**Earthworm abundance response to conservation agriculture practices in organic arable farming under Mediterranean climate**

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# Abstract:

Earthworms are one of the most important soil macrofaunal groups, and they play a major role in agricultural ecosystems*.* Agricultural practices, such as reduced tillage, the use of green manures and organic fertilization, can be beneficial for earthworm populations in agricultural systems. However, under a Mediterranean climate, not much is known regarding their response to agricultural management. The aim of this study was to analyse the effects of tillage type, organic fertilization, and green manures on the density and biomass of earthworms in organic arable dryland. The trial was conducted in a four-year crop rotation with a complete factorial design that combined tillage system (mouldboard ploughing vs. chisel), fertilization (composted farmyard manure vs. no fertilizer) and green manures (green manures vs. no green manures). Earthworms were assessed in each plot by the extraction of all individuals in soil areas of 33 cm × 33 cm that were excavated to a depth of 25 cm. Only five earthworm species were found in this trial, and the earthworm community was dominated by such endogeic ecotypes as *Aporrectodea rosea* and *Allolobophora georgii*, and the anecic ecotype *Aporrectodea trapezoides.* Endogeic species can benefit from soil inversion because of the incorporation of organic matter, but the anecic ones can be negatively affected by it. The results show that plots with farmyard manure had higher density and biomass of earthworms. We observed that the type of tillage significantly affected earthworm populations: plots that had been ploughed with mouldboard ploughing (soil inversion) the year prior to sampling presented more juveniles. The biomass of earthworms was significantly lower in plots with green manures and chiselling. Our results indicated that the combination of chiselling and green manures was not optimal for earthworm populations, but organic fertilization played a considerably more important role and enhanced their abundances.

**Keywords**: organic farming; Mediterranean soils; green manures; earthworms; chisel

# Introduction

Earthworms play a major role in ecosystem functioning because their burrowing and feeding activities modify the soil structure and several soil properties. In particular, earthworms increase soil macroporosity, relocate nutrients along the soil profile and form stable aggregates (Crittenden et al., 2014; Ernst and Emmerling, 2009; Metzke et al., 2007). The significant role of earthworms has been revealed by experiments in which they were eliminated in grass swards causing soil bulk density to increase, while organic matter, soil moisture and infiltration rate greatly decreased (Riley et al., 2008). Conversely, earthworm populations are influenced by soil moisture, organic matter, texture, pH and soil management (Crittenden et al., 2014). Soil tillage can modify the relative abundance of earthworm species and their community structure (Chan, 2001). Some studies concerning the impact of inversion tillage on the abundance of earthworm populations have found that the largest and most fragile earthworms (those with soft epitheliums) are most affected by intensive tillage, and species inhabiting the topsoil are at risk of being negatively affected by ploughing (Pelosi et al., 2014). The variability in burrowing and feeding behaviours can be important in determining the effects that tillage type can have on earthworms (Capowiez et al., 2009). According to Bouché (1972), earthworms can be divided into the following ecological groups based on soil habitats and feeding habits: (1) Epigeic species live and feed in the organic layers above the mineral soil surface. (2) Anecic species live in vertical burrows in mineral soil layers, but come to the surface to feed on leaf litter that they drag into their burrows (0–200 cm depth). (3) Endogeic species live in mineral soil layers and feed on soil organic matter. They make horizontal burrows through the soil that they sometimes reuse to feed and move around. Capowiez et al. (2009) and Ernst and Emmerling (2009) showed that soil layer inversion by mouldboard ploughing negatively affected the density of anecic earthworm species, while the density of endogeic species was enhanced.

The influences of other farming practices, such as crop rotation, crop residue management and fertilization, are also important for earthworm populations (Riley et al., 2008). Eriksen-Hamel et al. (2009) reported that the addition of crop residues to tilled soils could alleviate some of the negative impacts of tillage on earthworms, thus improving their growth and maintaining more stable populations. While many studies demonstrate the role of cover crops in decreasing soil erosion and improving weed control and soil fertility (Ward et al., 2012), few investigate the effect of cover crops on earthworms. Farmyard manure is an organic amendment alternative to mineral fertilizers that can be beneficial for earthworm populations in arable fields (Andersen, 1979). Brown et al. (2004) reported that organic manures benefit earthworms both directly and indirectly by providing additional food resources and shelter (through the mulching effect), and stimulating plant growth and litter return.

