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Research article

Earthworm community in conventional, organic and direct seeding with living mulch cropping systems

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Abstract – The loss of biodiversity by intensification of agricultural practices is a major environmental issue that calls for the design of new cropping systems. For instance, negative effects of tillage on earthworm populations have been reported. However, few field studies have compared full cropping systems. Here, we assessed diversity, density and biomass of earthworm populations for 3 years. We use a combined method involving a diluted solution of allyl isothiocyanate to expel earthworms followed by hand sorting. In a long-term trial, we compared 3 systems: (1) a conventional system, (2) a direct seeding living mulch-based cropping system, named a living mulch cropping system, and (3) an organic system. These three cropping systems differed in terms of soil tillage, pesticide and nitrogen use, and crop biomass production. The results showed that measured variables, except diversity, varied depending on the year of sampling. Further, anecic and epigeic density was 3.2–7.2 times higher in the living mulch cropping system than in the conventional and organic systems. There were 3.4–12.5 times more anecic and epigeic earthworm biomass in the living mulch cropping system. The conventional and organic systems showed, respectively, 2.8 and 2.2 times more earthworm density, and 1.9 and 1.8 times more endogeic earthworm biomass than in the living mulch cropping system. Shannon-Wiener and equitability indices were superior in the living mulch cropping system compared with the conventional and organic systems. Cropping systems thus modified specific and functional diversity as well as earthworm community biomass. On the other hand, the organic and conventional systems did not differ in their earthworm density, biomass or diversity.

cropping system / earthworms / soil tillage / pesticide / organic farming / conventional / conservation agriculture

1. INTRODUCTION

Earthworms represent a large proportion of soil organism biomass and have important agro-ecological functions since they influence organic matter dynamics and soil structure (Edwards and Bohlen, 1996; Sims and Gerard, 1999). They are ecosystem engineers because they directly or indirectly influence the availability of resources to other species (Jones et al., 1994) and they are taken to be soil quality indicators (Paoletti et al., 1999). Distinct ecological groups can be recognised among the Lumbricidae. Bouché (1972) separated earthworms in France into three categories, based mainly on morphological and behavioural characteristics. Epigeic species, dark in colour and small, are litter dwellers and decomposers. Anecic earthworms, also called deep burrowing species, are large coloured earthworms living in permanent vertical burrows in soil but feeding from the surface litter. Endogeic species, or horizontal burrowers, are small to medium in size and not coloured. They live in temporary horizontal burrows and feed on the soil.

Under conventional agriculture, many of the beneficial effects of earthworms on soil structure and nutrient cycling are replaced by ploughing and fertiliser use, both of which being strongly dependent on external non-renewable energy inputs. With the increasing interest in alternative crop management systems such as organic farming or conservation agriculture, earthworms play a central role in the ecological functioning of agroecosystems (Chan, 2001).

Many studies have been carried out to assess the effects of different agricultural practices on earthworm populations (Lee, 1985; Edwards and Bohlen, 1996): e.g., soil tillage (review in Chan, 2001), pesticide use (Kula and Kokta, 1992) or organic and inorganic fertilisation (Whalen et al., 1998). The overall aim of this study was to carry out a field-based comparison of entire cropping systems. Three systems were compared: an organic system without manure or inorganic fertilisation, a "direct seeding living mulch-based cropping system" (living

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Table I. Soil characteristics of the experimental plots. "Living mulch" refers to the "direct seeding living mulch-based cropping system".

Cropping system	Conventional	Living mulch	Organic
Clay (g kg^{-1})	179.5	163.8	173.5
Silt (g kg^{-1})	629.8	531.0	604.5
Sand (g kg^{-1})	215.5	305.5	224.0
Organic matter (g kg^{-1})	17.3	21.7	16.5
$CaCO_3$ (g kg^{-1})	0.9	0.9	0.9
C/N ratio	10.6	11.2	10.6
pH	7.4	7.0	7.3
Rotation	Oilseed rape, wheat, pea, wheat	Pea, wheat, maize, wheat	Oilseed rape, wheat, pea, wheat, lucerne, wheat

mulch cropping system) involving no ploughing and a permanent living cover crop, and a conventional system.

