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Earthworm activity affects soil aggregation and organic matter dynamics according to the quality and localization of crop residues – An experimental study (Madagascar)

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Abstract

Soil organic matter (SOM) plays a central role in the functioning of ecosystems, and is beneficial from agronomic and from environmental points of view. Alternative cultural systems, like direct seeding mulch-based cropping (DMC) systems, enhance carbon (C) sequestration in agricultural soils and lead to an increase in soil macrofauna. This study aimed at evaluating in field mesocosms the effects of earthworms on soil organic matter dynamics and aggregation, as influenced by residue quality and management.

In the highlands of Madagascar, buckets were filled with 2 mm sieved clayey Inceptisol. The effects of earthworm addition (*Pontoscolex corethrus*), residue addition (rice, soybean, and no addition), and localization of the residues (mulched or buried) were studied. After five months, soil from mesocosms with earthworms had significantly lower C concentration and higher proportion of large water-stable macroaggregates (> 2000 µm) than those without earthworms, because of the production of large macroaggregates by earthworms. Earthworm effect on soil aggregation was greater with rice than with soybean residues. Casts (extracted from mesocosms with earthworms) were slightly enriched in C and showed significantly higher mineralization than the non-ingested soil (NIS), showing that at the time scale of our study, the carbon contained in the casts was not protected against mineralization. No difference in microbial biomass was found between casts and NIS.

Complementary investigations are necessary to assess long-term effects of earthworm addition on SOM dynamics, the conditions of occurrence of physical protection, and the impact of earthworms on the structure of the microbial community.

Key words: *Pontoscolex corethrus*; Carbon mineralization; Carbon protection; Microbial biomass; Residue management; Direct seeding mulch-based cropping systems

1 Introduction

Soil organic matter (SOM) plays a crucial role in the functioning of terrestrial ecosystems and agroecosystems, and thus is closely linked with a wide range of ecosystem services (Swift et al., 2004). Increasing SOM levels is often considered beneficial. From an agronomic point of view, high SOM content enhances soil aggregation (Six et al., 2002). This leads to improved soil aeration and root penetration, and modifies soil hydrodynamic properties (Balesdent et al., 2005). As a result of improved structural stability, SOM also prevents soil erosion (Barthès and Roose, 2002). SOM also plays the role of a long-term nutrient reserve for plants, progressively released through the decomposition process (Powlson et al., 2001). Moreover, SOM provides food and substrate for soil organisms, ranging from macroinvertebrates to heterotrophic bacteria (Lavelle et al., 2001). This is of great importance, given that soil biota are increasingly recognized to play a major role in soil functions. From an environmental and global point of view, increasing soil carbon (C) sequestration might be a tool to mitigate the increasing concentration in atmospheric CO₂ (and other greenhouse gases) due to anthropogenic emissions. High atmospheric CO₂ concentration is now recognized as a major factor of global climate change. The soil can be considered as a sink of C: at the global scale, soil C stock in the 0–100 cm horizon amounts to 1567 GtC (Carter and Scholes, 2000), which is about twice the amount of C in the atmosphere and three times the amount of C in the vegetation (IPCC, 2001).

The conversion of natural soils to arable soils generally leads to a decrease in soil C stocks (Guo and Gifford, 2002). According to Lal (2004), agriculture can reduce the soil C pool by a factor of two to three. This is particularly important in tropical regions, where SOM turnover is faster than in temperate regions (Six et al., 2002). In some cases, the decrease in SOM due to agricultural practices can threaten the sustainability of agricultural production. Thus, alternative agricultural practices such as direct seeding mulch-based cropping (DMC) systems have been proposed in the tropics (Raunet and Séguy, 1998). These cropping systems are characterized by no-tillage and permanent soil cover: the soil is covered by a mulch either formed by residues or by a living cover crop. The capacity of DMC systems to sequester soil C was recently evaluated in the highlands of Madagascar. Results showed DMC systems caused a significant storage of soil C (Razafimbelo, 2005). For both tropical and temperate agrosystems, Six et al. (2002) evaluated the mean soil C sequestration under no-tillage systems to $0.3 \pm 0.1 \text{ MgC ha}^{-1} \text{ y}^{-1}$. However, the biological processes underlying this C sequestration are still poorly understood.