Diversified crop rotation and green manures are used to manage weeds and pests, and the use of less intensive soil tillage (such as reduced tillage with no soil inversion) can reduce soil erosion, thus ensuring the sustainability of farming systems (Pelosi et al., 2014). Due to the potential beneficial effect of reduced tillage, green manures and organic fertilization on earthworms, a sensible hypothesis could be that the integration of conservation agriculture techniques into organic farming systems should increase their populations and diversity. For instance, some authors have indicated that conservation agriculture and organic farming can increase the abundance of all soil organisms, including earthworm populations (Henneron et al. 2014, Pelosi et al., 2014). Several studies have found higher biodiversity in organically managed systems than in conventional systems (Scullion et al., 2002), Padmavathy and Poyyamoli (2013) reported higher earthworm populations in organically managed fields. Organic farming is fundamentally different from conventional systems due to the exclusion of synthetic pesticides and fertilizers. However, notably few studies provide results confirming that earthworm populations and diversity increase in arable cropping systems with a combination of conservation agriculture techniques and organic farming.

The aim of this study is to analyse the individual and collective effects of tillage type, organic fertilizer and green manures on the density and biomass of earthworms in organic arable cropping systems in the Mediterranean region. Indeed, there is a lack of studies of earthworm populations in Mediterranean agricultural areas. Monitoring earthworms in these areas can be challenging because environmental conditions strongly limit earthworm distribution. Frequently, earthworms are distributed in small patches because many species have narrow ecological requirements that are determined by the high spatial variability of soil and soil water regimes in many Mediterranean landscapes (Gutiérrez-López et al., 2016).

The hypotheses of this study are that (1) the application of farmyard manure as fertilizer will increase earthworm density and biomass; 2) mouldboard ploughing will decrease earthworm populations, specially anecic species; 3) the incorporation of cover crops into the soil as green manures can increase earthworm density and biomass; and 4) the combination of conservation agriculture techniques in organic farming systems could help increase the abundance of earthworms in arable fields under a Mediterranean climate.

To answer these questions, we took advantage of a trial designed to evaluate the effects of tillage, fertilization and green manures on a Mediterranean rainfed crop rotation and measured the abundance of earthworm populations in relation to these factors.

# Materials and Methods

## Experimental site and design

In November of 2011, a midterm field experiment was established in Gallecs, a rural area of Catalonia, Spain. This location is a peri-urban agricultural area of 753 ha situated in the region of Vallès Oriental, 15 km North of Barcelona (41°33'31.9"N 2°11'59.5"E). It has a Mediterranean climate; the mean annual temperature and precipitation are 14.9 °C and 647 mm, respectively. At the beginning of the experiment, soil properties of the field were evaluated. On average, the mineral fraction consisted of 43.3 ± 6.9 % sand, 26.9 ± 4.7 % loam and 29.7 ± 3.7 % clay; the texture was classified as loamy-clay (Soil Survey Staff, 1998); and the soil type was a Haplic Cambisol (IUSS Working Group WRB, 2015). At the beginning of the experiment, the average soil organic matter was 1.5 ± 0.1 % (Walkley-Black) and the pH (H2O) was 8.1 ± 0.1.

The trial consisted of a four-year crop rotation in a strip strip block design (Federer & King, 2006) comprising three factors: tillage system (mouldboard ploughing (P) vs. chisel (C)), fertilization (composted farmyard (+F) vs. no fertilizer (-F)) and green manures (with green manures (+G) vs. no green manures (-G)). Tillage treatment was laid out in strips, and fertilization in perpendicular strips across tillage strips; the tillage strips were split into subplots for the green manure treatment (nested within each combination of tillage and fertilization). In total 32 plots measuring 13 m × 12 m were established, comprising four replicates of each treatment combination (Figure 1), although the design implied that the effects of tillage and of fertilization were assessed with less accuracy owing to the blocking structure (see statistical analysis). The field had been under organic management for five years prior to the trial, with a typical dryland Mediterranean crop rotation that alternated cereals and legumes for human consumption. The crop rotation of this trial consisted of spelt (*Triticum spelta* L., 2011–2012), chickpeas (*Cicer arietinum* L., 2013) winter wheat (*Triticum aestivum* L., 2013-2014) and lentils (*Lens culinaris* Medik., 2015).

