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Positive effects of alternative cropping systems on terrestrial Oligochaeta (Clitellata, Annelida)

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Abstract

Agricultural intensification has reduced soil biodiversity in cultivated fields. Alternative cropping systems have been implemented to limit the harmful environmental effects of intensive conventional systems. This study aims at (i) assessing the impact of alternative systems on abundance and biomass of earthworms and enchytraeids, which are involved in key agro-ecological functions, and (ii) investigating the relationship between these soil organisms, under field conditions. Field data were collected in spring 2014 in two long-term agricultural sites near Paris, France. In Site 1, two types of organic amendments i.e. animal manure (MAN) and composted sludge (SLU) were compared to a control system (CONT) without organic fertilization. In Site 2, four different cropping systems were compared: a conventional (CONV), an integrated (INT), an organic (ORG) and a living mulch-based cropping system (LIV). They differed mainly in soil tillage, pesticide and fertilizer use, crop rotation and crop biomass production. In both sites, higher earthworm abundance was found in the alternative systems except in the INT system i.e. SLU, MAN vs CONT and LIV, ORG vs CONV, even if differences were not always significant. For enchytraeids, we found no significant effect of organic amendments but a higher abundance in LIV and ORG systems than in CONV and INT systems in Site 2. Positive effects of alternative systems on earthworm and enchytraeid communities could be explained by the organic amendments in Site 1 and the permanent plant cover, the absence of pesticide use and tillage and the crop rotations in Site 2. Finally, no significant correlation was found between enchytraeid and earthworm abundance or biomass. We concluded that under environmental favorable conditions, earthworms and enchytraeids could coexist without competitive exclusion.

Keywords Enchytraeids | Potworms | Earthworms | Organic matter | Agricultural practices

1. Introduction

Nutrient cycling, soil structure, and other chemical, physical and biological soil properties and processes are substantially regulated by the activity of a highly diverse community of microbes and invertebrate animals (Lavelle et al. 2006, Ponge et al. 2013). It is generally acknowledged that farming may alter soil biodiversity, thus modifying these regulations (Altieri 1999, Ponge et al. 2013). Conventional agriculture has been particularly criticized for its dependency on external inputs and the associated environmental problems affecting soil ecosystems (Holland 2004, Stoate et al. 2001). Over the last decades, research efforts have focused on developing more sustainable agricultural practices to limit environmental damages such as pollution by nitrates or pesticides, the loss of organic matter and the decrease in biodiversity.

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Bioindicators are biological processes, species, or communities that are used to assess the quality of the environment and how it changes over time (Holt & Miller 2011). Among terrestrial Oligochaeta, the use of earthworms as bioindicators of cultivated soils is largely documented (Daugbjerg et al. 1988, Paoletti 1999) but enchytraeids have been more recently recognized as indicators of soil quality (Graefe & Beylich 2002, Jänsch et al. 2005) and chemical stress in terrestrial ecosystems (Didden & Römbke 2001). Functional roles of enchytraeids and earthworms are quite similar, but at different scales (Didden et al. 1994, Van Vliet et al. 1993). Enchytraeids, being part of soil mesofauna (organisms with a body size or body diameter for worms between 100 µm and 2 mm), are involved in the evolution of soil structure (Didden 1990, Graefe & Beylich 2002, Langmaack et al. 2001, Van Vliet et al. 1993, 1995), thus increasing pore volume and water infiltration (Linden et al. 1994). They are also involved in the decomposition of plant residues (Golebiowska & Ryszkowski 1977, Hendrix et al. 1986) and in the mixing and transport of mineral and organic soil particles due to their burrowing activity, fecal pellet production, and feeding habit i.e. uptake of both organic food and inorganic particles. Earthworms, belonging to macrofauna (body size or body diameter for worms between 2 and 20 mm), represent a large proportion of soil living biomass and play important agro-ecological functions (Edwards & Bohlen 1996, Sims & Gerard 1999). They mechanically break up organic matter and mix it with mineral matter, thus homogenizing the distribution of nutrients in the soil matrix and making them more available for plant roots and bacteria (Blouin et al. 2013, Syers & Springett 1983). They are also involved in the stabilization of soil aggregates and the general improvement of soil physical condition (Pérès et al. 2010, Zhang & Schrader 1993). Furthermore, apart from their action on soil structure, earthworms also have important effects on the chemical and biological fertility of soils (Blouin et al. 2013, Edwards & Bohlen 1996, Lee 1985).