Parmelee et al. (1990) found that in temperate agrosystems, earthworm biomass and densities were 70% greater under no-tillage systems than under conventional agriculture. Preliminary studies in Madagascar also showed significant increases in the abundance, biomass and diversity of soil macrofauna (especially earthworms and coleopteran larvae)

under DMC systems compared with conventional systems. Under rice-soybean crop rotation, earthworm density was on average 19 individuals (ind) m⁻² under conventional tillage and 109 ind m⁻² under DMC (unpub. data). Soil macroinvertebrates are known to play a major role in soil biofunctioning, particularly in SOM dynamics. Some species, such as earthworms and termites in tropical regions, produce biogenic structures, and thus modify the physicochemical environment of other organisms (Blanchart et al., 1999; Brown et al., 2000). They have been called soil engineers *sensu* Jones (Jones et al. 1994; Lavelle et al., 1997). They modify soil aggregation and hydrodynamic properties (Blanchart et al., 1999). They also control biomass, diversity and activity of soil microorganisms (Doubé and Brown, 1995). Soil microorganisms, mainly fungi and bacteria, are the main actors of the transformations of organic molecules in soils, and their activity is thus a key factor in SOM dynamics.

Organic matter (OM) inputs in the soil system also greatly influence the soil functioning. In DMC systems, the major OM inputs are in the form of agricultural residues. The chemical characteristics of residues, and particularly their C/N ratio, lignin and polyphenol contents, are major determinants of OM decomposition, nutrient release, and finally, soil fertility (Palm et al., 2001). Soil aggregation has also been shown to be affected by the localization of the residues (Coppens et al., 2006).

The present study aimed at characterizing the effects of the endogeic earthworm *Pontoscolex corethrurus* on soil aggregation and SOM dynamics, and in particular, at comparing SOM dynamics between casts and non-ingested soil (NIS), in a clayey Inceptisol from Madagascar highlands. The study also aimed at examining whether earthworm effects on aggregation and SOM were influenced according to residue nature (rice vs. soybean) and localization (mulched vs. buried).

2 Materials and methods

2.1. Study site and mesocosm experiment

The study was carried out at Andranomanelatra, Antsirabe region, in the highlands of Madagascar (19°47'S, 47°06'E, 1600 m above sea level). The climate is an altitude tropical climate, with a dry and cold season from April to October and a wet and hot season from October to April. The mean annual rainfall is 1300 mm and the mean annual temperature 16°C. The soil is an andic Dystrustept (Soil Survey Staff, 1999), with 62% clay mainly as 1:1 minerals, but presents andic characteristics. Bulk density was 0.76 g cm⁻³ for the 0–10 cm layer. The pH_{H2O} was 5.7 (Razafimbelo, 2005).

The study was conducted between January and June 2005. The 0-10 cm depth layer of a soil cultivated under DMC systems for 6 years was collected, air-dried and roughly sieved at 2000 µm. Each plastic bucket with a diameter of 25 cm were filled with about 8 kg of this soil and were then introduced into the soil so that surface level was similar inside and outside

the buckets. Before buckets were filled with soil, their bottoms were drilled (8 holes with 1 cm diameter) and covered with a mosquito net so that water could flow but earthworms could not escape. In half of the buckets, six adult or sub-adult earthworms belonging to the species *Pontoscolex corethrurus* Müller 1856 (Glossoscolocidae), sampled near the study site, were added. This endogeic geophagous earthworm is a tropical peregrine earthworm (Fragoso et al., 1997) abundant in the study area, feeding and living within the soil. Residues were added at rates of 18.3 g rice residues per bucket (i.e. 364.7 g m⁻²) and 14.6 g soybean residues per bucket (i.e. 293.3 g m⁻²), which corresponded to usual residue inputs in local DMC systems. The soybean residues were essentially in the form of twigs, with a C/N ratio ranging between 16 and 23 (Gilmour et al., 1998; Abiven et al., 2005), whereas rice residues were constituted of leaves and stalks, with a C/N ratio ranging between 45 and 64 (Gilmour et al., 1998; Abiven et al., 2005). Residues were cut into 2 to 3 cm long pieces. Residues were either mulched (left at the soil surface) or buried (manually mixed with the soil before incorporation in the bucket) (Figure 1). Each of the ten treatments was replicated five times, and mesocosms were randomly located in the experimental plot.

