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## Earthworms in a 15 years agricultural trial

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## Abstract

Alternative cropping systems have been proposed to enhance sustainability of agriculture, but their mid and long-term effects on soil biodiversity should be studied more carefully. Earthworms, having important agro-ecological functions, are regarded as indicators of soil biological health. Species composition, abundance, and biomasses of earthworms were measured in autumn 2005, 2006, 2007 (period 1) and in 2011, 2012, 2013 (period 2) in a trial initiated in 1997 near Paris, France. A conventional, an organic and a direct seeded living mulch-based cropping systems were compared. Earthworms were sampled in a wheat crop by combining the application of a chemical expellant and hand-sorting.

In period 1, earthworm abundance did not usually differ in the three cropping systems, but sometimes it was higher in the conventional system. Mean total abundance was 122, 121 and 149 individuals  $m^{-2}$  in period 1 and 408, 386 and 216 in period 2 in the organic, living mulch and conventional systems respectively. While earthworm abundance and biomass increased slightly in the conventional system between the two periods, they at least tripled in the other two systems. This was mainly due to the species *Aporrectodea caliginosa* and *A. longa* in the living mulch cropping system, and to *A. caliginosa*, *Lumbricus castaneus*, *L. terrestris* and *A. longa* in the organic system.

After at least 14 years, organic and living mulch cropping systems contained between 1.5 and 2.3 times more earthworms than the conventional system. Considering the inter-annual variations in earthworm communities due to climatic conditions and cultural practices, earthworm communities should be assessed over several years before conclusions can be drawn. Moreover, since changes in cultural practices may take a long time to affect earthworm communities, mid and long-term trials are needed to assess the effects of cropping systems on soil biodiversity.

**Keywords:** Conservation agriculture; Conventional system; Living mulch; Organic farming; Pesticide; France.

## **1. Introduction**

Earthworms are known to improve soil physical, chemical and biological components (Eriksen-Hamel and Whalen, 2007; Fragoso et al., 1997; Jégou et al., 2000; Lavelle, 1997). Moreover, they are sensitive to crop management, easily collected by both specialists and farmers, and thus can be used to evaluate different environmental pressures (Blouin et al., 2013; de Lima and Brussaard, 2010; Daugbjerg et al., 1988; Jamar et al., 2010). They are thus considered as soil quality indicators (Doran and Parkin, 1996). According to Paoletti (1999), “in rural environments different farming systems can be assessed using earthworm biomass and numbers”.

In the current context of soil protection and chemical input reduction in agrosystems (Médiène et al., 2011), effects of alternative cropping systems on biodiversity should be studied. Cropping systems without plowing are known to rapidly promote earthworm communities, especially the larger individuals (Chan, 2001; de Oliveira et al., 2012). The presence of a living mulch at the soil surface generally increases earthworm abundance and biomass (Brevault et al., 2007; Fonte et al., 2009; Tebrügge and Düring, 1999), encouraging species which feed at the soil surface, i.e. mainly epigeics and anecics. The effect of systems without chemical inputs, and in particular without pesticides is less obvious. Although pesticides may have deleterious effects on earthworms under laboratory conditions (Yasmin and D’Souza, 2010), field studies which compare effects of conventional and organic farming on earthworm communities show variable results (Hole et al., 2005; Nuutinen and Haukka, 1990). These results can be difficult to interpret because of several factors varying at the same time, e.g. manure inputs and soil tillage (Hole et al., 2005). Moreover, effects of cropping systems on earthworm communities are sometimes studied at only one sampling date in trials whose duration is not precisely specified (Crittenden et al., in press; Liebzig and Doran, 1999; Scullion et al., 2002) or which have been set up only a few years before the sampling

(Nuutinen and Haukka, 1990). Few studies have been made on mid and long-term trials, with sampling intervals of several years.

Pelosi et al. (2009a) assessed earthworm species richness, abundance and biomass in three cropping systems which differ mainly in soil tillage, pesticide and fertilizer applications, and crop biomass production. These cropping systems were a conventional system, a direct seeded living mulch-based cropping system, called “living mulch cropping system” and an organic system. They were part of a long-term cropping system comparison trial (Debaeke et al., 2009), initiated in 1997. Samplings were made in this trial after 8, 9 and 10 years, i.e. in 2005, 2006, and 2007 (period 1). The authors concluded that the different cropping systems did not influence total earthworm abundance but they modified specific and functional diversity as well as earthworm community biomass due to soil tillage and organic resource availability. In the living mulch cropping system, with a permanent plant cover and no plowing, diversity indices as well as anecic and epigeic abundance and biomass were higher than in organic and conventional systems. Endogeic earthworms, however, were more numerous in the last two systems. Organic and conventional systems did not differ in their earthworm abundance, biomass or diversity. To explain these results, authors mentioned lower yields and available trophic resources in the organic system than in the conventional one. Moreover, according to LD50 of active substances (ANSES, Agritox, 2005), it seemed that pesticides sprayed in the conventional system had no pronounced effect on earthworm mortality. Pelosi et al. (2009a) also reported variations in earthworm total abundance and biomass from one year to another, i.e. between autumn 2005, 2006 and 2007. However, this variability over time was not significant and did not influence effects of cropping systems on earthworms.

Here we assess earthworm communities in the same long-term trial in 2011, 2012 and 2013 (period 2), after 14, 15 and 16 years, on the same crop i.e. winter wheat. Our objectives

are to answer the following questions: Are the differences observed at period 1 in earthworm abundance and biomass between conventional, organic and living mulch cropping systems maintained in period 2? Did switching from conventional to organic systems have any effect on the earthworm community? For that purpose, we compare earthworm communities in the long-term trial between the period 1 (2005, 2006, 2007) and the period 2 (2011, 2012, 2013).