According to some authors, intensive tillage and pesticide use generally reduce earthworm abundances and biomasses and modify species diversity and composition (Holland 2004, Pelosi et al. 2013). Conversely, reduced tillage and use of organic fertilization can have positive effects on earthworm communities (Röhrig et al. 1998). For enchytraeids, for which the effects of alternative farming systems have been assessed to a lesser extent than for earthworms, Van Capelle et al. (2012) argued that 'in contrast to earthworm-induced processes, the preservation of enchytraeid-driven processes depends on a minimum of soil loosening action'. Some authors suggested that, in conventionally cultivated arable lands, enchytraeids are even of greater importance than earthworms because they are more active metabolically and less sensitive to ploughing (Hendrix et al. 1986, Nowak 2004). Earthworms and enchytraeids could thus be differently influenced by alternative agricultural practices and cropping systems. Unfortunately, this has been rarely studied for enchytraeids and very few studies focus specifically and simultaneously on these two groups of terrestrial annelids. Despite the proven importance of these two keystone soil organisms, the relationships between them are not well understood (Karaban & Uvarov 2014). A negative effect of earthworms on enchytraeids has repeatedly been documented (Huhta & Viberg 1999, Räty 2004, Räty & Huhta, 2003, Tao et al. 2011). For instance, Schlaghamerský et al. (2014) found that earthworm invasion altered enchytraeid community composition and individual biomass in northern hardwood forests of North America. Conversely, a reduction of earthworm abundance in presence of enchytraeids has also been recorded (Haukka 1987, O'Connor 1967, Sandor & Schrader 2012). Neutral interactions (Makulek & Pilipiuk 2000) or positive effects of earthworms on enchytraeid abundances due to the production of casts have also been shown (Schrader & Seibel 2001). Unfortunately, most of these experiments were conducted under laboratory conditions or had not been conducted in cultivated systems. Topoliantz et al. (2000) showed an opposite distribution of earthworm and enchytraeid biogenic structures according to agricultural practices, estimated from micromorphological data. More information is thus needed on the effects of alternative farming systems on terrestrial annelids as well as on the interactions between earthworm and enchytraeid communities in cultivated fields.

The aim of this preliminary study was to carry out a field-based comparison of earthworm and enchytraeid populations in order to (i) understand if they respond similarly to different agricultural practices i.e. organic fertilization, and cropping systems i.e. conventional, organic, integrated and living mulch cropping systems, and (ii) to investigate the relationship between these soil organisms under field conditions.

2. Materials and methods

2.1. Sites and cropping systems

Field data were collected in March–April 2014 in two long-term trials near Paris, France, 12 km far away from each other. The climate in both study areas is oceanic temperate with a mean annual precipitation of 630 mm and a mean annual temperature of 10–11°C.

Site 1 (Tab. 1), located 33 km North-West of Paris (48°52’ N, 1°58’ E) and initiated in 1998 (QualiAgro experiment, INRA-VERI partnership), was situated in a wide cereal plain. It is a long-term investigation site where physico-chemical, agronomic and biological data are regularly measured (Annabi et al. 2011, Cambier et al. 2014). This site was sampled at the end of March 2014. Cumulated precipitations during the two months preceding sampling were 119mm. The trial tested the effects of repeated amendments of organic waste products under optimal mineral fertilization and conventional tillage i.e. ploughing every year. Plots with a farmyard manure (MAN) or a compost containing sludge from a wastewater treatment plant (joint composting of sludge from urban wastewater treatment with green waste) (SLU) were compared to control plots (CONT), without organic fertilization. Four replicates of 450 m² each (45 m long and 10 m wide) were studied for each of the three systems. Apart from organic amendments, all agricultural practices were identical between the three systems. The site is cultivated with a wheat-maize rotation. Only maize residues are restituted in soils and wheat straw is exported. Spreading of organic amendments was carried out every two years on the wheat stubble, with compost and manure doses calculated so as to input 4 tons of organic carbon ha^{-1}. The last application before our experiment was done in September 2013.