After five months in the field, mesocosms were removed, and their content was separated into a 0-10 and a 10-20 cm soil layer. The soil was roughly disaggregated to check earthworm presence, and air-dried. The earthworms were all living, and since they were mostly present in the 10-20 cm layer, all analyses were performed in this layer.

2.2. Soil aggregate fractionation

Soil aggregate fractionation was realized as described by Yoder (1936). Eighty grams of roughly fragmented soil were placed at the top of two stacked sieves (20 cm diameter), with 2000 and 200 µm meshes, respectively, which were located in a tank filled with about 8 L of deionised water. The set of sieves was lowered so that the soil sample was immersed into water for 30 min, then it was mechanically raised and lowered during 10 min to allow wet-sieving (stroke length was 32 mm and frequency 30 cycles min⁻¹). After wet-sieving, fractions > 2000 and 200-2000 µm were collected and oven-dried at 40°C. The suspension remaining in the tank (< 200 µm) was sieved with a 20 µm mesh, and dried at 40°C. For one sample per treatment (one out of five replicates), the suspension < 20 µm was oven-dried at 40°C. Each fraction was weighed, and results were expressed as proportions of the initial oven-dried soil.

For the treatments with earthworms, the fractions 20–200 and 200–2000 µm were then grouped, and the fraction 20-2000 µm was considered as representative of the soil that had not been ingested by earthworms (non-ingested soil, NIS). This is an approximation, since the fraction < 20 µm and the part of the fraction > 2000 µm that was not produced by earthworm activity were excluded. However, this approximation was reasonable, given that

the excluded fractions only represented a very little proportion of the total soil. Moreover, the fraction < 20 µm contained C that had been solubilized during the fractionation procedure, originating from both casts and NIS.

2.3. Cast extraction

In the treatments with earthworms, the fraction > 2000 µm contained earthworm casts, large aggregates whose origin was uncertain, and plant residues. Manual collection of casts was performed according to their globular shape using a binocular lens, casts being identified by smooth surface and circular section.

2.4. Soil respirometry

Respiratory activity was measured in a 28-day standard laboratory incubation. Four grams of intact casts or NIS fraction were placed in a container located in 500 mL air-tight glass jars linked with connection pipes. When the amount of extracted casts was less than 4 g for a given replicate, its casts were grouped with those of another replicate from the same treatment before incubation. Corresponding NIS were similarly grouped in the same proportions. Hence, depending on the treatment, three or four replicates per treatment were incubated. Soil moisture was maintained at 60% of field capacity, which had been determined previously. The jars were placed in an incubator at 28°C. Measurements of CO₂ emissions were realized after 0, 2, 4, 7, 9, 12, 17, 20, 22, 25, and 28 days of incubation, using a gas chromatograph MTI Agilent M 200 with 8m Poraplot Q column and microcatharometer detector. At each date of measurement, CO₂ content in the jar was determined before and after opening and air washing the jar. After opening the jar, soil water content was adjusted and the jar was rinsed with an air stream. For a given sample, the amount of CO₂ produced between dates d and d+1 was calculated as the difference between the first measurement at date d+1 and the second measurement at date d. Results were expressed as cumulated values in 28 days, in mgC-CO₂ g⁻¹C fraction.

2.5. Carbon analysis

Total C was determined on total soil samples, incubated casts and NIS fractions, by dry combustion of an aliquot ground at 200 µm. Total soil samples were first sieved at 2000 µm and large organic residues were removed before grinding. The analysis was performed with an Elemental Analyzer CHN (Fisons/Carlo Erba NA 2000). Since the soil did not contain carbonates, measured C was assumed to be organic.

2.6. Microbial biomass measurements

At the end of the incubation, microbial biomass in incubated casts and NIS fractions was measured using a fumigation extraction method (Vance et al., 1987). Four g of fractions were fumigated with chloroform, extracted with KCl 1 M and filtered at 0.22 μm (Millex GP, Millipore). Microbial biomass was determined from the amino acid concentration measured using the ninhydrin reaction. Microbial N was calculated as the difference in N-amino acid concentration with and without chloroform fumigation. It was translated into C biomass by multiplying by 21 (Öhlinger, 1995).

2.7. Statistical analyses

Data were checked for normality and homocedasticity, and transformed with the CoxBox transformation when necessary.