## **2. Materials and Methods**

### **2.1 Site and cropping systems**

Our experimental site was located 15 km south-west of Paris (48°48' N, 2°08' E). The experiment began in 1997 and the site, which is not irrigated, was under conventional agriculture before this date. The soil is a deep luvisol (FAO classification), with a neutral pH and an average texture of 58% silt, 25% sand and 17% clay. The climate is temperate, with a mean annual precipitation of 640 mm and a mean annual temperature of 10.4°C.

The three experimental cropping systems are a living mulch cropping system, a conventional system and an organic one. In the conventional system, where weeds and pests were controlled with pesticides, the soil was plowed every year except after the leguminous crops. In the living mulch cropping system, the permanent plant cover was killed with herbicides in order to plant the crop with economic interest e.g. wheat. In the organic cropping system no manure or other external organic amendments were added. As in the conventional system, plowing was done every year in this last system except after the leguminous crops. The organic system was managed following the rules of the AB France label, without any use of synthetic pesticides or mineral fertilizers. Weeds were limited by soil tillage, crop succession, crop density, and changes in crop sowing date. Nutrient exports were limited by straw restitution. Soil P and K levels were recorded throughout the experiment. Because they were always high enough, no supplements were added.

The trial site was divided into two replicates. In each replicate, a 1 ha plot of each system was divided into two subplots in which the rotation was established, such that for every year, one of the two subplots had a winter wheat crop (Fig.1) Management of the three systems, as well as crop rotations, C/N ratios of the top 20 cm of soil and organic matter contents are shown in Table 1. Calcium carbonate (CaCO<sub>3</sub>) content of soils was 0.9 g kg<sup>-1</sup> and pH varied between 7.0 and 7.5 in the three cropping systems. Between 1997 and 2008, alfalfa was not used in the living mulch cropping system, and was used one year out of five in the organic system. Since 2009, this crop has been used two years out of three in the two mentioned systems.

## **2.2 Earthworm sampling**

Species composition, abundances and biomasses were assessed in the subplots involving the same crop, i.e. winter wheat, to limit potential effects of the crop. Samples were taken at the end of October or early beginning of November 2011, 2012 and 2013 and compared with data obtained from period 1 (2005, 2006, 2007, Pelosi et al., 2009a). Pelosi et al. (2009a) provided data on total earthworm abundances and biomasses and by ecological categories in 2005, 2006 and 2007, but some information not published before will be analyzed in this study (i.e. abundance and biomass at the species level). Autumn is a period of high activity for numerous earthworm species (Bouché, 1972) and samples of periods 1 and 2 were all taken at that time to limit effects of seasonal variations. Rainfall and temperature data before sampling are summarized in Table 2. Five samples (40 x 40 cm), randomly located but at least 3 meters from the plot border, were made on each of the two replicates of each cropping system (Fig.1). The total sampled area for each system is not very large compared to the size of the plots but it is similar to that in the literature (de Oliveira et al., 2012; Krück et al., 2006; Topoliantz et al., 2000).

Earthworm samplings were made using both chemical extraction and hand-sorting of earthworms (Bouché, 1972). A diluted expellant solution of allyl isothiocyanate (AITC) was applied to the soil within a 40 x 40 cm metal frame i.e., 0.16 m<sup>2</sup>. AITC was first diluted with isopropanol (propan-2-ol) to obtain a 5 g L<sup>-1</sup> solution (Pelosi et al., 2009b; Zaborski, 2003). This solution was then diluted with water to give a concentration of 0.1 g L<sup>-1</sup>. After collecting emerging individuals, a 40 cm x 40 cm x 20 cm-depth block of soil (where expellant solution was applied) was excavated and remaining earthworms were hand-sorted. They were then preserved in 4% formalin solution, weighed without emptying their gut content, counted and identified at the species level. The development stage of each earthworm was noted. Earthworms were considered sub-adult if they had full tubercula pubertatis but no clitellum, and adult if they were clitellate (Sims and Gerard, 1999). They were considered juvenile if they had neither tubercula pubertatis nor clitellum. Sub-adults and adults were identified at species level according to the identification key of Sims and Gerard (1999). Juveniles were also identified at species level thanks to morphological characteristics of the species and to the specific form they take in formalin in comparison with that of identified adults. When identification at the species level was impossible, individuals were classified as 'unidentified'.

### **2.3 Statistical analysis**

Sample data of earthworm abundance and biomass were expressed per square meter. Given that the trial has only two replicates, we considered the five sampling points in each of the two replicate plots as true replicates. Despite the limited numbers of treatment replicates, this trial is worthwhile as it is a long-term trial and the plot size is realistic (0.5 ha), managed under real agricultural practices unlike many studies which use small or very small experimental plots (Blanchart et al. 2006; Birkhofer et al. 2008; Postma-Blaauw et al. 2010; Wardle et al. 2001). The possibility that the sample plots were affected by confounding

factors due to limited randomization cannot be excluded but was limited as the trial was evenly affected by the same management before the trial setup, the preexisting topographic and pedologic gradients were controlled by blocking, and a preliminary assessment of the trial spatial heterogeneity was found very low within-block soil heterogeneity. This trial has already been used to evaluate the effects of cropping systems on soil biodiversity (Pelosi et al. 2009a; Henneron et al., 2014). Samples were analyzed independently for each year. When the normality and the homoscedasticity of variance were not respected, data were transformed by  $f(x) = \ln(x + 1)$ . After that, when the conditions were satisfied, ANOVA and Tukey's test were used to analyze the results and ascertain differences between systems. When the data did not satisfy the conditions, non-parametric tests were used, i.e. Kruskal-Wallis tests and post-hoc tests for mean comparisons (R Development Core Team, 2011).