Site 2 (Tab. 1), located 15 km South-West of Paris (48°48’N, 2°08’ E) and initiated in 1997, was situated close to grass strips and forest of Versailles palace garden. It is also a long-term investigation site where physico-chemical, agronomic and biological data are regularly acquired (Debaeke et al. 2009, Henneron et al. 2015, Juarez et al. 2013, Pelosi et al. 2009b, 2015). It was sampled at the end of April 2014. Cumulated precipitations during the two months preceding sampling were 46.5 mm. It compared four different cropping systems, replicated twice (0.5 ha each) and differing mainly in soil tillage, pesticide and fertilizer use, crop rotation, and crop yield. In the conventional system (CONV), the soil had been ploughed three out of four consecutive years, the exception being after a leguminous crop. Weeds and pests were controlled mainly with herbicides and fungicides, insecticides and molluscicides being more rarely applied. In the organic system (ORG), no chemical inputs or fertilizers (organic or inorganic) were used. In this system, the soil had been ploughed each year except after a leguminous crop. The integrated system (INT) was characterized by a significant reduction of chemical input and a ploughing every two years. Finally, the direct seeding living mulch-based cropping system, named ‘living mulch cropping system’ or LIV, was a no-tilled system with a permanent plant cover sown simultaneously with the main crop and controlled with herbicides.

Soil texture, pH, organic matter content, crop rotation and date of the last ploughing in the different systems in the two sites are shown in Tab. 1. For soil moisture, three soil cores per plot were sampled using a 5 cm Ø soil auger, at 0–10 cm and 10–20 cm depth. Soil samples were fresh weighted placed in a stove for 72h at 105°C and dry weighted.

Table 1. Soil characteristics, crop rotations and date of the last ploughing in the experimental sites. In Site 1, MAN refers to plots with farmyard manure, SLU to plots with a compost containing sludge from a wastewater treatment plant, and CONT to control plots, without organic fertilization. In Site 2, CONV refers to conventional system, ORG to organic system, LIV to living mulch cropping system and INT to integrated system.

Cropping system	Site 1			Site 2			
	CONT	SLU	MAN	CONV	ORG	LIV	INT
Clay (g kg^{-1})	151.5	140.5	145.5	184.5	170.5	155.5	167.0
Silt (g kg^{-1})	781.5	790.8	782.5	599.5	598.0	518.0	546.0
Sand (g kg^{-1})	67.0	68.8	72.0	216.0	231.0	326.5	273.5
Organic matter (g kg^{-1})	17.9	26.8	24.7	18.4	17.4	21.1	18.6
CaCO3 (g kg^{-1})	< 1	< 1	< 1	< 1	< 1	< 1	< 1
C/N ratio	10.5	10.4	10.8	10.3	10.2	10.5	10.2
pH	6.6	6.8	7.2	7.5	7.1	7.2	7.3
Crop rotation	Wheat, maize			Oilseed rape, wheat, pea	Wheat,wheat, lucerne, lucerne	Wheat, lucerne, wheat+lucerne	Oilseed rape, wheat, pea
Date of the last ploughing	December 2012			October 2012	October 2013	-	October 2012

2.2. Earthworms

Three samplings, randomly located, were done once in spring 2014 on each of eight plots (four systems × two replicates) in Site 1 and twelve plots (three systems × four replicates) in Site 2. Earthworm sampling was carried out using a combination of chemical and hand-sorting extraction (Pelosi et al. 2009a). After removing the vegetation from the ground surface, two applications of 3.2 l of a diluted expellant solution of allyl isothiocyanate (AITC) were performed at 10-min intervals within a 40 cm × 40 cm metal frame. AITC was first diluted with isopropanol (propan-2-ol) to obtain a 5 g l^{-1} solution (Pelosi et al. 2009a, Zaborski 2003). This solution was then diluted with water to reach a concentration of 0.1 g l^{-1}. After collecting individuals from the soil surface during 20 min, a 40 cm × 40 cm × 20 cm-depth block of soil was excavated. Remaining earthworms were hand-sorted from this block. Earthworms were preserved in a 4% formalin solution. All individuals were weighed without emptying their gut content, identified at species level according to Sims & Gerard (1999) identification key and counted.