Conservation tillage generally improves earthworm density and biomass and modifies species diversity and composition (review in Holland, 2004). Actually, the absence or the reduction of soil tillage as well as the high availability of organic matter on the soil surface is favourable to earthworms, which can live in an undisturbed biotope with abundant available food (Edwards and Bohlen, 1996; Chan, 2001). Studies conducted by Schmidt et al. (2001, 2003) in the United Kingdom and Ireland showed that more earthworm density, biomass and species number were found in direct-drilled wheat-clover intercropping systems than in ploughed wheat monocropping systems. In this study, we asked how density, biomass and diversity were modified when surface organic matter increases and mechanical disturbance is reduced in the living mulch cropping system. However, large amounts of pesticides such as herbicides, fungicides and molluscicides are applied in this cropping system in order to control weeds and pests, and their toxicity towards earthworms is, in most cases, not clear or unknown. The beneficial effects of no tillage and the presence of the living mulch may therefore be counterbalanced by the adverse effect of the pesticides.

The organic system was chosen because although it is recognised that earthworms play an important role in organic farming, the benefit of this cropping system for earthworm communities as well as the factors which positively influence earthworms are still debatable. Bengtsson et al. (2005) described the effects of organic agriculture on biodiversity and abundance using a meta-analysis. They explained that 12 of 13 studies found a significant positive effect of organic farming on earthworms. Nevertheless, Hole et al. (2005) reported more contrasted results. They reviewed that some authors found a positive effect of organic farming on earthworm populations compared with conventional systems. They explained that such differences are likely to result from the prohibition of pesticides (Pfiffner and Mäder, 1998) and primarily from the use of farmyard manures in organic systems, which provide an important food resource (Berry and Karlen, 1993; Pfiffner and Mäder, 1998). However, other authors found no significant difference (Nuutinen and Haukka, 1990; Foissner, 1992) or even a greater abundance and/or biomass in conventional systems (Czarnecki and Paprocki, 1998; Yeates et al., 1997). In this study, we set out to assess the impact of organic farming on earthworm communities and to resolve the ambiguity concerning the role of the prohibition of pesticides on earthworm populations by using no organic fertilisers. A long-term experiment was chosen for this study. Treatments were in place for eight years before our first measurements began because switching from conventional to alternative systems involves a critical transition period over several years during which the system moves towards a new state of equilibrium (Tebrügge and Düring, 1999).

2. MATERIALS AND METHODS

2.1. Site and cropping systems

Field data were collected each year between 2005 and 2007 in a long-term trial initiated in 1997 and located 15 km southwest of Paris (48°48' N, 2°08' E). According to the FAO classification, the soil is a deep Luvisol, with on average 58% of silt, 25% of sand and 17% of clay (Tab. I). This soil has a neutral pH and the climate at this site is temperate, with a mean annual precipitation of 640 mm and a mean annual temperature of 10.4 °C. Weather conditions during the three years of sampling are presented in Figure 1.

The three experimental cropping systems are a living mulch cropping system, a conventional system and an organic one. They differ mainly in soil tillage, pesticide and fertiliser applications (Tab. II), and crop biomass production. On average over the last five years, the yield of wheat was 5.6 t ha^{-1} in the organic farming, 9.3 t ha^{-1} in the conventional system and 6.1 t ha^{-1} in the living mulch cropping system. In the conventional system, the soil was ploughed in three out of the four years, the exception being after the leguminous crops. Weeds and pests were controlled with pesticides. In the living mulch cropping system, the permanent plant cover was planted simultaneously with the main crop and was controlled with herbicides. The organic cropping system used neither chemical inputs nor organic or inorganic fertilisers. In this system ploughing was done every year except after the leguminous crops, primarily for reasons of weed management. This system was conducted following the rules of the AB France label with an increase in crop diversity (including lucerne) in the rotation, compared with the other two systems. Weeds were also limited by soil tillage, weed smothering by crop density, and changes