### **3. Results**

The proportion of juveniles was 58% in the living mulch cropping system, 61% in the organic system, and 69% in the conventional system in period 1, when averaged over the three years (Pelosi et al., 2009a). In period 2, it was 66%, 82%, and 61% for the three mentioned systems, respectively.

The seven species sampled in period 1 were also found in period 2 (Table 3). *Aporrectodea giardi* was found in the three systems in 2005 and 2006 but it was absent from organic and conventional systems in the other four years. Conversely, many individuals of the anecic *A. longa*, that was very scarce in period 1, were found in period 2 (Table 3).

In period 1, total earthworm abundance did not differ in the three systems, except in 2007 when it was higher in the conventional system than in the other two systems (Fig. 2). Conversely, in period 2, abundance in the organic system was significantly higher (between 1.6 and 2-fold) than in the conventional system (Fig. 2). Earthworm abundance in the living

mulch system was similar to the organic system and it was significantly different from conventional system only in 2013. Between periods 1 and 2, mean (over the three years, N=30) total abundance increased from 122 to 408 individuals  $m^{-2}$  in the organic system, from 121 to 386 individuals  $m^{-2}$  in the living mulch cropping system, and from 149 to 216 individuals  $m^{-2}$  in the conventional system, all these differences being significant. Total biomass was significantly the highest in the living mulch cropping system in period 1, the organic and conventional systems being similar (Fig. 3). In period 2, it increased in the living mulch cropping system as well as in the organic one, even if differences with the conventional system were not always significant (i.e. in 2012 and 2013) (Fig. 3). Total precipitations during the six month before the samplings were slightly higher in 2007, 2012 and 2013 (Table 2). Correlation coefficients ( $R^2$ ) between rainfall (sum of September and October) and total abundance of earthworms for the six years of sampling (n=6) were 0.86, 0.81 and 0.60 for the living mulch, the organic and the conventional systems, respectively. For total biomass,  $R^2$  were 0.84, 0.70 and 0.53 for the three mentioned systems, respectively.

Regarding ecological groups, in period 1, abundance and biomass of anecics and epigeics were higher in the living mulch cropping system than in the other systems, while abundance and biomass of endogeics tended to be higher in the organic system and even more so in the conventional system (Table 3 and 4). In period 2, anecic abundance and biomass were always significantly higher in the living mulch system than in the conventional system. These variables were generally higher in the organic system than in the conventional system but differences were not always significant. Many more epigeics were found in the organic system than in the other two systems in 2011 and 2012. In 2013, more epigeics were found in the two alternative systems (i.e. organic and living mulch cropping systems) than in the conventional one. Because adult anecics are big earthworms, total biomass in the living mulch cropping system significantly exceeded the biomass of the other two systems at both periods.

Total biomass in the organic system was generally higher than in the conventional system but the differences were significant only in 2011 (Table 4). Endogeic abundance was not different in 2011 in the three systems. Conversely, it increased significantly in the alternative systems, and mainly in the organic system, in 2012 and 2013 compared to the conventional system. Increase in earthworm abundance and biomass was not the same in the two alternative systems or for the different species. The increase in abundance was significantly due to the species *Aporrectodea caliginosa* and *A. longa* in the living mulch cropping system, and to *A. caliginosa*, *Lumbricus castaneus*, *L. terrestris* and *A. longa* in the organic system (Table 3 and 4). Conversely, abundance of *A. chlorotica* did not increase in the living mulch cropping system and slightly in the organic system. Similarly, *A. icterica* increased significantly but slightly in both systems.

Year-to-year variations in earthworm abundance and biomass occurred within both sampling periods. However, differences between the three systems were consistent within each period. For instance, in period 1, we noticed inter-annual variability in earthworm abundance and biomass but the differences were maintained over the three years, being significant or not: anecic and epigeic abundance and biomass were higher in the living mulch cropping system than in organic and conventional systems and endogeic earthworms were more numerous in the last two systems.

#### **4. Discussion**

Earthworm communities (abundance and biomass) greatly increased between the two sampling periods in the two alternative cropping systems while they increased much less in the conventional system. Several assumptions related to climatic conditions and cultural practices in the cropping systems may be proposed to explain these results. First, taking into account the high correlation between rainfall (during the two months preceding earthworm

samplings) and total abundance and biomass of earthworms in the three cropping systems, there was a possible effect of rainfall on temporal changes between the two periods. The correlation was lower in the conventional system than in the other two systems. This highlights that the different systems can make moisture more or less available to earthworms. The rainfall may thus have been less beneficial to earthworms in the conventional system than in the two others. Moreover, in the organic and the living mulch cropping systems, alfalfa has been used more intensively since 2009. Some authors considered that alfalfa is a low-quality food for earthworms (Boström and Lofs-Holmin, 1986) but others explained that it can benefit earthworm activity and abundance (Bradley et al., 2011; Gallagher and Wollenhaupt, 1997; Kautz et al., 2010; Shipitalo et al., 1988). In the living mulch system, a growing number of endogeic *A. caliginosa* individuals were recorded between periods 1 and 2. Similarly anecic *A. longa*, which was not present in period 1, represented 25% of the total abundance and 40% of the total biomass, on average over period 2. Since this last species was observed in the field margins (during earthworm collections for laboratory studies) of the experimental trial, we could hypothesize that it colonized the trial from field edges and thrived thanks to the absence of mechanical disturbance. This species was also found in the organic and conventional systems, despite the plowing which generally affects big individuals. It seems that *A. longa* has replaced *A. giardi* in these two systems since this latter species had been found in 2005 and 2006 but not after. Being anecic, these two species may have been in competition for space and resources, leading to the disappearance of *A. giardi*, maybe less competitive. The increase in earthworm abundance and biomass as well as the changing pattern of earthworm species assemblages following a switch from one management system to another (less intensive) has already been described by Decaëns et al. (2011) and Pizl (1999) in grasslands and abandoned arable fields, respectively. This underlines that these findings are site specific because potential colonization of fields by new species depend on the landscape