For all studied variables, all treatments were first compared using a two-ways ANOVA followed with a post-hoc Tukey HSD test. We then studied the effects of the different factors (earthworm addition, nature of the residues and localization of the residues) with factorial ANOVAs. Logically, no data concerning the localization of the residues were available when no residues were added (fig. 1). Hence, we did the analysis in two steps. For the variables “proportion of aggregates > 2000 μm ” and “total soil C”, we performed : (i) considering all treatments, a two-way ANOVA with earthworm addition and residue management (soybean buried, soybean mulched, rice buried, rice mulched, no addition) as factors; (ii) considering treatments with residue addition only, a three-way ANOVA with earthworm addition, residue nature and residue localization as factors. For the variables “fraction C concentration”, “cumulated fraction C-CO₂ emissions” and “fraction microbial biomass”, which compared casts and non-ingested soil (NIS), (i) considering all treatments, a two-way ANOVA with “fraction” (cast or NIS) and residue management (soybean buried, soybean mulched, rice buried, rice mulched, no addition) as factors; (ii) considering treatments with residue addition only, a three-way ANOVA with fraction, residue type and residue localization as factors. Statistical analyses were performed using R software.

3 Results

3.1. Soil aggregate fractionation

Considering the 10 mesocosms where all fractions were separated (i.e. including that < 20 μm), the cumulated yield of fraction masses averaged 101.8%. Considering all 50 mesocosms, the cumulated yield of fractions > 20 μm averaged 100.2%. On average, fractions > 2000, 200-2000, 20-200 (n = 50) and < 20 μm (n = 10) accounted for 9%, 60%, 31%, and 1% of the total soil, respectively.

Data on the proportion of the fraction > 2000 μm for each treatment are presented in Table 1. We found that on average, the fraction > 2000 μm was 2.5-fold and significantly larger with than without earthworm addition (13.5% vs. 5.4%, $p < 0.001$, Table 3). It was significantly affected neither by the localization nor by the nature of the residues though it tended to be larger with rice than with soybean residues (10.5% vs. 9.3%, $p = 0.053$, Table 4). An interaction was found between earthworm presence and the nature of the residues (rice and soybean): the increase in the proportion of aggregates > 2000 μm in the presence of earthworms was significantly greater with rice than with soybean residues (2.8- vs. 2.3-fold increase, $p = 0.036$, Table 2).

3.2. Carbon content of total soil

Data on the total soil C for each treatment are presented in Table 1. We found that carbon content of the total soil was lower with than without earthworm addition (49.3 vs. 50.3 gC kg^{-1} , $p = 0.004$, Table 3). It was significantly higher with rice than with soybean residues (50.3 vs. 49.4 gC kg^{-1} ; $p = 0.012$, Table 4) and with buried than with mulched residues (50.3 vs. 49.4 gC kg^{-1} , $p = 0.015$).

3.3. Carbon content of the fractions (casts and non-ingested soil (NIS))

Data on the C concentration of the fractions (casts and NIS) for each treatment are presented in the Table 2. C concentration tended to be higher in casts than in NIS, but the difference was not significant (48.4 vs. 47.1 gC kg^{-1} , $p = 0.071$, Table 5). Nature and localization had no significant effect on fraction C concentration (Table 6).

3.4 Soil respirometry

Data on the cumulated C-CO₂ emitted during the 28-day incubation for each treatment are presented in Table 2. Considering the average of all treatments, cumulated C-CO₂ emitted during the 28-day incubation was 16.5% higher for earthworm casts than for NIS (23.3 vs. 20.0 $\text{mgC-CO}_2 \text{g}^{-1}$ fraction C, $p = 0.009$, Table 5). Cumulated C-CO₂ was significantly higher with rice than with soybean residues (23.3 vs. 20.1 $\text{mgC-CO}_2 \text{g}^{-1}$ fraction C, $p = 0.018$, Table 6) and with buried than with mulched residues (23.1 vs. 19.9 $\text{mgC-CO}_2 \text{g}^{-1}$ fraction C, $p = 0.022$).

3.5 Microbial biomass

Data on the microbial biomass for each treatment are presented in Table 2. We found no difference in microbial biomass between treatments or between fractions (Table 5). Fraction nature (casts vs. NIS, Table 5), residue addition, residue nature or localization had no significant effect on microbial biomass (Table 6).