Two tillage systems were used: a mouldboard plough (P) (soil inversion at 25 cm depth; EG 85-240-8, Kverneland) plus a rotary harrow (5 cm depth; HR3003D, Kuhn); and a chisel plough (C) (no soil inversion at 25 cm depth; KCCC 1187 - A00, Kverneland) plus a rotary harrow (same as for plough). The fertilization (+F) treatment utilized six-month-long composted cow farmyard manure sourced near the field. The farmyard manure was applied every year before sowing the main crop. The total amount of manure applied differed per the nutritional demands of each crop. The year before sampling, c. 38 Ton ha-1 (138.28 kg ha-1 Ntot) of farmyard manure was applied before winter wheat was sown. The organic fertilizers were mixed in the soil by means of a chisel or mouldboard plough in accordance to the tillage treatment. In September 2012 and 2014, green manure (+G) was sown in the corresponding 16 plots. It consisted of a mixture of oat (*Avena sativa* L.), white mustard (*Sinapis alba* L.*),* bitter vetch (*Vicia ervilia* (L.) Willd.) and common vetch (*Vicia sativa* L.). At the end of March of the following year, green manure was incorporated into the soil by disc harrowing.

## Earthworm sampling

In February 2015, after three years of crop rotation, earthworms were assessed during green manure or stubble (depending on the type of treatment). Three sampling frames of 33 cm × 33 cm were placed 2 m from the edge of each plot, with two on the mid-line and one on the centre of a randomly chosen side and were manually excavated to a depth of 25 cm. All earthworm and cocoons were hand sorted and preserved alive with moist soil at 4 °C until their fixation. In the laboratory, the earthworms were fixed using formalin (4 % formaldehyde) and preserved in alcohol (90 %) (Kuntz et al., 2013; Peigné et al., 2009). They were counted and sorted by adults (with a clitellum), juveniles (without a clitellum and tubercula pubertatis) and cocoons. Adults and juveniles were identified following Bouché (1972) and weighed (conserved weight in alcohol with gut contents).

## Statistical analysis

The individual and combined effects of the type of tillage (P vs. C), fertilization (+F vs. -F) and green manure (+G vs. -G) on adult and juvenile earthworm density and biomass was evaluated using linear mixed-effects models. The three treatments were used as fixed factors with tillage and fertilization blocks introduced as random factors (independent random effects for the fertilization blocks and the tillage blocks). The normality of residuals was verified by the Shapiro-Wilk test and homoscedasticity was assessed using the Bartlett test. To meet the normality and homoscedasticity requirements we used logarithmic transformation on data when necessary. The differences in mean density and biomass among the treatments resulting from the combination of all three factors were verified using Tukey’s HSD test. The same statistical procedure was followed for the analysis of the effect of tillage, fertilization and green manures on the densities of the main earthworm species*.* All the analyses were performed in R version 3.2.2 (R Core Team, 2015) with the packages lme4 (Bates et al., 2011) for linear mixed effects model fitting and multcomp (Hothorn et al., 2008) for post hoc multiple comparisons.