2.3. Enchytraeids

Four samplings, randomly located, were done once in spring 2014 on each of eight plots (four systems × two replicates) in Site 1 and twelve plots (three systems × four replicates) in Site 2. Sampling was conducted based on the ISO 23611-3 (2007), with a 5 cm Ø soil auger, at 0–10 cm and 10–20 cm depth because most enchytraeids generally stay in the uppermost soil layers, but in cropping systems they can be redistributed in depth by soil tillage (Röhrig et al. 1998, Van Vliet et al. 1995). Samples were transferred to the laboratory in plastic bags, weighed and kept at 4°C for a maximum of 15 days. Enchytraeids were extracted from soil using a modified wet-funnel method (O'Connor 1962). Each sample was placed on a screen fabric into a funnel connected to a plastic tube. They were covered with water and placed under a heat source (40 watt bulbs) for five hours. After two hours, sediments containing enchytraeids were collected from the tube and filtered through a fine sieve (mesh size 50 µm). The sieve content was then placed in a petri dish. A second collection was carried out after three more hours. To estimate abundances, enchytraeids were counted alive under a binocular microscope. All individuals were photographed. Because weighing individual enchytraeids was difficult, individual fresh biomasses were indirectly estimated from the volume of each individual multiplied by a factor of 1.051 (Phillipson et al. 1979). For volume calculation, enchytraeids were considered as cylinders. Individual lengths were measured from the pictures using the software Image J (Schneider et al. 2012). Because some very small individuals were lost during manipulations (less than 5%), a length of 0.5 mm was attributed to individuals that could not be measured on pictures. As diameter did not vary importantly between individuals, a mean diameter value was obtained by measuring the body diameter of approximately 300 individuals per system, leading to an average value of 0.119 mm. Individuals of the different systems were identified at the genus level but only after this sampling event, in September 2014.

2.4. Statistical analysis

Sample data were first converted to abundances and biomasses per square meter. In Site 1, given that the trial has only two replicates, we considered the three (for earthworms) or four (for enchytraeids) 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, 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. 2009b, 2015, Henneron et al. 2015).

When normality and homoscedasticity of variances were not respected, data were transformed by $f(x) = \ln(x + 1)$. After that, if conditions were satisfied, ANOVA and Tukey tests were used to ascertain differences between agricultural systems in each of the two sites and between practices in Site 2. When data did not satisfy the conditions, non-parametric tests were used, i.e. Kruskal-Wallis tests and post-hoc comparisons between groups.

In Site 2, in addition to the comparison of earthworm and enchytraeid communities between systems, we considered that the four cropping systems differed mainly by three agricultural practices i.e. soil tillage, pesticide application and permanent cover crop. We know that other agricultural practices such as fertilization or crop rotation might influence Oligochaeta communities and that it was difficult to draw conclusions on the effect of agricultural practices independently of each other when studying entire cropping systems. However, we wanted to test these effects, keeping in mind the limits of this approach. For that purpose, the following rules were used (Tab. 2): soil tillage was considered of 'high' intensity if it was done each year except after a leguminous crop, as in CONV and ORG systems. It was considered of 'medium' intensity in the INT system which was ploughed every two years. Finally, it was considered 'low' in the no-tilled LIV system. Pesticide application was considered as 'high' in CONV and LIV systems in which weeds and pests were controlled mainly with herbicides and fungicides, 'medium' in the INT system in which the use of chemical inputs (also mainly herbicides and fungicides) was significantly reduced compared to CONV and LIV systems, and 'low' in the ORG system in which no pesticides were used. Finally, the permanent plant cover was present in the LIV system and only partially in the ORG system (two years of alfalfa in the crop rotation) but absent in the two others.

A rank correlation (Spearman's coefficient) test was used to study correlations between variables and notably between the abundance and biomass of earthworms and enchytraeids on the one hand and environmental variables known to control their populations (soil moisture and soil organic matter content) on the other hand (R Development Core Team 2011).

3. Results

3.1. Earthworms

In Site 1, only three species were found in the three systems: one anecic species i.e. *Lumbricus terrestris*, and two endogeic species i.e. *Aporrectodea caliginosa* and *Allolobophora rosea*. Total earthworm abundance was significantly higher in the system amended with composted sludge (SLU) than in the CONT system (Tab. 3). Abundance in the MAN system was close to that in the SLU system. While abundance was more than two times higher in the MAN than in the CONT systems, the difference was not significant. A significant and positive correlation between earthworm abundance and soil organic matter content was found ($R = 0.81$; $p < 0.01$; $n = 12$). Total earthworm biomass was not significantly different between the three systems but it was higher in the system amended with farmyard manure (MAN) than in SLU and CONT systems (Tab. 3). The average individual biomass in the MAN and the CONT systems (0.44 and 0.48 g wet weight, respectively) was more than twice as high as in the SLU system (0.21 g wet weight).