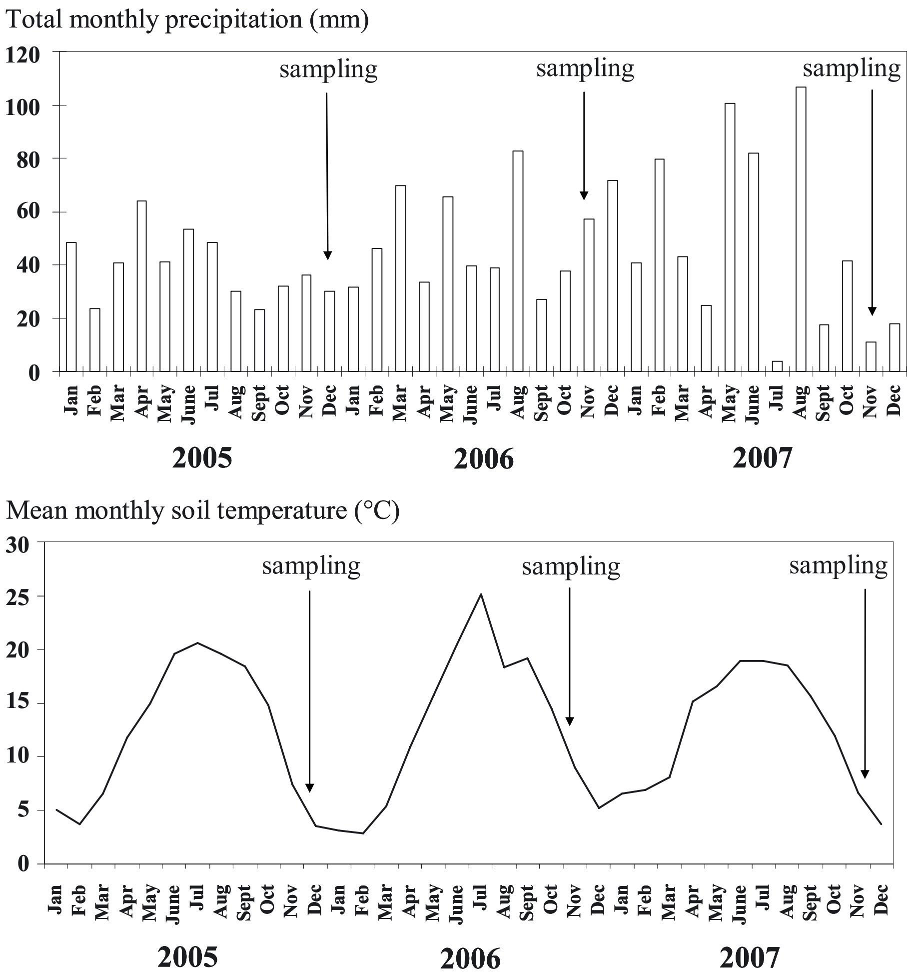

Figure 1. Monthly precipitation (mm) in Versailles, and soil temperature (°C) at 10 cm depth, in Grignon (15 km further west); same type of soil.

in crop sowing date. Nutrient exportations were limited by straw restitution. P and K levels were recorded throughout the experiment; however, because they were always high enough, no supplements were added.

The trial site was divided into six 1-ha plots, corresponding to two replicates of the three cropping systems. The plots were assigned randomly to treatments. Each replicate was divided into two subplots in which the rotation was established such that, for each year, one of the two subplots had a winter wheat crop. Crop management of the wheat crop in the three systems during the three years is shown in Table II.

2.2. Earthworm sampling method

Each year, at the end of autumn, which is known to be a period of great earthworm activity (Bouché, 1972), earthworm populations were sampled in the subplots involving the winter wheat crop. Five samplings, randomly located, were performed on each of the two replicates of each cropping system. A sampling method combining a chemical extraction and hand-sorting (Bouché, 1972) was used. Firstly, a diluted expellant solution of allyl isothiocyanate was applied to the soil within a 40 cm × 40 cm metal frame. Following Zaborski (2003) and Pelosi et al. (2008a), allyl isothiocyanate was first diluted with isopropanol (propan-2-ol) to obtain a 5 g L^{-1} solution. This solution was then diluted with water to reach a concentration of 0.1 g L^{-1}. After that, a 40 cm × 40 cm × 20 cm-deep block of soil was excavated and earthworms were extracted by hand and preserved in 4% formalin solution. They were weighed, counted and identified at the species level according to the identification key of Sims and Gerard (1999). The development stage of each earthworm was noted. Earthworms are considered sub-adult if they have a full tubercula pubertatis but no clitellum, and adult if they are clitellate (Sims and Gerard, 1999). They are considered juvenile if they have neither tubercula pubertatis nor clitellum.

2.3. Statistical analysis

Sample data were first transformed to record earthworm number and biomass per square metre. When the

Table II. Wheat crop management in the three systems during the three years of sampling. "Living mulch" refers to the "direct seeding living mulch-based cropping system".