# Results

## Overview of earthworm diversity

Overall, five earthworm species were found: *Aporrectodea trapezoides* (Dugès, 1828), *Aporrectodea rosea* (Savigny, 1826), *Allolobophora georgii* (Michaelsen, 1890), *Octodrilus complanatus* (Dugès 1828) and one unidentifiable specimen belonging to the family Hormogastridae(Michelsen, 1900). In this study, we have focused on the three most abundant species: *A. rosea*, *A. georgii* and *A. trapezoides*. The first two areendogeic ecotypes (Bouché, 1972), but the latter is quite variable in its behaviour. Some authors have considered it an endogeic ecotype that sometimes feeds on the surface (Lee, 1985) but is primarily considered to be anecic (Fernández et al. 2010, Gutiérrez-López et al., 2016). Only one specimen of *Octodrilus complanatus* (anecic) and one unidentified Hormogastridae (endogeic) were observed*.* The most abundant species was *A. rosea* (mean 53.5 ± 6.6 ind m-2) followed by *A. georgii* (mean 29.8 ± 3.2 ind m-2), and *A. trapezoides* (mean 24.1 ± 3.3 ind m-2). However, the highest earthworm biomass was of *A.* *trapezoides* (14.80 ± 2.46 g m-2), followed by *A.* *rosea* (7.56 ± 0.91 g m-2) and *A.* *georgii* (5.59 ± 0.96 g m-2).

## Effect of organic fertilization, tillage and green manures on the density and biomass of earthworms

Fertilization was the main factor that influenced earthworm populations (Table 1). The density and biomass of earthworms were significantly enhanced by farmyard manure (Figure 2 I and 2 II). The densities of juveniles, adults and total earthworms were significantly higher in plots with farmyard manure (mean ± standard error of total density: +F= 146.4 ± 11.7 ind m-2; -F= 68.4 ± 6.7 ind m-2) (Table 2). This pattern is related to *A. rosea* and *A. georgii*, but not *A. trapezoides* (Table 1 and Table 2). Similarly, biomass was significantly enhanced by farmyard manure (mean ± standard error of total biomass: +F= 39.8 ± 4.4 g m-2; -F= 16.2 ± 2.7 g m-2). In contrast, total and adult densities were not affected by the type of tillage, but the density of juveniles was significantly higher in plots with mouldboard ploughing (Table 1). Total biomass of earthworms was not affected by the type of tillage. The presence of green manures did not affect the density of earthworms; however, significant differences were found for total and adult biomass (Table 1). The highest biomass was found in plots without green manures and chiselling (49.10 ± 10.01 g m-2). Furthermore, there was a significant interaction between tillage and green manure factors due to the significant decrease of total earthworm biomass in plots with chiselling and without green manures when no farmyard manure was incorporated (Table 1).

## Effects of the combination of conservation agriculture practices and organic fertilization on the biomass and abundance of earthworms

Contrasting results were found regarding the combined effects of the factors on density and biomass of earthworms. The combination of the two techniques of conservation agriculture (C and +G) was not the best combination for earthworm populations, regardless of fertilization (Figure 2 I). Total biomasses were significantly lower in plots with green manure and chiselling in comparison to those managed without green manure; the incorporation of green manures seems to affect negatively the total biomass of earthworms in unfertilized plots managed with chisel (Figure 2 II).

# DISCUSSION

## Overview of earthworm diversity

Our results show a low diversity of earthworms, with only four species identified in the study area, similarly to other studies in agricultural soils in the Mediterranean (Andriuzzi et al., 2017; Rosas-Medina et al., 2010; Pérès et al., 2010). Studies in agricultural systems of temperate climates had shown higher species richness (9-13 species) compared to our study in the Mediterranean region (Kuntz et al., 2013; Peigné et al., 2009). Furthermore, the earthworm community is dominated by only three species: two endogeic (*Aporrectodea rosea* and *Allolobophora georgii*) and one anecic ecotype (*Aporrectodea trapezoides*)*.* According to some studies *A. trapezoides* is very variable in its behaviour, similar to *Aporrectodea caliginosa* (Bouché, 1972, cited as *Nicodrilus caliginosus meridionalis*), being basically anecic but with some endoanecic (or even endogeic) populations (Fernández et al. 2010; Gutiérrez López et al., 2010); although sometimes juveniles behave as epigeics and adults as anecics (Gutiérrez López et al., 2016). In the case of the populations found in this study, the morphological characteristics correspond to a clearly anecic ecological category, with a medium-large size and a darker pigmentation in the dorsal anterior area and especially with the back somewhat flattened (with which they anchor in the galleries of the ground while feeding on surface).