elements surrounding the plots as well as the species found in these elements. Finally, in the living mulch cropping system, pesticide use did not prevent an increase in the earthworm communities, maybe due to the large amount of available organic matter and the absence of soil disturbance.

As in the living mulch cropping system, a sharp increase in total abundance and biomass occurred in the organic system between periods 1 and 2, mainly due to an increase of the number of juveniles (representing 82% of the total abundance in period 2) and to the species *A. caliginosa*, *L. terrestris*, *A. longa*, and *L. castaneus*. This result is striking because no differences were found between organic and conventional systems in period 1 (except once, in favor of the conventional system). It thus took more than 9 years for differences in abundance to appear between these two cropping systems. To explain differences between systems we can hypothesize that earthworm species have benefited from the alfalfa (Bradley et al., 2011; Gallagher and Wollenhaupt, 1997; Kautz et al., 2010; Shipitalo et al., 1988) that was grown cropped in the organic than in the conventional system. Another assumption could be a mid-term harmful influence of the numerous pesticides applied before the establishment of the trial, as they were used at significant doses and with active ingredients involving high acute and chronic risks for earthworms e.g., carbendazim (Cluzeau et al., 1990; De Silva et al., 2009). Their breakdown products, that can be highly toxic (Gasser et al., 2007; Sinclair and Boxall, 2003), as well as the bound residues, may explain the mid-term effects. These compounds may affect earthworm survival as well as their individual growth and fecundity (Pelosi et al., 2013a; Yasmin and D'Souza, 2010), leading to mid or long-term effects on earthworm populations (Daugbjerg et al., 1988). Pelosi et al. (2013b) showed that species living in the soil's surface layer were the most affected by pesticides. In the organic system, earthworms (mainly epigeic species) could have colonized the plots and thrived thanks to a N rich form of organic matter (i.e. alfalfa) and a gradual decrease in the harmful effects of

pesticides. The use of pesticides in the conventional system may deter some species from colonizing the plots. As earthworms do not disperse much when conditions are favorable (Mathieu et al., 2010), it would have taken several years to re-colonize the organic plots after the establishment of the trial, maybe helped by passive dispersal by agricultural machinery.

The change in earthworm communities in organic and living mulch cropping systems between the two periods highlights that a modification of cropping systems and thus of cultural practices takes some years to affect earthworms. This underlines (i) the potential slow response of the earthworm communities to agricultural management changes and (ii) the need for mid and long-term trials to study the effects of consistently managed alternative cropping systems on biodiversity. This was also emphasized by Tebrügge and Düring (1999) and Irmeler (2010) who criticize the fact that most studies are restricted to short investigation periods. Whilst sampling over several years is important, the age of trials in which alternative systems are assessed is perhaps even more so. In the literature, it was often difficult to find information on trial duration and/or this information is generally not very precise (Liebig and Doran, 1999; Yeates et al. 1997). Hole et al. (2005) reported conflicting results in their literature review of the effect of organic farming compared to conventional farming. The different crops as well as the cultural practices used to compensate for lack of fertilizer nitrogen (i.e. manure inputs) and to control weeds (i.e. longer rotation and tillage) in organic systems may affect earthworm populations and may thus partly explain these conflicting results. The totality of these practices should be considered when comparing organic and conventional systems because considerable variations can exist between one organic or conventional system and another.

In the literature, when the duration of the trial is given, it varies greatly from one study to another. For instance, Pfiffner and Mäder (1998) compared the impact of different cropping systems in a long-term trial (since 1978), and found a benefit of organic farming for

earthworms. Conversely, Nuutinen and Haukka (1990) sampled « the three last years of the seven-year experiment » and found no significant difference in earthworm abundance or biomass between organic and conventional farming systems. Similarly, Tarrant et al. (1997) found no abundance, biomass or species composition differences between a «current farm practice» and a «reduced input approach in which inputs have been reduced by 50% and no insecticides used». Authors explained these results by the short duration of their trial, i.e. six years. Long-term monitoring (more than 15 years) is thus needed before conclusions can be drawn.

## **5. Conclusions**

Our study highlighted that abundance and biomass of earthworm communities are still increasing in the alternative cropping systems after 14 years of differentiation. The organic system, that did not differ from the conventional system in terms of earthworm abundance, biomass and diversity after 10 years of differentiation, displayed a sharp increase in earthworm communities after 14 years, maybe due primarily to the alfalfa crop and to the cessation of pesticide use. Taking into account the year-to-year variations in earthworm communities and the time required for organisms to adapt to new soil conditions, it would be preferable to determine temporal patterns of community change over several years (at least three consecutive years) rather than comparing different systems at a fixed point in time. This highlights the need to conduct mid and long-term trials to assess the effects of cropping systems on soil biodiversity.