4 Discussion

4.1 Effect of earthworm on the total soil

The fraction $> 2000 \mu\text{m}$ was on average 2.5-fold larger with than without earthworms. It means that earthworms enhanced the formation of water-stable macroaggregates. This is consistent with the classical results on the effects of endogeic earthworms on soil aggregation. With *P. corethrurus*, several studies reviewed in Blanchart et al. (1999) indicated a positive effect of earthworm addition on the proportion of large macroaggregates. This increase in the proportion of large aggregates was mainly due to cast production by earthworms. Casts are first pasty and unstable, but stabilize with ageing (Marinissen and Dexter, 1990). Different mechanisms can explain the high structural stability of earthworm casts when dried. Mucus produced by the earthworm and microbial-derived binding agents like polysaccharides play a role of binding agent (Martin and Marinissen, 1993; Brown, 1995; Six et al., 2000). Fungi hyphae also directly stabilize casts (Marinissen and Dexter, 1990; Jastrow and Miller, 1991).

In the present study, the soil used for the mesocosms had been sieved at $2000 \mu\text{m}$, thus a low proportion of aggregates $> 2000 \mu\text{m}$ was expected in the mesocosms without earthworms. However, the amount of aggregates $> 2000 \mu\text{m}$ was not negligible in treatments without earthworms ($54 \text{ g kg}^{-1} \text{ sol}$). It might be due to imprecise $2000 \mu\text{m}$ sieving prior to mesocosm implementation, to soil crusting at the contact between the soil and the bucket, or to aggregate formation through dry-wet cycles (Denef et al., 2001).

We found a significant interaction between the proportion of aggregates $> 2000 \mu\text{m}$ and the nature of the residues. It means that earthworms produced more casts with rice than with soybean addition. Soybean residues were mainly woody twigs, whereas rice residues were smaller straws. It can be hypothesized that earthworms were less able to ingest soybean residues, and therefore were less active in the buckets with soybean residues. This lower activity has led to a lower proportion of aggregates $> 2000 \mu\text{m}$.

Concerning the C of the total soil, earthworm addition significantly decreased C content of total soil in the 10-20 cm layer of the mesocosms. In our system, earthworm activity would not be a factor in the increase in soil C content under DMC. However, we aimed at understanding mechanisms linking earthworms to SOM dynamics rather than the impacts of earthworms on C sequestration at the field-scale, which would have required another experimental setup. For example, we only studied the 10-20 cm layer, where earthworms were predominantly active, whereas most soil C accumulated in DMC systems is in the 0-10 cm layer.

Literature showed contrasting results regarding the effects of earthworms on soil C content. Mc Cartney et al. (1997), Burtelow et al. (1998), Desjardins et al. (2003) found a

negative effect of earthworm addition on soil C content, whereas Gilot (1997) found an opposite effect. An abundant literature, reviewed by Parmelee et al. (1998), focuses on the mechanisms relating earthworms to C mineralization and stocks, and highlights the importance of the time scale considered. Earthworms have direct effects on soil C: respiration, mucus and tissue decomposition represent a C loss for the soil system. However, in our experiment, the earthworms were all alive at the end of the experiment, and tissue production did not participate in the C loss associated with earthworm addition. Earthworms also have indirect effects on soil C as determinants of microbial activity. They have been reported to enhance mineralization by fragmenting SOM, mixing together SOM, mineral particles and microorganisms, and thus by creating new surfaces of contact between SOM and microorganisms (Parmelee et al., 1998). In addition to this mixing effect, mucus production associated with water excretion in the earthworm gut is known to enhance the activity of microorganisms (Barois, 1987). This enhanced microbial activity decreases when the casts dry, and aggregation is then reported to physically protect SOM against mineralization, and thus to decrease C mineralization at a longer term (Martin, 1991; Lavelle and Martin, 1992).

The integrative effect of earthworms on C mineralization has been described to depend on the time scale considered (Martin, 1991; Lavelle et al., 1998). After a long period of time, the overall earthworm impact on soil C is determined by the relative importance of the short-term enhanced mineralization and of the longer-term C protection. In our study, short-term enhanced mineralization was higher than long-term C protection.