Arable cropping systems with annual rotation schemes, high rates of soil disturbance and habitat simplification likely contribute to low species richness. Smith et al. (2008) reported low species richness in a study in Michigan, US, dominated by the genus *Aporrectodea*. These researchers relate the findings to the fact that this genus is relatively tolerant to agricultural activities, as it is able to persist deeper in the subsoil than other species.

Boström (1995) suggested that the large amount of organic matter ploughed under the soil, which served as food for the earthworms, together with the supply of cocoons allowed the endogeic earthworms to make a fast recovery. Capowiez et al. (2009) reported that *Aporrectodea* *caliginosa* increased with mouldboard ploughing due to an increase in food resources (buried organic matter) and a decrease in competition for food with anecic species. Anecic species, on the other hand, could be the most negatively affected by intensive and repeated soil disturbance because of direct physical damage and an indirect effect on food resources (burial of surface organic matter) and their habitat (destruction of burrows) (Capowiez et al., 2009).

Pelosi et al. (2014) suggested that anecic species are less abundant, or even absent, in ploughed fields. Though several studies have shown that ploughing reduced the number of the large-bodied anecic species (Chan, 2001; Ernst and Emmerling, 2009; Pelosi et al., 2014), our results indicated *A. trapezoides* were not significantly affected by the type of tillage. Kuntz et al. (2013)reported that the implementation of reduced tillage in an organically managed clay soil over a six-year crop rotation enhanced the density and biomass of earthworms while also influencing their community structure. In this four-year experiment, we did not find that the type of tillage significantly affected earthworm communities, perhaps indicating that either the response of earthworm populations occurs over a longer time period or that *A. trapezoides* experiences the same effects under mouldboard ploughing than under chisel ploughing, which translates into a lack of effect in our experiment.

## Effect of organic fertilization, tillage and green manures on the density and biomass of earthworms

It is known that fertilizers have long-term benefits for earthworm populations through increased input of nutrients, organic matter and enhanced production of litter material, which ultimately result in more food for earthworms. Furthermore, farmyard manure is considered most suitable for earthworm population growth (Brown et al., 2004; Curry, 2004, 1976). Our study supports the claim that farmyard manure enhances earthworm growth and reproduction.

The effect of fertilization is significant for both density and biomass of adults and juveniles, while the effect of tillage is only significant for the density of juveniles. We hypothesize that the high number of juveniles in plots using mouldboard ploughing might relate to a better survival of earthworms in conventionally tilled soils. Several studies have shown that tillage with soil inversion can reduce earthworms by 70% both in numbers and biomass, but populations generally recover within a year if the disturbance is not repeated (Boström, 1995). Eriksen-Hamel et al. (2009) reported that ploughing reduced the earthworm population by 73-77%, but one year later, there were five times as many earthworms, and the biomass was similar to the pre-tillage level. This could be explained because disturbance may increase survival partly due to soil loosening and improvement of soil physical conditions (Chan, 2001). In this experiment, earthworm sampling was carried out six months after the soil was disturbed, probably leaving enough time for their recovery.

In this study, the highest biomass was found in plots without green manures, which is contrary to our initial hypothesis that the incorporation of organic matter from green manure should favour earthworms. However, few studies are available which analyse the effects of green manures and their interaction with tillage systems on earthworm populations, and thus our results are difficult to frame within existing evidence. Stroud et al. (2016) reported that *Lumbricus terrestris* L. was not enhanced by oilseed radish as cover crop in a long-term rotation. Valckx et al. (2011) also studied *Lumbricus terrestris* food and habitat preferences for cover crops and have found that rye grass was the preferred food resource, but no preference or repellence was found for mustard (*Sinapis alba).* However, they found an increasing trend for repellence against oats (*Avena sativa*) over time, suggesting that the allelopathic effect of oats may affect earthworms indirectly by changing habitat and food preference. Since we used oats and white mustard for the green manure mix, this could explain our results, although we cannot confirm that there is a repellent effect of green manures on earthworms. Furthermore, we found no studies regarding the food and habitat preferences of *Aporrectodea rosea*, *A.* *trapezoides* or *Allolobophora* *georgii.* There is a need to study in more detail whether there are some allelopathic effects of oats or mustard as cover crops that may affect earthworms.