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Table 1. Crop rotation before 2009, crop management and yields (averaged over the last four years) for the period 2 and C/N ratios of the top 20 cm of soil and organic matter contents in the three cropping systems. Crop management for period 1 has been precisely described in Pelosi et al., 2009. “Living mulch” refers to the “direct seeded living mulch-based cropping system”.

Cropping system	Living mulch	Organic	Conventional
Crop rotation before 2009	Pea, wheat, maize, wheat	Oilseed rape, wheat, pea, wheat, alfalfa, wheat	Pea, wheat, oilseed rape, wheat
Crop rotation between 2010 and 2013	Wheat/alfalfa, alfalfa, alfalfa, wheat/alfalfa	Wheat, barley/pea, alfalfa, wheat	Pea, wheat, oilseed rape, wheat
Permanent plant cover	Alfalfa	No	No
Plowing (in autumn)	-	Each year	Each year, except after pea
Fertilisers	N, P, K, S	No	N, P, K, S
Pesticide use	Herbicides, fungicides, insecticides, molluscicides	No	Herbicides, growth regulator, fungicides, insecticides, molluscicides
Wheat yield (t ha <sup>-1</sup> )	5.9	7.0	9.8
C/N ratio	10.6	10.6	11.2
Organic matter (g kg <sup>-1</sup> )	17.3	16.5	21.7

Table 2. Monthly precipitation (mm) and soil temperatures (°C) at 10 cm depth, in Grignon (15 km further west from experimental plots; same type of soil), in September and October (i.e. the two months preceding earthworm samplings) and total precipitations during the six months before sampling.

	2005		2006		2007		2011		2012		2013	
	Sept.	Oct.										
Mean of minimal soil temperature (°C)	16.2	13.3	16.9	13.0	14.1	10.9	15.8	12.2	14.3	12.4	15.8	13.9
Mean of maximal soil temperature (°C)	20.6	16.2	21.5	15.8	17.2	13.1	18.9	14.5	17.8	13.6	17.6	14.8
Total of precipitation (mm)	24.5	33.0	24.8	39.8	10.2	27.2	41.0	16.0	49.5	75.5	90.5	40.5
Total of precipitation during the 6 months before sampling (mm)	217.5		239.4		346.8		258.5		363.0		384.0	

Table 3. Average abundance (number m<sup>-2</sup>) and standard deviation (between brackets) of earthworm species in the three cropping systems (living mulch cropping system, organic and conventional systems) in autumn 2005, 2006, 2007 (Pelosi et al. 2009a), 2011, 2012 and 2013. N=10. Abundance values in a column followed by the same letter are not significantly different at P = 0.05.