Carbon content of the total soil was significantly higher with rice than with soybean residue addition. This could be due to a slower decomposition of rice residues, which have a higher C/N ratio. Another explanation could be a difference in initial inputs. Initial inputs were higher with rice than with soybean residues (18.3 vs. 14.6 g residues per bucket, respectively). However, C content of rice and soybean stems being 44.8 and 44.4%, respectively (Abiven et al., 2002), the difference in initial C input was 0.21 gC kg⁻¹ soil only, whereas the observed difference in total soil C between rice and soybean treatments reached 0.95 gC kg⁻¹. Finally, soil sample preparation might explain a part of the lower C content of total soil with soybean than with rice addition: before being ground, total soil samples were sieved at 2000 µm and large residues were removed. Soybean residues were larger than rice residues, thus one can assume that more soybean residues were removed.

Furthermore, we found more C in the 10-20 cm layer with buried than mulched residues. Without earthworm and using labelled residues in a laboratory experiment, Coppens et al. (2006) found similar amounts of ¹³C (originating from the residues) in the soil of the mesocosms with mulched and incorporated residues. However, with mulched residues, they found that the ¹³C was rather located in the upper layer (0-5 cm), which might

logically mean that in this case, C was less concentrated in the deeper layer, which is consistent with our results.

4.2 Properties of casts and NIS

We found that on average casts had a higher C concentration than NIS, but the difference was not significant. Whereas several studies concerning casts of anecic earthworms are available (Guggenberger et al., 1996; Vikram Reddy et al., 1997; Mora et al., 2003; Hedde et al., 2005), few data exist concerning the C content of belowground casts of endogeic earthworms. We found that casts tended to be enriched in C compared with NIS, but this difference was not significant. It means that earthworms did not select their food and did not feed in OM-rich patches. Barois et al. (1999) observed that in Martinique, the endogeic earthworm *Polypheretima elongata* had different feeding strategies according to the soil OM content. Casts were enriched in C in OM poor soil, but not in richer soils. If these results can be applied to *P. corethrurus*, it is consistent with the high OM content of the Dystrustept of our study site.

A major hypothesis for the mechanism of soil C storage is that aggregates physically protect SOM against mineralization. After 28 days, we found a greater C mineralization in casts than in NIS, and this effect was stronger with rice than with soybean residues.

Martin (1991) incubated casts of the tropical geophagous earthworm *Millsonia anomala* (Omodeo) and a 2-mm sieved control Ultisol during 420 days, and found on average a 4-times lower mineralization in casts than in NIS. She suggested that OM in casts was physically protected against mineralization on the long term. However, the casts from our study were less than 5-month old, and did not protect soil C against mineralization. Gilot et al. (1997) found that protective effects of aggregates increased with time. Hence, no conclusion regarding long-term C protection within earthworm casts could be drawn from our study. The higher mineralization in casts compared with NIS might indicate a higher concentration in labile compounds. This difference tended to be stronger with rice than with soybean residues, indicating that the enrichment in decomposable compounds was higher with rice than with NIS, which may be consistent with the quality of rice and soybean residues: rice straws contain 28.9% soluble C and 3% lignin, whereas soybean residues contain 24.2% soluble C and 12% lignin (Abiven et al, 2005).

This increase in mineralization in casts compared with NIS, probably associated with enrichment in labile compounds, resulted from an increase in the activity of the microbial community. However, this increase was not associated with an increase in microbial biomass. Most reviewed literature compared the microbial biomass of total soil with or without earthworms, and showed contrasting results. Postma-Blaauw et al. (2006) found no effect of earthworms on microbial biomass, whereas Araujo et al. (2004) found an increase in

microbial biomass with earthworm addition and Wolters and Joergensen (1992), Bohlen and Edwards (1995) and Scheu et al. (2002) found a decrease in microbial biomass when earthworms were added. The question arises whether the increased mineralization was associated with a change in the specific structure of the microbial community. Scheu et al. (2002) showed that endogeic species not only affected biomass but also the physiological characteristics of microorganisms. However, our study did not provide any direct information about the structure of the microbial community. However, on the same experimental setup, Kechenin, Villenave, Rabary and Blanchart (in prep.) found a significant increase in the abundance of microbivorous nematodes but no significant changes in the nematode community structure between treatments with and without earthworms. It might indicate that earthworm addition did affect the development of bacteria and fungi but not their relative abundances.

5 Conclusions

At the scale of the total soil, we found that earthworms decreased total soil C content. Thus, it could not be stated that the increase in the biomass and density of earthworms was responsible for the observed soil C accumulation under DMC systems. However, our experimental study aimed at determining mechanisms linking earthworm and C dynamics, and did not take into account all factors for assessing the impact of earthworms on C sequestration. For example, we did not focus on the superficial soil layer or on the long-term effects of earthworms.