Cropping system		Conventional			Living mulch			Organic		
Year		2005	2006	2007	2005	2006	2007	2005	2006	2007
Preceding crop		Oilseed rape	Pea	Pea	Maize	Pea	Wheat	Lucerne	Oilseed rape	Oilseed rape
Permanent plant cover		–	–	–	White clover	Bird's-foot trefoil	None	–	–	–
Fertlisers (kg ha^{-1})	N	181	181	208	152	151	157	0	0	0
	P_2O_5	0	75	0	0	50	0	0	0	0
	K_2O	0	75	0	0	50	0	0	0	0
Pesticides (number of treatments)	Herbicide*	2	2	3	2	3	4	0	0	0
	Fungicide**	3	2	2	1	0	1	0	0	0
	Molluscicide***	1	0	0	0	0	1	0	0	0
	Insecticide	0	0	0	0	0	0	0	0	0
Soil tillage (number of passages)	Crushing	2	0	1	0	1	0	0	1	1
	Ploughing (autumn)	1	0	0	0	0	0	0	1	1
	Stubble ploughing	0	3	2	0	0	0	0	3	4
	Harrowing	0	0	0	0	0	0	1	1	0
	Hoeing	0	0	0	0	0	0	0	1	1
	Rolling	0	0	0	0	0	1	0	0	0

* In the conventional system: clopyralid, 2,4-MCPA, diflufenicanil, chlortoluron, glyphosate, isoproturon, mesosulphuron- and iodosulphuron-methyl-sodium. In the living mulch cropping system: metsulphuron methyl, mesosulphuron- and iodosulphuron-methyl-sodium, glyphosate, clodinafop-propargyl, cloquintocet-mexyl, amidosulphuron, diflufenicanil, ioxynil bromoxynil, fluroxypyr.
** In the conventional and the living mulch cropping systems: epoxiconazole, fenpropimorphe, azoxystrobine, tebuconazole, bromuconazole.
*** In the living mulch cropping system: metaldehyde.

homoscedasticity of variance was not respected, data were transformed by $f(x) = \ln(x + 1)$. Data from the five samplings taken each year on each of the two replicates of each cropping system were pooled. The results were compared by ANOVA using the statistical program R (version 2.6.1, 2007). When treatments were significantly different by ANOVA, a Tukey test was used to determine which treatments were different. Treatment means were compared within years. They were also gathered over the three years to compare differences between cropping systems independently of the year. Lastly, the three treatment means were pooled by year to assess earthworm community differences in function of the year.

The Shannon-Wiener index was calculated as follows (Lacoste and Salanon, 2005):

$H' = -\sum_{i=1}^{S} p_i \log_2 p_i$, where $p_i = n_i/N$ is the proportional abundance of each species, and S is the total number of species. The Shannon-Wiener index is commonly used to characterise species diversity in a community. It accounts for both abundance and evenness of a species and can vary from 0.5 (low diversity) to 5 (high diversity) (Lacoste and Salanon, 2005).

An equitability index, also called evenness ($J' = H'/H_{\max}$, where $H'_{\max}$ is the $\log_2$ of the total number of species) was calculated (Lacoste and Salanon, 2005). This index can range from 0 to 1; it is minimum when a large proportion of the total community is represented by a small number of species.

3. RESULTS AND DISCUSSION

We undertook to assess diversity, density and biomass of earthworm populations once a year during three consecutive years in a long-term trial that compared a conventional system with a "direct seeding living mulch-based cropping system" (living mulch cropping system) and with an organic system without fertilisation.

3.1. Earthworm species and diversity

The same six species were found in each of the three cropping systems (Tab. III). This number of species is consistent with other studies in arable fields. In Switzerland, Pfiffner and Mäder (1998) found between five and nine species in a Luvisol under organic and conventional cultivation, depending on the period. However, Schmidt et al. (2001) found between one and five more earthworm species in British and Irish low-input wheat-clover intercropping systems than in conventional wheat monocropping ones. The living mulch cropping system of our study was home to the same number of species as the other two systems but the ecological groups were not represented in the same way. In the living mulch cropping system, anecics and epigeics represented 64% of total density, while in the conventional and organic systems more than 75% of the earthworms were endogeics, mainly *A. caliginosa* and *A. chlorotica*, which are among the common and dominant species in agricultural lands (Schmidt et al., 2001, 2003). Cropping systems thus modify functional composition of earthworms and, as a consequence, the effects it has on soil chemical and physical properties. These results are in agreement with Ivask et al. (2007), who explained that the specific composition of an earthworm community indicates the intensity of agricultural activity in the field.

There was no significant effect of year on the diversity indices. Shannon-Wiener and equitability indices were

Table III. Earthworm species found in the experimental trial under study and their contribution (in %) to the total density. "Living mulch" refers to the "direct seeding living mulch-based cropping system".