In Site 2, seven species were found, with five common to the four systems, one anecic i.e. *L. terrestris*, three endogeics i.e. *A. caliginosa*, *A. rosea* and *Aporrectodea icterica,* and one epigeic i.e. *Lumbricus castaneus*. The anecic species *Aporrectodea longa* was not found in the organic system and the endogeic *Allolobophora chlorotica* was found only in CONV and ORG systems. In LIV and ORG systems, earthworms were 1.8 and 2.2 times more abundant than in CONV and INT systems, respectively, but the differences were not significant (Tab. 3). Total biomass was significantly higher in the LIV system than in the other three systems. Biomass appeared to be positively influenced by a low intensity of soil tillage and a permanent plant cover (Tab. 4). A temporary plant cover such as in the ORG system, or a reduction in the tillage intensity such as in the INT system did not induce a significant increase in the earthworm biomass (Tab. 4). The average individual biomass in the ORG system (0.12 g wet weight) was 2.7 times lower than in the LIV system (0.32 g wet weight). In the CONV and INT systems, average wet weights of 0.17 and 0.25 g, respectively, were measured.

Comparing both experimental sites, total earthworm abundance was 42 % lower in Site 1 (114 ind. m^{-2} on average over the three systems) than in Site 2 (196 ind. m^{-2} on average over the four systems) but biomass was similar, close to 40 g m^{-2} (Tab. 3).

3.2. Enchytraeids

We found no correlation between soil sample fresh weight and enchytraeid abundance. Moreover, no significant correlation was found between enchytraeid and earthworm abundance ($R = -0.14$) or biomass ($R = 0.26$) ($n = 20$).

In Site 1, four genera were found in all three systems: *Fridericia, Enchytraeus, Henlea* and *Achaeta*, with a majority of *Fridericia*, then *Enchytraeus,* and more sporadically *Henlea* and *Achaeta*. The genus *Mesenchytraeus* was found only in the CONT system, but scarcely. Total enchytraeid abundance and biomass were not significantly different between the three systems (Tab. 3).

In Site 2, the same four genera as in Site 1 were found in the four systems, and in the same proportion: a majority of *Fridericia*, then *Enchytraeus,* and more sporadically *Henlea* and *Achaeta*. A significant effect of the cropping system was found on enchytraeid abundance, which was higher in the LIV system than in CONV and INT systems. Abundance in the ORG system was 2 and 3.2 times higher than in CONV and INT systems, respectively, but differences were not statistically significant. Enchytraeid

Table 2. Rules used to test the effect of agricultural practices in the Site 2: soil tillage, pesticide application and permanent cover crop. For cropping systems, CONV, ORG, LIV and INT mean conventional, organic, living-mulch and integrated system, respectively.

Cropping systems	Intensity of soil tillage	Intensity of pesticide application	Permanent plant cover
CONV	High	High	No
ORG	High	Low	Partial
LIV	Low	High	Yes
INT	Medium	Medium	No

Table 3. Abundance (number m^{-2}) and biomass (g m^{-2}) of the total community of earthworms and enchytraeids in the experimental sites. In Site 1, MAN refers to plots with farmyard manure, SLU to plots with a compost containing sludge from a wastewater treatment plant, and CONT to control plots, without organic fertilization. In Site 2, CONV refers to conventional system, ORG to organic system, LIV to living mulch cropping system and INT to integrated system. Abundance and biomass values in a column followed by the same letter are not significantly different at P = 0.05. One statistical analysis per site.

	Earthworms		**Enchytraeids**	
	Abundance (individuals m^{-2})	Biomass (g m^{-2})	Abundance (individuals m^{-2})	Biomass (g m^{-2})
Site 1				
CONT	61.9 (a)	29.5 (a)	22 536 (a)	4.52 (a)
SLU	147.4 (b)	31.3 (a)	19 576 (a)	4.95 (a)
MAN	133.3 (ab)	58.5 (a)	27 438 (a)	3.93 (a)
Site 2				
CONV	142.7 (a)	24.1 (a)	4 380 (a)	0.68 (a)
ORG	261.5 (a)	30.4 (a)	9 015 (ab)	1.89 (a)
LIV	261.5 (a)	83.0 (b)	14 821 (b)	2.34 (a)
INT	116.7 (a)	29.4 (a)	2 903 (a)	0.39 (a)

Table 4. Effects of soil tillage, pesticide application and permanent cover crop on abundance (number m^{-2}) and biomass (g m^{-2}) of earthworms and enchytraeids in Site 2. Abundance and biomass values in a column followed by the same letter are not significantly different at P = 0.05. One statistical analysis per agricultural practice.