Concerning the mechanisms at the scale of the aggregates, we found that the casting activity of earthworms increased the proportion of aggregates > 2000 μm and might lead to an enrichment of casts in C. This relocation of organic C underlines an incorporation of organic C by earthworm, and was higher with smaller rice residues than with larger and more woody soybean residues.

At the time scale of our study, casts did not provide physical protection of organic C against mineralization. Mineralization was increased in casts compared to NIS, indicating a higher microbial activity, and probably an enrichment in more labile compounds. However, this increase in microbial activity was not associated with an increase in microbial biomass.

Several topics require further investigation to better understand the relationships between earthworms, residues, soil aggregation, OM dynamics, and microbial communities. Conditions of occurrence of physical protection by casts must be determined. Effects of earthworms on C dynamics must be investigated in the field on the long term. The origin of CO_2 emitted during the incubations (SOM vs. residues) could be determined by using labelled residues. Finally, more research is needed to better understand the effect of

earthworms and crop residues on the structure of the microorganism community and the determinants of their activity.

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Table 1. Proportion of aggregates > 2000 μm and total soil carbon as affected by earthworm addition and residue addition, type and localization (mean \pm standard error SE, N = 5 for each of the 10 treatments).

Treatments			Mass of the fraction > 2000 μm (% of total soil)	Carbon content of the total soil (gC kg^{-1})
Earthworm addition	Residue type	Residue localization		
Yes	Rice	Buried	15.0 \pm 1.2	50.1 \pm 1.0
No	Rice	Buried	5.8 \pm 1.5	51.3 \pm 0.7
Yes	Rice	Mulched	16.0 \pm 2.2	49.9 \pm 0.7
No	Rice	Mulched	5.3 \pm 2.1	49.9 \pm 1.8
Yes	Soybean	Buried	13.4 \pm 2.1	42.9 \pm 0.9
No	Soybean	Buried	6.7 \pm 1.2	50.6 \pm 1.1
Yes	Soybean	Mulched	12.4 \pm 2.2	48.5 \pm 0.7
No	Soybean	Mulched	4.7 \pm 0.7	49.2 \pm 0.6
Yes	No residues		10.5 \pm 4.1	48.5 \pm 1.7
No	No residues		4.5 \pm 0.3	50.3 \pm 0.9

Table 2. Carbon concentration of the fraction, cumulated CO_2 emitted during fraction incubation, and microbial biomass at the end of incubation for casts and non-ingested soil (NIS) as affected by residue addition, type and localization (mean \pm SE, N = 3 or 4).

Treatments			C concentration of the fraction (gC kg^{-1} fraction)	Cumulated CO_2 emission ($\text{mgC-CO}_2 \text{ kg}^{-1}$ C fraction)	Microbial biomass (mgC kg^{-1} fraction)
Fraction	Residue type	Residue localization			
Casts	Rice	Buried	50.9 \pm 3.5	25.4 \pm 2.2	161.3 \pm 38.9
NIS	Rice	Buried	48.2 \pm 2.2	25.4 \pm 4.8	155.8 \pm 44.3
Casts	Rice	Mulched	47.9 \pm 0.95	21.3 \pm 2.6	172.7 \pm 19.9
NIS	Rice	Mulched	47.5 \pm 1.2	19.6 \pm 2.2	173.8 \pm 56.5
Casts	Soybean	Buried	48.1 \pm 0.8	23.9 \pm 2.9	171.6 \pm 26.3
NIS	Soybean	Buried	46.4 \pm 0.9	17.8 \pm 3.5	197.8 \pm 40.8
Casts	Soybean	Mulched	46.6 \pm 2.3	22.0 \pm 1.4	163.8 \pm 37.5
NIS	Soybean	Mulched	47.2 \pm 2.0	16.8 \pm 1.9	134.3 \pm 27.2
Casts	No residues		48.5 \pm 1.5	23.2 \pm 3.8	171.9 \pm 18.0
NIS	No residues		45.5 \pm 1.9	20.1 \pm 3.1	201.7 \pm 31.3

Table 3. Effect of earthworm addition and residue management on the proportion of aggregates > 2000 μm and on total soil carbon.