		Cropping system		
Ecological groups	Species	Living mulch	Organic	Conventional
Anecics	*Lumbricus terrestris*	31	9	6
	Aporrectodea giardi	19	5	7
Endogeics	*Aporrectodea caliginosa*	30	56	61
	Aporrectodea icterica	4	6	6
	Allolobophora chlorotica	1	20	19
Epigeics	*Lumbricus castaneus*	14	3	2

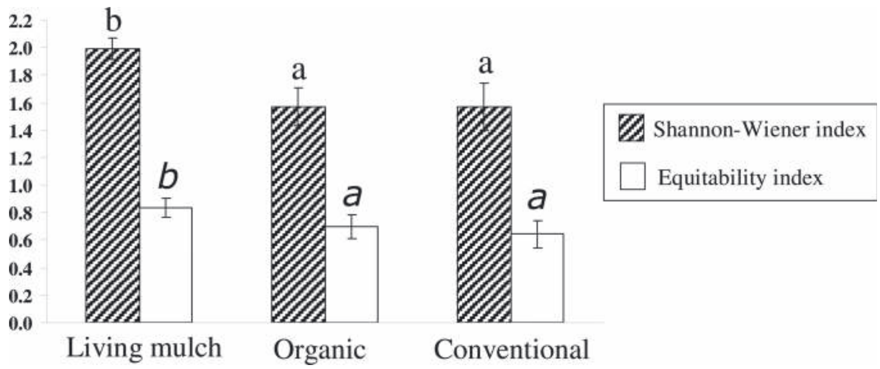

Figure 2. Shannon-Wiener and equitability indices of the three cropping systems ("direct seeding living mulch-based cropping system", named living mulch cropping system, organic and conventional systems). Histogram bars are means of 6 values, i.e., 2 replicates of each plot × 3 years. Bars with the same letter are not different at $P = 0.05$; one ANOVA per diversity index. Vertical lines are standard deviations.

significantly higher ($P < 7.6 \times 10^{-5}$ and $P < 3.7 \times 10^{-3}$, respectively) in the living mulch cropping system than in organic and conventional systems on average over six values, i.e., two replicates of each plot × three years, but they were similar in the organic and conventional systems (Fig. 2). The values found in the two latter systems are consistent with Jones et al. (2001), who noted a Shannon-Wiener index close to 1.5 in pasture fields and 1.66 in arable fields and an evenness between 0.54 and 0.95. Nevertheless, the high values of diversity indices in the living mulch cropping system attest to the greater functional biodiversity and the higher potential resilience of the system, which are necessary elements in the sustaining of agrosystems and in the provisioning of specific agrosystem services (Moonen and Bàrberi, 2008).

3.2. Density and biomass of the total community

Density and biomass of the total community varied according to the year (Tabs. IV, V). Total density was higher in 2006 than in 2007; 2005 results were not significantly different from those of 2006 and 2007. Total earthworm biomass was significantly higher in 2006 compared with 2005 and 2007. However, the ratios between the highest and the lowest total density and biomass for the average of the three systems were only 1.7 and 1.6, respectively (Tabs. IV, V). These year-to-year variations could be partly related to the higher rainfall and higher soil temperature in autumn 2006 (Fig. 1) since earthworm population dynamics vary greatly with soil temperature and water content (Edwards and Bohlen, 1996; Sims and Gerard, 1999; Pelosi et al., 2008b). Variability in earthworm density from year to year, which is occasionally reported (Whalen, 2004), is confirmed in our study.

Total earthworm density ranged from 74.4 individuals m^{-2} in the organic system in 2007 to 193.1 individuals m^{-2} in the conventional system in 2006 (Tab. IV). Total biomass ranged from 17.7 $g\,m^{-2}$ in the conventional system in 2005 to 97.8 $g\,m^{-2}$ in the living mulch cropping system in 2006 (Tab. V). These results were close to the mean of 194 individuals m^{-2} and 36 $g\,m^{-2}$ obtained by Schmidt et al. (2001) in conventional wheat monocropping systems. However, their wheat-clover intercropping systems had a mean abundance of 548 individuals m^{-2} and 137 $g\,m^{-2}$, which represents 4.5 and 1.7 times more density and biomass than in the living mulch cropping system under study here, respectively. The authors reported that these means were exceptionally high for arable systems and that they were more comparable with levels reported from pastures and other perennial grassland-type habitats in Ireland and Great Britain. Our results were thus in the same range as those found in other studies under temperate conditions.