## Effects of the combination of conservation agriculture practices and organic fertilization on the biomass and abundance of earthworms

Density and biomass of earthworms respond differently to the combination of experimental factors, and the optimal combination of conservation agriculture practices is different depending on whether the focus is on density or on biomass. While biomass is favoured under reduced tillage, but not green manures, density is increased under mouldboard ploughing. These effects may relate to the developmental stage of earthworms: whereas juveniles condition the effects on density, the effects on biomass are dependent on adults, but in no case conservation agriculture techniques were the best combination for earthworm populations (Figure 2). Several studies have indicated that the use of conservation agriculture techniques increases populations of earthworms (Scullion et al., 2002). Our results showed that earthworms can have similar abundances with both tillage systems, and that the effects found may be transient, in relation to quick changes in the juveniles (Table 2). Perhaps in the Mediterranean region, where extreme climate conditions -particularly summer drought- play an important role on agroecosystems functioning, the use of all conservation agriculture techniques does not contribute to a more sustainable cropping system. Only organic fertilization seems to play an important role in these systems, and it could be a crucial factor to maintain and benefit earthworm populations in Mediterranean agricultural systems.

The highest density of earthworms was found in plots with mouldboard ploughing, fertilization and the presence of green manures. This can be explained due to a species-specific response. *Aporrectodea rosea*, which was the most abundant species, avoids compaction and reduced tillage seemed to create more compacted zones. Some authors have shown that minimum tillage and no-tillage result in more compacted soil than ploughing (Capowiez et al., 2009; Peigné et al., 2009). Compacted zones are created by wheel tracks, and in tilled plots those zones are fragmented into clods by the plough; but in reduced tillage plots only parts of the compacted zones are fragmented (Capowiez et al., 2009). In consequence, a soil structure with more macropores is obtained with mouldboard ploughing and, in the short term, earthworms are not able to improve soil macroporosity (Peigné et al., 2009). Furthermore, *A. rosea* lives mainly in the upper layers of the soil, feeding on soil organic matter. Therefore, this species could take advantage of increased availability of crop residues incorporated by inversion tillage (Chan, 2001). The highest total biomass was found in plots tilled with chisel and fertilization, but only minor differences related to green manures (Table 2). These results correlate to the response of *A. trapezoides*, the only anecic ecotype found in this experiment. Particularly the response of adults determines the overall pattern, since *A. trapezoides* is the largest species and had the highest biomass. Therefore, it may be more likely affected by soil inversion tillage (Gutiérrez-López et al., 2016), and thus reduced non-inversion tillage could benefit this species.

# CONCLUSIONS

Organic inputs, in the form of farmyard manure but not from green manures, have the strongest effects on earthworm populations. However, tillage systems are of less importance. The two tillage systems did not show significant differences in total density and biomass of earthworms, but they did show an important effect depending on the earthworm ecotype and stage of development. Endogeic species can benefit from soil inversion because of the incorporation of organic matter and enhanced survival of juveniles.

Our study did not show overall positive effects of the combination of conservation agriculture techniques on earthworms. It is important to understand how these different factors interact when designing a sustainable organic system. Different farming practices can shape earthworm communities by modulating the relative abundances of the different ecotypes. However, more information is needed about the biology of *Aporrectodea trapezoides, A. rosea* and *Allolobophora georgii*, which are abundant in many Mediterranean systems. There is a need for long-term studies under Mediterranean climate to understand their ecology and their responses to conservation agriculture techniques, as well as their potential in supporting a sustainable soil management.



Figure 1. Experimental design in a strip strip block with three factors of two levels each. P, mouldboard ploughing; C, chisel ploughing; + F, fertilization with farmyard manure, - F, not fertilized: + G, with green manure, - G, no green manure.

