa, as reported by Perner (2003). This finding suggests that these metrics and taxa are, *a priori,* equally suitable for use in studies aiming to detect cropping system effects.

However, they displayed very different patterns of response to the three cropping systems studied here. In this case, carabid activity-density, carabid CWM body size and spider genus richness were the variables distinguishing between cropping systems with the smallest sampling effort, whereas a much greater sampling effort was required for carabid richness. The very low carabid species richness values obtained, resulting from repeated disturbances selecting only the few adapted species, were similar in all cropping systems, potentially accounting for the inability of this metric to distinguish between cropping systems with a reasonable sampling effort. Furthermore, it was easier to demonstrate the effects of cropping system in oilseed rape crops than in cereals, in which this effects were more variable.

The sampling effort could be reduced by increasing the number of field-year replicates, but not equally for all metrics and taxa. The decrease was the strongest for CWM body size and for all metrics in oilseed rape crops. In cereals, in which the simulated cropping systems had a smaller effect on spider activity-density and body size than oilseed rape, a doubling of the number of field-year replicates was insufficient to halve the sampling effort per field, thus resulting in an overall increase in total sampling effort. In this case, the considerable variability between field-years could not compensate for the within-field variability. In light of these results, the characterization of these patterns of variance for the different metrics in preliminary experiments appears to be crucial, to optimize the sampling design.

In conclusion, the effects of three innovative contrasting cropping systems on arthropod communities were difficult to differentiate from other sources of variability within and between fields and years, although they were of a similar amplitude to those already reported for organic or conservation agriculture. Large trap size increased the sampling effort and increased the trapping of small species, whereas the type of preserving fluid (salt water vs. vinegar) had very little impact on the communities sampled. As expected, a high sampling effort was generally required for the comparison of activity-density and richness values across fields managed with

different cropping systems. Also consistent with our initial expectations, a smaller sampling effort was generally required for the comparison of functional composition (CWM body size in this study) between two communities, than for comparisons of taxonomic richness, because this metric is less sensitive to rare taxa or to the number of individuals trapped. Finally, we found that the minimum sampling effort was much smaller for comparisons of the effects of cropping system in oilseed rape crops than in cereals. Selection of the most appropriate trap design, metrics and crops for study therefore appeared to be the main levers for optimizing the trade-off between sampling effort and the ability to detect arthropod community responses to habitat management.

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**Authors’ contributions**

AG and MVM conceived the ideas and designed the methodology; AG collected and analyzed the data and led the writing of the manuscript. All authors provided critical input concerning the draft versions of the manuscript and approved the final version for publication.

**Conflict of interest**

The authors of this preprint declare that they have no financial conflict of interest with the content of this article.