The earthworm community was mainly represented by juveniles since they represented 58% in the living mulch cropping system, 61% in the organic system, and 69% in the conventional system, when averaged over the three years. These proportions are in the same range as Pfiffner and Mäder (1998) in organic and conventional systems in Switzerland, and as Schmidt et al. (2003) in conventional wheat and cereal-legume

Table IV. Density (number m^{-2}) of the total community and the three ecological groups in the three cropping systems ("direct seeding living mulch-based cropping system", named living mulch cropping system, organic and conventional systems) over three years. Abundance values in a column followed by the same letter are not significantly different at P = 0.05; small letters are used to compare density between cropping systems within a given year and capital letters are used to compare density between years, considering the three cropping systems.

			Ecological groups		
Year	Cropping system	TOTAL	Anecics	Endogeics	Epigeics
2005	Living Mulch	164.4 (a)	83.1 (b)	67.5 (a)	13.8 (b)
	Organic	103.8 (a)	19.4 (a)	84.4 (a)	0.0 (a)
	Conventional	117.5 (a)	10.0 (a)	105.6 (a)	1.9 (a)
	3 systems	128.6 (AB)	37.5 (A)	85.8 (A)	5.2 (A)
2006	Living Mulch	111.3 (a)	45.0 (b)	35.6 (a)	30.6 (b)
	Organic	188.8 (a)	27.5 (ab)	146.3 (b)	15.0 (ab)
	Conventional	193.1 (a)	21.3 (a)	169.4 (b)	2.5 (a)
	3 systems	164.4 (B)	31.3 (A)	117.1 (A)	16 (B)
2007	Living Mulch	86.3 (a)	45.6 (b)	35.6 (a)	5.0 (b)
	Organic	74.4 (a)	5.0 (a)	68.8 (ab)	0.6 (a)
	Conventional	135.6 (b)	16.9 (a)	116.3 (b)	2.5 (ab)
	3 systems	98.8 (A)	22.5 (A)	73.6 (A)	2.7 (A)
3 years	Living Mulch	120.7 (a)	57.9 (b)	46.2 (a)	16.5 (b)
	Organic	122.3 (a)	17.3 (a)	99.8 (b)	5.2 (a)
	Conventional	148.7 (a)	16.1 (a)	130.4 (b)	2.3 (a)

Table V. Biomass (g m^{-2}) of the total community and the three ecological groups in the three cropping systems ("direct seeding living mulch-based cropping system", named living mulch cropping system, organic and conventional systems) over three years. Biomass values in a column followed by the same letter are not significantly different at P = 0.05; small letters are used to compare biomass between cropping systems within a given year and capital letters are used to compare biomass between years, considering the three cropping systems.

			Ecological groups		
Year	Cropping system	TOTAL	Anecics	Endogeics	Epigeics
2005	Living Mulch	72.6 (b)	58.7 (b)	12.3 (a)	1.7 (b)
	Organic	39.0 (a)	24.6 (a)	14.4 (a)	0.0 (a)
	Conventional	17.7 (a)	6.1 (a)	11.4 (a)	0.2 (a)
	3 systems	43.1 (A)	29.8 (A)	12.7 (A)	0.6 (A)
2006	Living Mulch	97.8 (b)	82.7 (b)	9.9 (a)	5.2 (b)
	Organic	53.3 (ab)	26.5 (a)	25.7 (b)	1.2 (ab)
	Conventional	42.8 (a)	12.9 (a)	29.6 (b)	0.3 (a)
	3 systems	64.7 (B)	40.7 (A)	21.7 (B)	2.2 (B)
2007	Living Mulch	66.3 (b)	57.9 (c)	7.9 (a)	0.6 (b)
	Organic	20.3 (a)	7.3 (a)	13.0 (ab)	0.0 (a)
	Conventional	35.7 (ab)	19.1 (b)	16.5 (b)	0.1 (a)
	3 systems	40.8 (A)	28.1 (A)	12.5 (A)	0.2 (A)
3 years	Living Mulch	78.9 (b)	66.4 (b)	10.0 (a)	2.5 (b)
	Organic	37.6 (a)	19.5 (a)	17.7 (b)	0.4 (a)
	Conventional	32.1 (a)	12.7 (a)	19.2 (b)	0.2 (a)

intercropping systems in the United Kingdom. Cropping systems entailed much the same proportions of juveniles and subadults/adults and thus did not influence the repartition of the community into the different stages of development.