Table 1. Estimates of coefficients from the linear mixed models testing the effect of fertilization (F: + fertilization with farmyard manure, F: – not fertilized; T: tillage system (P: mouldboard ploughing, C: chisel ploughing); G: + with green manure, G: – no green manure), and the interaction between factors (F, T and G) on the different variables measured.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | F: + vs – | T: P vs C | G: + vs – | F×T | F×G | T×G |
| Total density§ | 0.390\*\* | 0.076 NS | -0.021 NS | 0.006 NS | 0.034 NS | 0.091 NS |
| Density adults§ | 0.480\*\*\* | -0.045 NS | -0.081 NS | 0.028 NS | 0.017 NS | 0.046 NS |
| Density juveniles§ | 0.305\* | 0.203\*\* | 0.042 NS | 0.042 NS | 0.103 NS | 0.100 NS |
| *A. rosea* total density | 19.984\* | 8.046 NS | 3.890 NS | 8.621 NS | 5.965 NS | 8.053 NS |
| *A. georgii* total density§ | 0.0323\* | 0.009 NS | -0.050 NS | -0.019 NS | -0.097 NS | -0.074 NS |
| *A. trapezoides* total density | 9.656 NS | -1.137 NS | -0.381 NS | -3.031 NS | 0.762 NS | 3.218 NS |
| Total biomass§ | 0.489\* | -0.091 NS | -0.151\* | -0.068 NS | 0.060 NS | 0.160\* |
| Biomass adults§ | 0.582\* | -0.166 NS | -0.232\* | -0.028 NS | 0.112 NS | 0.150 NS |
| Biomass juveniles | 1.209\* | 0.521 NS | 0.121 NS | -0.378 NS | 0.234 NS | 0.334 NS |
| *A. rosea* total biomass | 3.121\* | 0.215 NS | -0.221 NS | 0.509 NS | -0.090 NS | 0.903 NS |
| *A. georgii* total biomass§ | 0.408\* | -0.086 NS | -0.133 NS | 0.006 NS | -0.049 NS | 0.026 NS |
| *A. trapezoides* total biomass | 7.134 NS | -2.834 NS | -1.222 NS | -2.234 NS | 1.091 NS | 2.347 NS |

Total density and biomass refers to the sum of adults and juveniles. §:Logarithmic transformation was applied to meet homoscedasticity and normality of residuals. Significance levels according to the following codes: \*\*\* p < 0.001, \*\* p < 0.01, \* p < 0.05, NS not significant.



Figure 2. Mean (± SE standard error) total number of individuals (I) and total biomass (II) in each treatment: F: – not fertilized, F: + fertilization with farmyard manure; G: – no green manure, G: + sowed with green manure; T: tillage system (P: mouldboard ploughing, C: chisel ploughing). Bars with no letters in common are significantly different (Tukey HSD test, P < 0.05).