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## Supplementary material

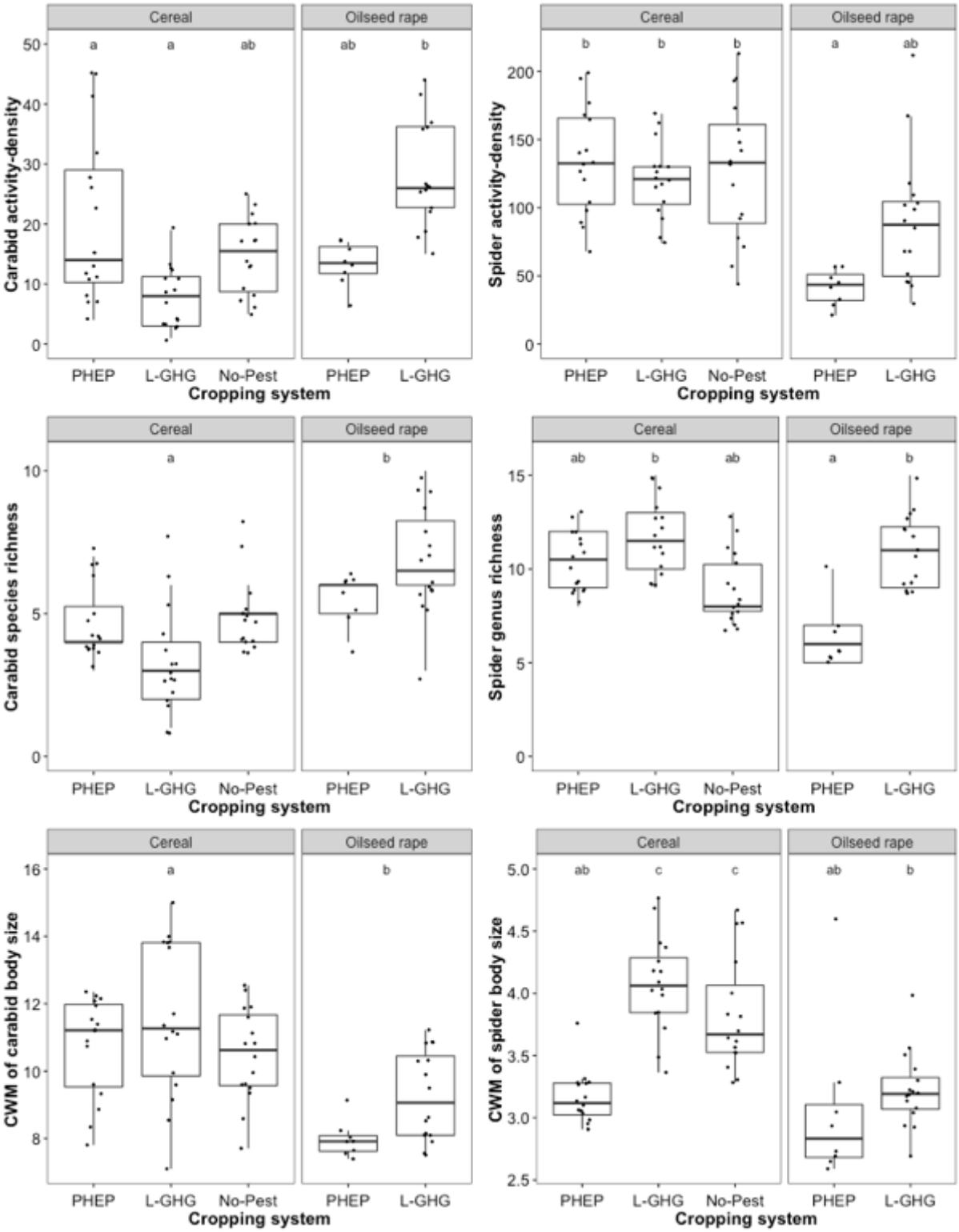


Figure S1: Effects of crop and cropping system on carabid and spider activity-densities, richness and CWM body size. The data shown here are for large pitfall traps filled with salt

water only, summed over three sampling dates ($n$ = 72 observations for each model). The letters above the boxplots indicate significant differences between trap types in post-hoc Tukey tests ($P$ = 0.05). See the materials and methods for the cropping system abbreviations.