Cropping systems had generally no significant effect on total density, except in 2007, where the conventional system had a greater density than the other two systems (Tab. IV). On the contrary, cropping systems had an effect on total biomass (Tab. V) since it was higher in the living mulch cropping system than in the two others, whatever the year, although the differences were not always significant (Tab. V). Our results are in agreement with those of Schmidt et al. (2001, 2003) in terms of total biomass, but they found a lower total density in conventional systems compared with a direct-drilled wheat-clover intercropping system. In the same way, Chan (2001) and Ivask et al. (2007) reported higher earthworm densities under conservation or organic practices, respectively, than under conventional management. In our study, cropping systems did not modify total density but total biomass was higher in the living mulch cropping system than in the other two systems.

Comparison of organic and conventional systems did not reveal density, biomass or diversity differences in any year or ecological group, except in two cases, when density and biomass were higher in the conventional system. These results are not in accordance with the general trend found in the literature, which shows a positive effect of organic farming on earthworm populations compared with conventional systems (Scullion et al., 2002; Bengtsson et al., 2005; Riley et al., 2008). Pfiffner and Mäder (1998) found that biomass and abundance of earthworms were higher by a factor of 1.3 to 3.2 in the organic plots compared with the conventional plots. It is important to note some authors conclude that such results are not always associated with lower pesticide inputs since they are likely to result mainly from the use of farmyard manure in organic systems. Pulleman et al. (2003) admitted that they could not differentiate between the effects of manure inputs and the absence of pesticides. In our study, there was no organic manuring and the results are in agreement with some studies using manure in the same quantities in organic and conventional systems, which found no differences between the two systems (Nuutinen and Haukka, 1990). Some authors even found a lower abundance and/or biomass in organic farming than in the conventional system (Czarnecki and Paprocki, 1998; Yeates et al., 1997). These differences might result in heavily tilled organic fields supporting lower populations than conventional fields (Czarnecki and Paprocki, 1998). Another possibility is that more crop residues are returned to the soil in the conventional system since the yields are generally greater than in organic farming. Indeed, on average over the last five years, the yield of wheat in the conventional system was twice as high as in the organic system in the trial under study here. The organic matter would therefore be more available to earthworms in conventional systems. Moreover, if pesticides used in conventional systems are not harmful and reasonably applied, earthworm populations would remain at a high level. On the contrary, i.e., if they are harmful and/or applied at high rates, earthworm populations would decrease in conventional systems. For instance, Siegrist et al. (1998), who used a similar quantity of farmyard manure on biological and conventional plots, found no difference in biomass but a higher density and diversity in organic plots compared with conventional systems. They admitted that the pesticides used on conventional fields, i.e., Dinoseb, Carbendazim and certain dithiocarbamates, had negative effects on earthworms. These chemicals are not authorised in France since they are moderately to strongly toxic to earthworms (Lee, 1985; Högger and Ammon, 1994; Edwards and Bohlen, 1996). Herbicides tend to be less toxic for earthworms (Edwards and Bohlen, 1996), but can cause community reduction by decreasing sources of organic matter on which earthworms feed. Molluscicides and certain fungicides can be noxious for earthworms (Lee, 1985) but the pesticides used in our trial at agriculturally recommended rates appeared not to be harmful for earthworm populations. Actually, 67% of the pesticides used in the trial under study have a DL50, that is to say, the lethal dose for 50% of exposed individuals of *Eisenia fetida*, superior to 1000 mg kg^{-1} of soil (AFSSA, Agritox, 2005). These chemicals are thus not dangerous for earthworms. Almost all the others have a DL50 lower than 1000 but higher than 200 mg kg^{-1} of soil (AFSSA, Agritox, 2005) and/or are recognised as being nontoxic for earthworms (Lee, 1985; Dalby et al., 1995; Edwards and Bohlen, 1996; Iglesias et al., 2003). This is in agreement with other studies such as that of Tarrant et al. (1997), who compared a current farm practice which represents typical levels of pesticide use in the United Kingdom, and a reduced input system in which inputs were reduced by 50% and no insecticides were used. They found no difference in earthworm density, biomass or composition between the two systems and they concluded that the two pesticide regimes caused no ecologically significant differences in earthworm populations during their period of investigation. Mele and Carter (1999) reported that application of post-emergent herbicides for two consecutive years, at double recommended rates, was associated with significant increases in earthworm densities the following spring, compared with the recommended rate.