Table 2. Mean (± SE standard error) density (ind m-2) and biomass (g m-2) of *Aporrectodea rosea*, *Allolobophora georgii* and *Aporrectodea trapezoides*, per developmental stage (adults and juveniles) and their sum per square meter: F+: fertilization with farmyard manure, F–: not fertilized, P: mouldboard ploughing, C: chisel, G+: with green manure and G–: no green manure.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **Species** | **F+** | **F–** |
|  |  | **P** | **C** | **P** | **C** |
|  |  | **G+** | **G–** | **G+** | **G–** | **G+** | **G–** | **G+** | **G–** |
| *Density* | *Adults* | *A. trapezoides* | 18.18 ± 9.17 | 12.88 ± 5.01 | 21.21 ± 9.17 | 25.00 ± 6.35 | 5.30 ± 2.27 | 7.58 ± 1.96 | 6.06 ± 4.29 | 14.39 ± 5.85 |
| *A. rosea* | 45.45 ± 17.19 | 38.64 ± 5.01 | 29.55 ± 5.45 | 37.12 ± 6.23 | 15.91 ± 3.58 | 12.88 ± 3.36 | 14.39 ± 6.11 | 18.18 ± 7.53 |
| *A. georgii* | 16.67 ± 4.37 | 34.09 ± 4.35 | 31.82 ± 12.89 | 25.00 ± 5.85 | 12.12 ± 3.91 | 9.85 ± 4.52 | 9.85 ± 0.76 | 9.09 ± 2.14 |
| **Total adults** | 80.30 ± 17.69 | 85.61 ± 11.23 | 82.58 ± 16.84 | 87.12 ± 11.76 | 33.33 ± 8.57 | 30.30 ± 6.19 | 30.30 ± 7.82 | 41.67 ± 7.04 |
| *Juveniles* | *A. trapezoides* | 13.64 ± 1.52 | 14.39 ± 5.01 | 15.15 ± 6.43 | 14.39 ± 5.72 | 14.39 ± 5.72 | 5.30 ± 1.45 | 0.76 ± 0.76 | 3.79 ± 1.91 |
| *A. rosea* | 62.88 ± 17.20 | 33.33 ± 6.89 | 28.79 ± 6.25 | 18.18 ± 6.19 | 22.73 ± 4.37 | 14.39 ± 2.27 | 9.85 ± 3.79 | 25.76 ± 7.87 |
| *A. georgii* | 7.58 ± 3.81 | 18.94 ± 6.23 | 12.88 ± 2.59 | 9.85 ± 3.36 | 12.12 ± 4.29 | 9.85 ± 3.79 | 8.33 ± 2.27 | 10.61 ± 4.01 |
| **Total juveniles** | 84.09 ± 17.34 | 66.67 ± 2.77 | 56.82 ± 9.69 | 42.42 ± 13.03 | 49.24 ± 10.95 | 29.55 ± 3.98 | 18.94 ± 2.59 | 40.15 ± 8.95 |
| *Biomass* | *Adults* | *A. trapezoides* | 17.96 ± 10.28 | 11.99 ± 4.59 | 20.51 ± 8.00 | 27.63 ± 7.45 | 4.08 ± 1.95 | 6.65 ± 1.93 | 4.45 ± 3.58 | 11.62 ± 5.00 |
| *A. rosea* | 8.66 ± 3.15 | 8.29 ± 1.86 | 6.71 ± 1.18 | 9.89 ± 2.18 | 3.10 ± 0.85 | 2.17 ± 0.50 | 3.11 ± 1.48 | 3.63 ± 1.64 |
| *A. georgii* | 3.50 ± 0.84 | 7.49 ± 1.68 | 7.23 ± 2.95 | 6.57 ± 1.58 | 2.49 ± 0.74 | 1.61 ± 0.67 | 1.63 ± 0.19 | 7.72 ± 6.09 |
| **Total adults** | 30.12 ± 9.34 | 27.77 ± 6.71 | 34.45 ± 9.09 | 44.08 ± 9.31 | 9.67 ± 3.07 | 10.42 ± 1.87 | 9.18 ± 4.19 | 22.97 ± 7.39 |
| *Juveniles* | *A. trapezoides* | 2.14 ± 0.43 | 1.63 ± 0.27 | 2.98 ± 0.51 | 2.89 ± 1.09 | 1.98 ± 0.73 | 1.42 ± 0.60 | 0.20 ± 0.20 | 0.28 ± 0.19 |
| *A. rosea* | 3.44 ± 1.10 | 2.42 ± 0.63 | 1.92 ± 0.52 | 1.38 ± 0.60 | 1.73 ± 0.51 | 1.30 ± 0.39 | 0.70 ± 0.24 | 2.02 ± 0.70 |
| *A. georgii* | 0.45 ± 0.30 | 1.37 ± 0.48 | 0.92 ± 0.30 | 0.76 ± 0.33 | 0.95 ± 0.48 | 0.73 ± 0.27 | 0.53 ± 0.05 | 0.77 ± 0.27 |
| **Total juveniles** | 6.03 ± 1.47 | 5.42 ± 0.35 | 5.83 ± 0.55 | 5.02 ± 1.67 | 4.67 ± 1.32 | 3.46 ± 0.17 | 1.43 ± 0.30 | 3.08 ± 0.78 |

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