Cultural practices	**Earthworms**		**Enchytraeids**	
	Abundance (individuals m^{-2})	Biomass (g m^{-2})	Abundance (individuals $m^{-}2$)	Biomass (g m^{-2})
Soil tillage				
Low	261.5 (a)	83.0 (b)	14 821 (b)	2.34 (a)
Medium	116.7 (a)	29.4 (a)	2 903 (a)	0.39 (a)
High	202.1 (a)	27.2 (a)	6 697 (ab)	1.29 (a)
Pesticides application				
Low	261.5 (a)	30.4 (a)	9 015 (a)	1.89 (a)
Medium	116.7 (a)	29.4 (a)	2 903 (a)	0.39 (a)
High	202.1 (a)	53.5 (a)	9 600 (a)	1.51 (a)
Permanent plant cover				
Yes	261.5 (a)	83.0 (b)	14 821 (b)	2.34 (b)
Partial	261.5 (a)	30.4 (a)	9 015 (ab)	1.89 (ab)
No	129.7 (a)	26.7 (a)	3 642 (a)	0.53 (a)

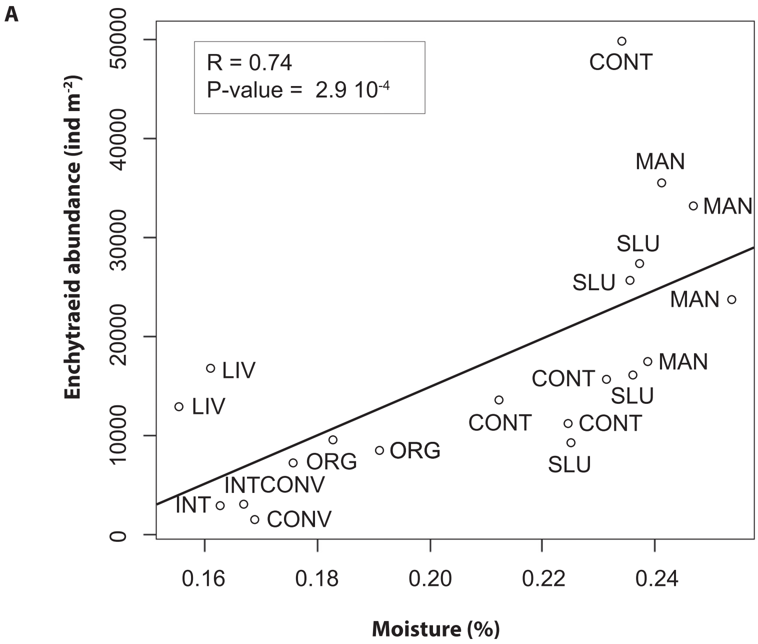

Figure 1. (**A**) Enchytraeid abundance (individuals m^{-2}) and (**B**) enchytraeid biomass (mg m^{-2}) in function of soil moisture (in %).

abundance and biomass were higher in the presence of a permanent plant cover than without it and were intermediate with a temporary one i.e. in the ORG system (Tab. 4). Enchytraeid abundance was significantly higher with no-tillage (LIV) than with reduced tillage (INT) but no significant difference was found between 'high' (ORG and CONV) and 'low' (LIV) or 'medium' (INT) intensity of soil tillage.

Comparing both experimental sites, enchytraeids were more abundant in Site 1 than in Site 2. The average total abundance in Site 2 (7,779 ind. m^{-2} on average over the four systems) was 3 times lower than in Site 1 (23,184 ind. m^{-2} on average over the three systems). Similarly, the average enchytraeid biomass was 4.5 g m^{-2} in Site 1 and 1.3 g m^{-2} in Site 2. It was thus 2.5 times higher in Site 1 than in Site 2. Significant correlations were found between soil moisture and enchytraeid abundance ($R = 0.74$; $p < 0.01$) (Fig. 1A) and biomass ($R = 0.68$; $p < 0.01$) (Fig. 1B) using data of both sites ($n = 20$).

Most of the enchytraeids (between 68 and 78 %) were extracted from the horizon 10–20 cm in the three systems in Site 1 i.e SLU, MAN, CONT and the ORG system in

Site 2, that were ploughed 6 months before. Conversely, 90 % of the individuals were extracted from the horizon 0–10 cm in LIV, CONV and INT systems that were last ploughed at least one year before sampling.

4. Discussion

4.1. Effect of alternative systems on earthworm and enchytraeid communities

Only three species of earthworms were found in Site 1. This low diversity could be explained by the intensive agricultural practices i.e. conventional ploughing, use of pesticides, crop rotation with two cereal crops, and by the location of the site within a wide cereal plain without nearby landscape elements nearby such as hedges, meadows or forests. On the contrary, the higher diversity found in Site 2 (7 species) could be due to the higher diversity of agricultural practices in the four cropping systems and the longer crop rotations. The four genera of enchytraeids found in both sites are relatively common in agricultural systems (Beylich & Graefe 2009, Postma-Blaauw et al. 2012).