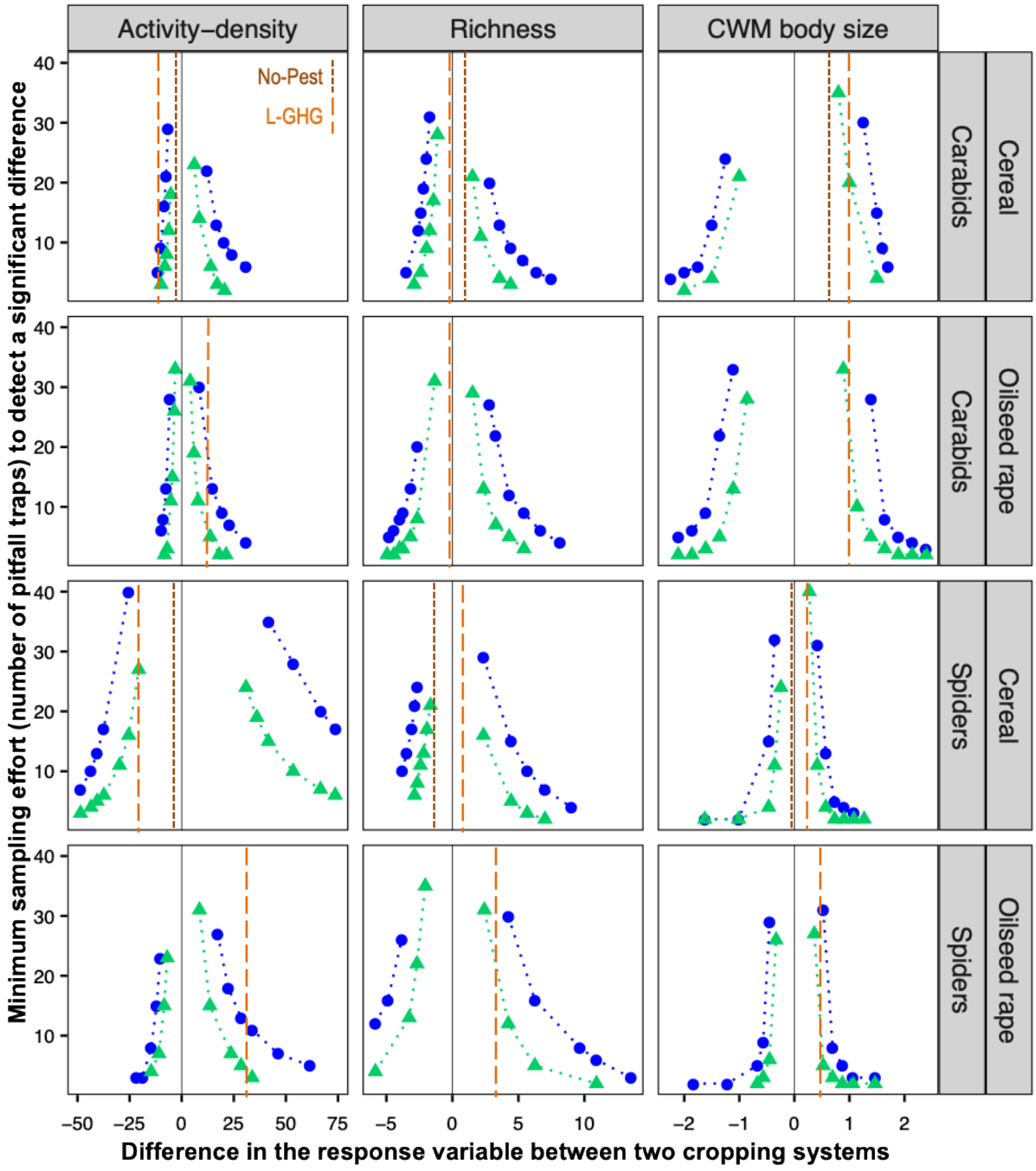


Figure S2. Minimum sampling effort (number of traps) required to detect an absolute difference in the response variable between a simulated cropping system and the productive cropping system taken as a reference (vertical full line). The response variables were carabid and spider activity-density, richness (number of carabid species or spider genera) and CWM body size (mm). Simulations were carried out with one (blue circles) or three (green triangles) field-year

replicates. The vertical dashed lines indicate the observed differences in the response variable in the cropping system experiment.

Table S1. Effect of the types of traps of carabid and spider activity-density, taxonomic richness, rarefied richness and CWM of body size. Data are from pitfall traps placed in May and June and before analysis. The "crop" and "cropping system" variables were introduced in all models as co-variables but their effects are not detailed here. Field-year and trap location effects were also accounted for as random effects. The estimates are given for the cereal crop in the productive cropping system (PHEP) taken as a reference here.