Species	Cropping system	Year sampling					
		Period 1			Period 2		
		2005	2006	2007	2011	2012	2013
<i>A. caliginosa</i>	Living Mulch	46.3 (30.1)	30.0 (16.1)	29.4 (19.3)	128.1 (99.5)	242.5 (128.3)	186.3 (57.0)
	Organic	53.1 (36.7)	98.8 (78.5)	41.3 (24.0)	115.6 (23.4)	268.1 (117.3)	186.3 (75.9)
	Conventional	69.4 (37.8)	120.0 (61.1)	50.0 (28.9)	100.0 (38.8)	111.9 (48.3)	105.0 (33.4)
<i>A. chlorotica</i>	Living Mulch	4.4 (9.8)	0.6 (2.0)	0.0 (0.0)	0.0 (0.0)	1.3 (4.0)	1.3 (4.0)
	Organic	15.6 (13.3)	45.0 (60.4)	14.4 (20.4)	3.8 (6.0)	69.4 (40.0)	60.6 (33.2)
	Conventional	11.9 (13.0)	28.1 (18.2)	31.3 (20.4)	26.3 (21.2)	42.5 (32.9)	46.9 (36.1)
<i>A. icterica</i>	Living Mulch	4.4 (8.4)	3.1 (4.4)	6.3 (5.9)	2.5 (4.4)	30.6 (17.3)	17.5 (15.0)
	Organic	2.5 (3.2)	0.6 (2.0)	11.9 (6.9)	13.8 (14.7)	26.3 (15.0)	21.3 (15.6)
	Conventional	5.0 (6.5)	15.0 (11.9)	5.6 (6.9)	0.0 (0.0)	51.9 (27.2)	9.4 (12.2)
Unidentified endogeics	Living Mulch	12.5 (22.8)	1.9 (3.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Organic	13.1 (16.5)	1.9 (4.2)	1.3 (4.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Conventional	19.4 (20.9)	6.3 (6.6)	29.4 (14.5)	0.0 (0.0)	2.5 (7.9)	0.6 (2.0)
<b>Total endogeics</b>	<b>Living Mulch</b>	<b>67.6 (56.4) a</b>	<b>35.6 (15.0) a</b>	<b>35.7 (19.1) a</b>	<b>130.6 (100.6) a</b>	<b>274.4 (135.1) ab</b>	<b>205.0 (65.3) ab</b>
	<b>Organic</b>	<b>84.4 (53.8) a</b>	<b>146.3 (136.7) b</b>	<b>68.9 (35.4) ab</b>	<b>133.2 (28.3) a</b>	<b>363.8 (157.4) b</b>	<b>268.1 (97.5) b</b>
	<b>Conventional</b>	<b>105.6 (55.7) a</b>	<b>169.4 (80.4) b</b>	<b>116.3 (50.5) b</b>	<b>126.3 (42.6) a</b>	<b>208.8 (82.8) a</b>	<b>161.3 (63.5) a</b>
<i>L. terrestris</i>	Living Mulch	48.8 (34.1)	32.5 (18.6)	26.3 (13.1)	30.0 (26.3)	36.3 (27.9)	49.4 (28.5)
	Organic	15.6 (15.9)	13.1 (11.9)	3.1 (3.3)	34.4 (27.2)	33.8 (41.1)	44.4 (22.9)
	Conventional	5.6 (7.5)	1.9 (3.0)	10.6 (9.3)	3.1 (5.3)	26.3 (21.0)	11.9 (13.0)
<i>A. giardi</i>	Living Mulch	34.4 (24.0)	12.5 (11.8)	19.4 (13.0)	16.9 (26.7)	15.6 (30.8)	20.6 (22.4)
	Organic	3.8 (5.3)	14.4 (10.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Conventional	4.4 (4.2)	19.4 (16.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
<i>A. longa</i>	Living Mulch	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	79.4 (53.0)	75.6 (49.7)	133.8 (30.4)
	Organic	0.0 (0.0)	0.0 (0.0)	1.9 (4.2)	42.5 (38.8)	14.4 (11.4)	50.0 (25.7)
	Conventional	0.0 (0.0)	0.0 (0.0)	6.3 (7.2)	5.0 (7.1)	26.9 (16.7)	15.6 (17.7)
<b>Total anecics</b>	<b>Living Mulch</b>	<b>83.2 (43.2) b</b>	<b>45.0 (21.2) b</b>	<b>45.7 (14.5) b</b>	<b>126.3 (85.2) b</b>	<b>128.1 (58.9) b</b>	<b>203.8 (55.0) c</b>
	<b>Organic</b>	<b>19.4 (19.9) a</b>	<b>27.5 (18.0) ab</b>	<b>5.0 (6.5) a</b>	<b>76.9 (39.8) b</b>	<b>48.1 (40.7) a</b>	<b>94.4 (34.3) b</b>
	<b>Conventional</b>	<b>10.0 (8.4) a</b>	<b>21.3 (7.0) a</b>	<b>16.9 (13.2) a</b>	<b>8.1 (7.8) a</b>	<b>53.1 (28.0) a</b>	<b>27.5 (23.4) a</b>
<i>L. castaneus</i> (Total epigeics)	<b>Living Mulch</b>	<b>13.8 (15.0) b</b>	<b>30.6 (31.8) b</b>	<b>5.0 (4.9) b</b>	<b>13.1 (10.8) a</b>	<b>39.4 (37.4) a</b>	<b>37.5 (20.8) b</b>
	<b>Organic</b>	<b>0.0 (0.0) a</b>	<b>15.0 (12.9) ab</b>	<b>0.6 (2.0) a</b>	<b>45.6 (30.2) b</b>	<b>162.5 (86.0) b</b>	<b>31.3 (20.6) b</b>
	<b>Conventional</b>	<b>1.9 (4.2) a</b>	<b>2.5 (4.4) a</b>	<b>2.5 (4.4) ab</b>	<b>23.1 (14.2) ab</b>	<b>31.3 (14.1) a</b>	<b>7.5 (10.1) a</b>

1 Table 4. Average biomass (g m<sup>-2</sup>) and standard deviation (between brackets) of earthworm  
 2 species in the three cropping systems (living mulch cropping system, organic and  
 3 conventional systems) in autumn 2005, 2006, 2007 (Pelosi et al. 2009a), 2011, 2012 and  
 4 2013. N=10. Biomass values in a column followed by the same letter are not significantly  
 5 different at P = 0.05.