In Site 1, organic amendments caused variations in soil organic matter content compared to the system without organic fertilization. This probably explains the higher abundance of earthworms in the amended systems, considering the positive correlation between soil organic matter content and earthworm abundance that has been found. Chan (2001) and Lee (1985) explained that among all possible adverse effects of cultivation on earthworms, the most important may be the loss of surface litter and the consequent decline in soil organic matter that leads to a reduction in food resources. Our results highlighted that earthworm populations can grow with increasing soil organic matter content, even in the absence of litter. Differences in earthworm individual biomass between the two amended systems i.e. MAN and SLU, suggest that the type of amendment influenced the species composition. Indeed, the big-sized anecic *L. terrestris* was more affected than the two endogeic species by the type of organic amendment. We found respectively 2 and 3 times more abundance and biomass of *L. terrestris* in MAN than in SLU systems (data not shown). The amendment quality could thus partially explain the difference in earthworm biomass between plots in our study. Conversely, we found no effect of these organic amendments on enchytraeid abundance and biomass. Nevertheless, enchytraeids feed directly on decomposing organic matter or indirectly by utilizing the casts of other edaphic animals, e.g., Lumbricidae or Collembola. Mitchell et al. (1978) and Andrén & Lagerlöf (1983) found that enchytraeid populations increased in plots amended with sludge and farmyard manure, respectively. We would have thus expected more enchytraeids in the amended plots.

In Site 2, earthworm and enchytraeid abundances were consistently higher in LIV and ORG than in CONV and INT systems, even if differences were not always significant. The earthworm species composition in LIV and ORG systems was not the same since small species were found in the ORG system and large species were sampled in the LIV system (data not shown). Large earthworms such as anecics generally benefit from the permanent plant cover and the absence of ploughing (Chan 2001, Parmelee et al. 1990, Pelosi et al. 2009b) while small epigeics and endogeics living in close contact to the soil surface, may benefit more from the absence of pesticide use (Pelosi et al. 2013, for more information on pesticide application practices in this site). Given that most pesticides applied in cultivated fields remain in the top 2.5 cm (Van Gestel 1992), we may assume that the suppression of pesticides in the ORG system benefited epigeic and some endogeic species more than the other, deeper-dwelling, earthworm species. Reduction in soil tillage in the INT system or introduction of a temporary plant cover in the ORG system was separately insufficient to induce a significant increase in total earthworm biomass. The large earthworm species sampled in the LIV system may benefit from the synergetic effect of both permanent plant cover and absence of ploughing (Pelosi et al. 2009b). Enchytraeids could be unaffected by tillage due to their small body size and great reproductive rates but the suppression of tillage may provide a more favorable environment for surface organisms by reducing moisture and temperature fluctuations and supplying a relatively permanent substrate for decomposers (House & Parmelee 1985). Moreover, Van Capelle et al. (2012) showed that a mulch layer strongly benefited enchytraeids but also a certain amount of soil tillage. We can here assume that the absence of tillage and the stratification of the organic matter favored enchytraeids in the first 10 cm of soil in the LIV system. The vertical distribution of enchytraeid communities was strongly influenced by ploughing and more precisely by the time lapse since last ploughing. Most enchytraeids were found in the 10–20 cm soil horizon in the systems ploughed 6 months before. Conversely, they were found in the first 10 cm of the soil in the systems that were last ploughed at least one year before sampling. Furthermore, enchytraeids, being indicators of chemical stress in terrestrial ecosystems, are sensitive to pesticides (Didden & Römbke 2001). Some authors reported that pesticides applied under field conditions decrease the abundance and biomass of enchytraeids. For instance, Parmelee et al. (1990) found that carbofuran reduced the annelid biomass, also describing the functional consequences in terms of soil organic matter stocks. Karnatak et al. (2007), who worked at promoting rice yields while limiting environmental damages, concluded that the 'application of pesticides imposed serious hazards on enchytraeid population'. They studied different common pesticides in rice ecosystem i.e. phorate, butachlor, quinalphos, caldan, and their combination. In Site 2, if soil tillage, plant cover and pesticides certainly influenced communities of terrestrial Oligochaeta, crop rotations, with more legumes, could also partly explain the higher earthworm and enchytraeid populations in ORG and LIV systems. Enchytraeids appeared to be particularly favored by the introduction of a plant cover, even a temporary one, since two years of alfalfa out of four years of crop rotation seemed sufficient to induce intermediate enchytraeid abundance and biomass in the ORG system. It could also be an effect of increased N-input due to this legume crop. Contrarily, they appeared to be less sensitive to tillage since intermediate abundance and biomass of enchytraeids were observed in the ORG system involving 'high' tillage intensity. They were also shown to be less sensitive to pesticide application considering the high abundance and biomass of enchytraeids observed in the LIV system where the intensity of pesticide applications is considered as 'high'.