| Taxa | Response variable | Explanatory variable | P-value | Factor levels | Estimate ± SE |
|---|---|---|---|---|---|
| **Carabids** | **Activity-density** | Intercept (ref = 9.5 cm salt water) | | | 2.72 ± 0.43 |
| | (lmer, log+1-transformed variable) | Type of trap | **< $10^{-4}$** | 5.5 cm salt water | -0.72 ± 0.12 |
| | | | | 5.5 cm salt vinegar | -0.54 ± 0.12 |
| | | Cropping system | 0.28 | *not detailled* | |
| | | Crop | 0.71 | *not detailled* | |
| | | Crop × cropping system | 0.10 | *not detailled* | |
| | **Richness** | Intercept (ref = 9.5 cm salt water) | | | 1.37 ± 0.11 |
| | (glmer, Poisson log-link function) | Type of trap | **$5.38 \cdot 10^{-3}$** | 5.5 cm salt water | -0.37 ± 0.12 |
| | | | | 5.5 cm salt vinegar | -0.21 ± 0.11 |
| | | Cropping system | **$2.11 \cdot 10^{-3}$** | *not detailled* | |
| | | Crop | 0.80 | *not detailled* | |
| | | Crop × cropping system | **$3.50 \cdot 10^{-4}$** | *not detailled* | |
| | **Rarefied richness** | Intercept (ref = 9.5 cm salt water) | | | 2.65 ± 0.31 |
| | (lmer) | Type of trap | **$3.87 \cdot 10^{-2}$** | 5.5 cm salt water | -0.43 ± 0.17 |
| | | | | 5.5 cm salt vinegar | -0.09 ± 0.07 |
| | | Cropping system | 0.22 | *not detailled* | |
| | | Crop | 0.83 | *not detailled* | |
| | | Crop × cropping system | 0.15 | *not detailled* | |
| | **CWM of body size** | Intercept (ref = 9.5 cm salt water) | | | 2.37 ± 0.08 |
| | (lmer, log-transformed variable) | Type of trap | **$2.72 \cdot 10^{-3}$** | 5.5 cm salt water | -0.12 + 0.04 |
| | | | | 5.5 cm salt vinegar | -0.05 ± 0.03 |
| | | Cropping system | 0.92 | *not detailled* | |
| | | Crop | $6.18 \cdot 10^{-2}$ | *not detailled* | |
| | | Crop × cropping system | 0.56 | *not detailled* | |
| **Spiders** | **Activity-density** | Intercept (ref = 9.5 cm salt water) | | | 4.42 ± 0.09 |
| | (glmer, Poisson log-link function) | Type of trap | **< $10^{-4}$** | 5.5 cm salt water | -0.98 ± 0.11 |
| | | | | 5.5 cm salt vinegar | -1.43 ± 0.11 |
| | | Cropping system | **$1.00 \cdot 10^{-2}$** | *not detailled* | |
| | | Crop | **< $10^{-4}$** | *not detailled* | |
| | | Crop × cropping system | **< $10^{-4}$** | *not detailled* | |
| | **Richness** | Intercept (ref = 9.5 cm salt water) | | | 2.31 ± 0.06 |
| | (glmer, Poisson log-link function) | Type of trap | **< $10^{-4}$** | 5.5 cm salt water | -0.45 ± 0.08 |
| | | | | 5.5 cm salt vinegar | -0.66 ± 0.09 |
| | | Cropping system | 0.15 | *not detailled* | |
| | | Crop | **< $10^{-4}$** | *not detailled* | |
| | | Crop × cropping system | **< $10^{-4}$** | *not detailled* | |
| | **Rarefied richness** | Intercept (ref = 9.5 cm salt water) | | | 2.95 ± 0.28 |
| | (lmer) | Type of trap | **< $10^{-4}$** | 5.5 cm salt water | -0.38 ± 0.09 |
| | | | | 5.5 cm salt vinegar | -0.25 ± 0.09 |
| | | Cropping system | 0.55 | *not detailled* | |
| | | Crop | 0.67 | *not detailled* | |
| | | Crop × cropping system | 0.64 | *not detailled* | |
| | **CWM of body size** | Intercept (ref = 9.5 cm salt water) | | | 1.06 ± 0.06 |
| | (lmer, log-transformed variable) | Type of trap | **$1.02 \cdot 10^{-2}$** | 5.5 cm salt water | -0.07 ± 0.03 |
| | | | | 5.5 cm salt vinegar | -0.006 ± 0.02 |
| | | Cropping system | 0.14 | *not detailled* | |
| | | Crop | 0.55 | *not detailled* | |
| | | Crop × cropping system | 0.95 | *not detailled* | |