Species	Cropping system	Year sampling					
		Period 1			Period 2		
		2005	2006	2007	2011	2012	2013
<i>A. caliginosa</i>	Living Mulch	8.3 (5.0)	8.0 (5.8)	6.0 (4.1)	32.1 (20.3)	49.0 (24.5)	40.3 (14.7)
	Organic	10.3 (7.1)	18.6 (12.4)	7.9 (4.5)	15.0 (6.5)	38.8 (10.2)	19.7 (6.2)
	Conventional	7.4 (4.0)	19.2 (9.8)	10.4 (5.5)	14.0 (5.1)	22.1 (13.6)	18.9 (7.9)
<i>A. chlorotica</i>	Living Mulch	0.4 (0.7)	0.1 (0.2)	0.0 (0.0)	0.0 (0.0)	0.1 (0.4)	0.2 (0.6)
	Organic	1.8 (1.6)	6.9 (8.8)	2.4 (4.0)	0.2 (0.4)	7.7 (5.0)	5.5 (3.5)
	Conventional	1.0 (1.1)	4.4 (2.9)	4.2 (2.6)	3.3 (2.4)	5.9 (4.3)	5.8 (5.1)
<i>A. icterica</i>	Living Mulch	1.8 (3.1)	1.7 (2.8)	1.9 (1.4)	1.1 (1.8)	8.0 (6.5)	3.5 (3.2)
	Organic	1.0 (1.4)	0.1 (0.4)	2.6 (1.6)	3.3 (3.4)	4.0 (4.3)	2.2 (2.0)
	Conventional	1.6 (2.3)	5.5 (4.9)	1.2 (2.0)	0.0 (0.0)	11.0 (6.1)	1.5 (2.3)
Unidentified endogeics	Living Mulch	1.8 (3.3)	0.2 (0.4)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Organic	1.4 (2.3)	0.1 (0.2)	0.1 (0.2)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Conventional	1.3 (1.9)	0.6 (0.6)	0.5 (0.6)	0.0 (0.0)	0.2 (0.8)	0.2 (0.6)
<b>Total endogeics</b>	<b>Living Mulch</b>	<b>12.2 (8.4) a</b>	<b>9.9 (6.1) a</b>	<b>7.9 (4.3) a</b>	<b>33.2 (21.2) a</b>	<b>57.2 (27.3) a</b>	<b>44.0 (16.3) b</b>
	<b>Organic</b>	<b>14.4 (8.7) a</b>	<b>25.7 (20.8) b</b>	<b>13.0 (5.7) ab</b>	<b>18.5 (7.0) a</b>	<b>50.5 (13.5) a</b>	<b>27.4 (6.8) a</b>
	<b>Conventional</b>	<b>11.4 (5.1) a</b>	<b>29.6 (14.9) b</b>	<b>16.5 (7.6) b</b>	<b>17.3 (4.9) a</b>	<b>39.2 (18.8) a</b>	<b>26.4 (10.7) a</b>
<i>L. terrestris</i>	Living Mulch	34.8 (18.0)	69.6 (44.8)	34.5 (22.9)	40.9 (28.9)	61.1 (46.2)	57.1 (25.3)
	Organic	19.9 (25.9)	14.7 (17.1)	5.4 (8.9)	22.6 (20.2)	31.7 (39.2)	25.4 (25.0)
	Conventional	3.3 (5.9)	1.6 (4.0)	11.8 (15.5)	1.4 (4.0)	15.5 (14.0)	10.5 (13.4)
<i>A. giardi</i>	Living Mulch	23.8 (17.5)	13.1 (11.7)	23.4 (25.2)	25.8 (38.2)	29.3 (60.3)	39.6 (44.5)
	Organic	4.7 (7.6)	11.7 (9.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Conventional	2.8 (5.6)	11.3 (10.6)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
<i>A. longa</i>	Living Mulch	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	67.8 (42.9)	87.3 (70.1)	116.4 (36.0)
	Organic	0.0 (0.0)	0.0 (0.0)	1.9 (4.0)	17.1 (15.6)	9.8 (8.4)	17.8 (11.6)
	Conventional	0.0 (0.0)	0.0 (0.0)	7.3 (8.8)	2.6 (4.5)	13.4 (10.2)	8.2 (9.1)
<b>Total anecics</b>	<b>Living Mulch</b>	<b>58.7 (19.3) b</b>	<b>82.7 (43.7) b</b>	<b>57.9 (30.7) c</b>	<b>134.5 (69.1) c</b>	<b>177.7 (63.5) b</b>	<b>213.1 (70.4) b</b>
	<b>Organic</b>	<b>24.6 (29.1) a</b>	<b>26.5 (19.1) a</b>	<b>7.3 (9.8) a</b>	<b>39.7 (19.3) b</b>	<b>41.5 (38.1) a</b>	<b>43.1 (24.0) a</b>
	<b>Conventional</b>	<b>6.1 (7.1) a</b>	<b>12.9 (11.5) a</b>	<b>19.1 (15.7) b</b>	<b>4.0 (5.4) a</b>	<b>28.9 (17.2) a</b>	<b>18.7 (17.2) a</b>
<i>L. castaneus</i> (Total epigeics)	Living Mulch	1.7 (1.7) b	5.2 (5.5) b	0.6 (0.6) b	1.9 (1.8) a	1.8 (2.2) a	5.5 (5.8) b
	Organic	0.0 (0.0) a	1.2 (1.0) ab	0.0 (0.0) a	3.0 (2.5) a	21.6 (11.2) b	1.3 (0.9) a
	Conventional	0.2 (0.3) a	0.3 (0.4) a	0.1 (0.1) a	1.8 (1.3) a	2.3 (1.2) a	0.5 (0.6) a

7

8

## Figure captions

Fig.1. Schematic representation of the experimental trial.

Fig.2. Total abundance (number  $m^{-2}$ ) of earthworms in the three cropping systems (“direct seeded living mulch-based cropping system”, called “Living mulch”, organic and conventional systems) in autumn in autumn 2005, 2006, 2007 (already published in Pelosi et al., 2009a), 2011, 2012 and 2013. N=10. Abundance values with the same letter are not significantly different at  $P = 0.05$  (one statistical analysis per year).

Fig.3. Total biomass ( $g m^{-2}$ ) of earthworms in the three cropping systems (“direct seeded living mulch-based cropping system”, called “Living mulch”, organic and conventional systems) in autumn in autumn 2005, 2006, 2007 (already published in Pelosi et al., 2009a), 2011, 2012 and 2013. N=10. Biomass values with the same letter are not significantly different at  $P = 0.05$  (one statistical analysis per year).

Fig.1.