Finally, the classification of the four system was similar for earthworm and enchytraeid abundance and biomass: LIV > ORG > CONV > INT. Abundances of terrestrial Oligochaeta in CONV and INT systems were very close, suggesting that just a reduction in chemical inputs and soil tillage does not necessarily benefit earthworms and enchytraeids. A complete cessation of these practices would thus be needed to see an effect on Oligochaeta communities. This is in accordance with Metzke et al. (2007) who explained that some authors reported a higher earthworm abundance and biomass in organic rotations compared to conventional or integrated farming systems. However, other sampling events would be necessary to assert all these results.

4.2. Comparison between agricultural sites and relationship between earthworms and enchytraeids

In Site 1, we found low earthworm diversity and abundance but several genera and a high number of enchytraeids. It was the contrary in Site 2 where we found more species and a higher abundance of earthworms but a lesser abundance of enchytraeids. The highest abundance of enchytraeids in Site 2 i.e. in LIV, was lower than the lowest abundance value in Site 1 i.e. in SLU. In terrestrial ecosystems, the average annual abundance of enchytraeids lies between 20,000 and 60,000 individuals m^{-2} but is subject to strong seasonal fluctuations (Jänsch et al. 2005). In agricultural systems, reported enchytraeid abundances range from 650 (Willard 1974) to 30,000 individuals m^{-2} (Didden 1991). The abundances found in our study are situated in the upper limit in Site 1, and much less in Site 2. Many studies have shown that enchytraeid abundances are higher in soils rich in organic matter, but can also be strongly influenced by other environmental factors (O'Connor 1957). Our results showed that, at the date of our samplings, soil moisture was positively correlated with enchytraeid abundance, as it was suggested by Beylich & Achazi (1999). This could partly explain the differences in enchytraeid abundance and biomass between the two sites, since soil moisture was higher at Site 1 than at Site 2. Higher precipitations before sampling in Site 1 may explain these differences in soil moisture. Another factor could have been the presence of earthworms but no correlation was found between enchytraeid and earthworm variables. In the sites we studied, these two soil organisms seemed to have a neutral relationship (Makulek & Pilipiuk 2000). This is in accordance with Severon et al. (2012) who explained that '[…] no clear relationship, neither positive nor negative, was found with respect to enchytraeid vs. earthworm abundances'. According to Beylich & Graefe (2009), under generally favorable conditions (pH, food resources), high numbers of earthworms are compatible with enchytraeids. Our results tend to confirm these findings but it is difficult to draw definitive conclusions on the relationships between earthworms and enchytraeids in our study because the absence of correlation between them could be due to the predominant effect of soil moisture. Nevertheless, more information should be collected at the species level to better understand the relationship between these two groups of terrestrial Oligochaeta.

5. Conclusion

This preliminary study has highlighted the overall positive effect of alternative agricultural practices and cropping systems on terrestrial Oligochaeta. Organic amendments appeared to favor earthworms but not enchytraeids, which were rather influenced by soil moisture in the agricultural sites under study. The different cropping systems include a variety of practices that vary simultaneously i.e. soil tillage, fertilizer and pesticide use, crop rotation, etc., that affect the abundance, biomass, species composition and vertical distribution of enchytraeids and earthworms. If both earthworms and enchytraeids appeared to benefit from a synergetic effect of the absence of ploughing and the introduction of a plant cover, large earthworms would thrive under very favorable conditions whereas enchytraeids would withstand less favorable conditions in terms of soil tillage intensity or continuity in time of the plant cover. The lack of correlation between enchytraeids and earthworms seems to indicate an absence of strong mutualistic or competitive relations among these two groups of soil organisms. This neutral relationship is rather positive to soil functioning which can benefit from the presence of these two groups of terrestrial Oligochaeta. New data at the species level could allow a better understanding of the relationship between these two groups of terrestrial Oligochaeta and of the potential consequences on soil functioning.

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