To conclude, our study revealed that the organic system without organic inputs did not provide better conditions for earthworms than a conventional system.

3.3. Density and biomass of the three ecological groups

Anecic earthworms were significantly more numerous in the living mulch cropping system than in the other two systems in 2005 and 2007 (Tab. IV). In 2006, density in the living mulch cropping system, significantly higher than in the conventional system, was 1.6 times higher than in the organic system, although this difference was not significant. After more than eight years of no tillage and the presence of a permanent cover crop, the living mulch cropping system had, on average over the three years, an anecic density 3.3 and 3.6 times higher than the organic and conventional systems, respectively (Tab. IV). Epigeics were more abundant in the living mulch cropping system than in the other two systems every year, but not significantly so in 2006 and 2007. Endogeics were significantly less numerous in the living mulch cropping system compared with the other two systems in 2006 and on average over the three years. In 2007, fewer endogeic earthworms were found in the living mulch cropping system than in the conventional system and their density in the living mulch cropping system was only half that in the organic system, although this difference was not significant. In 2005, density was 1.3 and 1.6 times lower in the living mulch cropping system than in the organic and conventional systems, respectively (Tab. IV). Thus, our study did not reveal differences in total density between systems because the high density of endogeics in the organic and conventional systems counterbalanced the higher density of anecics and epigeics in the living mulch cropping system.

Anecic and epigeic biomass was higher in the living mulch cropping system than in the two others, whatever the year, although the difference with the organic system was not significant in 2006 (Tab. V). On average over the three years, anecic and epigeic biomass was 3.4 and 6.3 times higher in the living mulch cropping system than in the organic system and 5.2 and 12.5 times higher than in the conventional one, respectively (Tab. V). In 2005, no differences in endogeic biomass were found between the three cropping systems while, in 2006, endogeic biomass was lower in the living mulch cropping system. In 2007, it was higher in the conventional system, followed by the organic system, and finally by the living mulch cropping system.

Anecic and epigeic earthworms, despite the numerous pesticide applications to control the cover crop and slug populations, may be favoured in the living mulch cropping system by the absence of soil tillage and the abundant surface organic matter (Tab. I) (Tebrügge and Düring, 1999; Chan, 2001). Actually, the permanent plant cover as well as the absence of organic matter incorporation into the soil profile allow more resource availability for anecic and epigeic earthworms in the living mulch cropping system. Edwards and Bohlen (1996) reported that the most important factor affecting the influence of cropping on earthworm populations is the proportion of the plant material that is returned to the soil. Moreover, pesticides, applied at recommended rates in the living mulch cropping system, were not harmful to earthworms (Lee, 1985; Edwards and Bohlen, 1996; AFSSA Agritox, 2005). Anecics and epigeics should be less abundant or even absent in ploughed fields because ploughing can affect earthworms directly through mechanical damage or exposure to predation as well as indirectly through consequent changes in soil environment, including destruction of burrows, loss of surface organic matter, and changes in soil physical conditions such as water content and temperature (Edwards and Bohlen, 1996; Chan, 2001). The endogeic density was higher in the organic system than in the living mulch cropping system and even more so in the conventional one. This ecological group seems favoured by ploughed plots in this trial, which is in agreement with other studies (Wyss and Glasstetter, 1992; Nuutinen, 1992). Endogeics, living in the top 20 centimetres of the soil, may profit from crop residues in the soil made available to them through incorporation (Chan, 2001).

In conclusion, the differences in ecological group composition account for the higher total biomass in the living mulch cropping system and portend different effects on the soil structure and organic matter mineralisation.

4. CONCLUSION

The sampling of earthworm communities for three consecutive years highlighted variations in earthworm total density and biomass from one year to another, although this temporal variability was not very significant. Comparison of three cropping systems showed that total densities were generally not significantly different, while the living mulch cropping system presented a higher biomass compared with the other two systems. The study of the distribution of ecological groups within the earthworm community revealed that more anecics and epigeics but less endogeics were found in the living mulch cropping system. These variations in ecological group distribution accounted for biomass differences between systems.

An increase in anecic density matched an absence of soil tillage and the presence of living mulch in the living mulch cropping system. This led to a higher biomass in that system. Conversely, in the organic system without organic manure, abandoning pesticide use did not favour earthworms, maybe because of the lower available trophic resources due to the lower yields.

Soil tillage and organic resource impact quantification thus appears primordial for the study and modelling of the effects of cropping systems on earthworm populations.

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