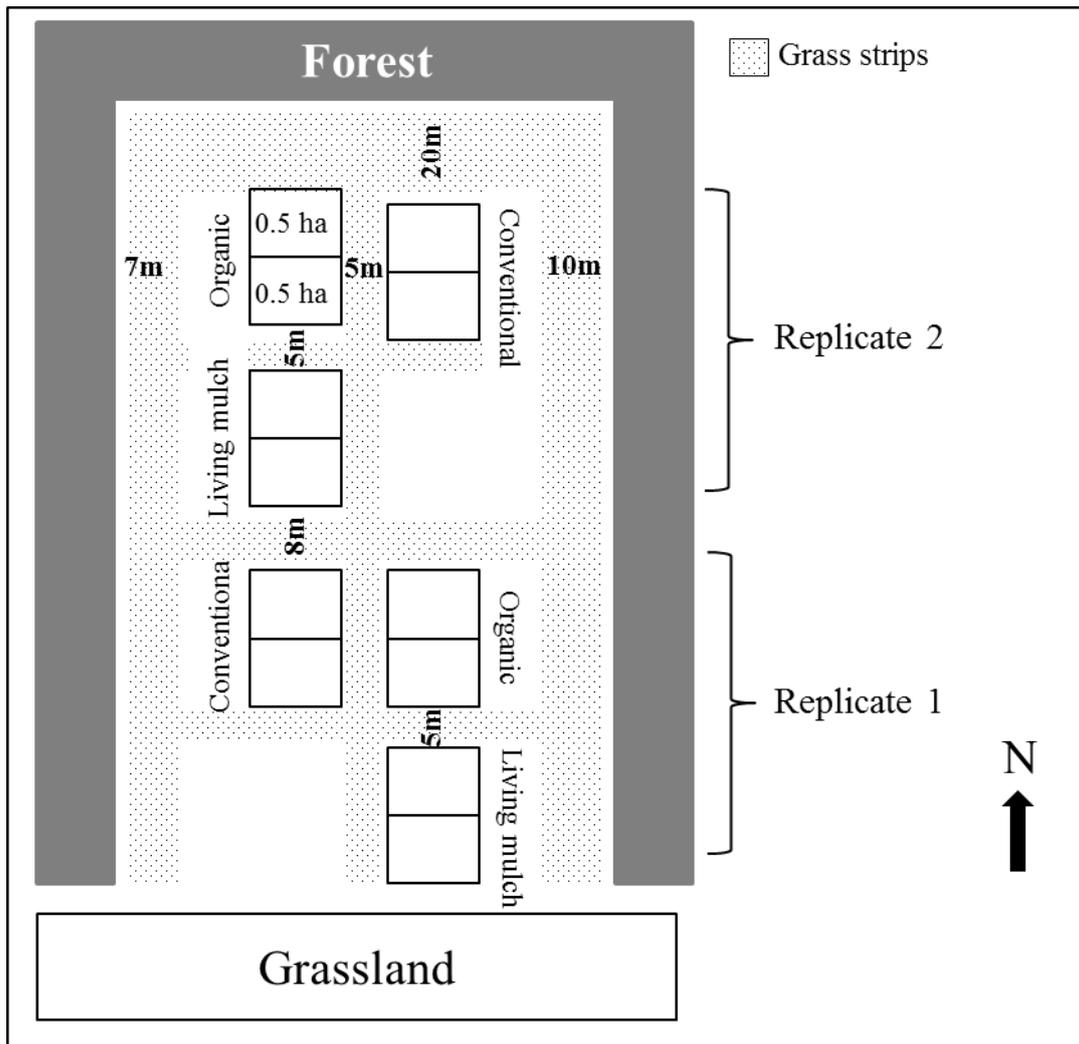


Fig.2.

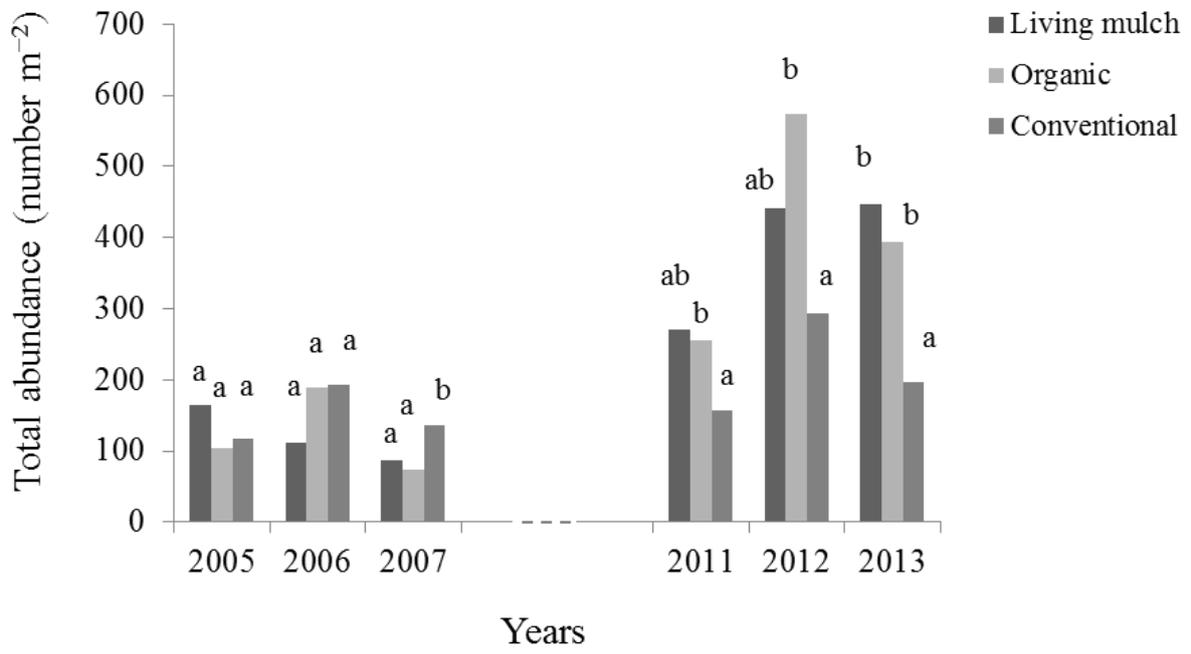


Fig.3.