Variable	Source of variance	df	Sum of squares	Mean square	F-value	P-value
Mass of the fraction > 2 mm	Earthworm	1	16.26	16.26	189.78	< 0.001
	Residue management	4	1.35	0.34	3.94	0.009
	Earthworm \times Residue management	1	0.49	0.12	1.42	0.24
	Residuals	40	3.43	0.086	-	-
C content of the total soil	Earthworm	1	4.83 E ⁺¹⁰	4.83 E ⁺¹⁰	9.57	0.004
	Residue management	4	7.45 E ⁺¹⁰	1.86 E ⁺¹⁰	3.69	0.012
	Earthworm \times Residue management	1	1.42 E ⁺¹⁰	3.55 E ⁺⁹	0.70	0.59
	Residuals	39	1.97 E ⁺¹¹	5.05 E ⁺⁹	-	-

Table 4. Effect of earthworm addition, nature of the residue and localization of the residues on the proportion of aggregates > 2000 μm and on total soil carbon

Variable	Source of variance	df	Sum of squares	Mean square	F-value	P-value
Mass of the fraction > 2 mm	Earthworm	1	736.82	736.82	195.75	< 0.001
	Nature	1	15.11	15.11	4.01	0.054
	Localization	1	3.84	3.84	1.02	0.32
	Earthworm \times Nature	1	18.02	18.02	4.79	0.036
	Earthworm \times Localization	1	3.87	3.87	1.03	0.32
	Nature \times Localization	1	7.47	7.47	1.98	0.17
	Earthworm \times Nature \times Localization	1	0.11	0.11	0.03	0.87
	Residuals	32	120.45	3.76	-	-
C content of the total soil	Earthworm	1	0.064	0.064	5.04	0.032
	Nature	1	0.091	0.091	7.18	0.012
	Localization	1	0.084	0.084	6.65	0.015
	Earthworm \times Nature	1	0.0068	0.0068	0.54	0.47
	Earthworm \times Localization	1	0.022	0.022	1.74	0.20
	Nature \times Localization	1	0.0019	0.0019	0.15	0.70
	Earthworm \times Nature \times Localization	1	0.0014	0.0014	0.11	0.74
	Residuals	32	0.41	0.013	-	-

Table 5. Effect of nature of the fraction (cast or NIS) and residue management on the C concentration of the fractions, the cumulated CO₂ emissions and the microbial biomass

Variable	Source of variance	df	Sum of squares	Mean square	F-value	P-value
C concentration of the fractions	Fraction	1	17.19	17.19	3.52	0.071
	Residue management	4	38.02	9.51	1.95	0.13
	Fraction × Residue management	4	16.67	4.17	0.85	0.50
	Residuals	28	136.84	4.89	-	-
Cumulated CO ₂ emissions	Fraction	1	97.99	97.99	7.93	0.009
	Residue management	4	166.65	41.66	3.37	0.024
	Fraction × Residue management	4	47.90	11.97	0.97	0.44
	Residuals	26	321.35	12.36	-	-
Microbial biomass	Fraction	1	89	89	0.050	0.83
	Residue management	4	8050	2012	1.12	0.37
	Fraction × Residue management	4	4417	1104	0.62	0.65
	Residuals	28	50147	1791	-	-

Table 6. Effect of nature of the fraction (cast or NIS), nature of the residues and localization of the residues on the C concentration of the fractions, the cumulated CO₂ emissions and the microbial biomass

Variable	Source of variance	df	Sum of squares	Mean square	F-value	P-value
C concentration of the fractions	Fraction	1	8.84	8.84	1.77	0.20
	Nature	1	19.97	19.97	4.00	0.057
	Localization	1	9.75	9.75	1.96	0.17
	Fraction × Nature	1	2.07	2.07	0.41	0.53
	Fraction × Localization	1	10.23	10.3	2.05	0.17
	Nature × Localization	1	4.55	4.55	0.91	0.35
	Fraction × Nature × Localization	1	0.004	0.004	< 0.001	0.98
	Residuals	24	119.73	4.99	-	-
Cumulated CO ₂ emissions	Fraction	1	83.70	83.70	7.34	0.013
	Nature	1	74.70	74.70	6.55	0.018
	Localization	1	69.32	69.32	6.08	0.022
	Fraction × Nature	1	44.56	44.56	3.91	0.061
	Fraction × Localization	1	0.22	0.22	0.019	0.89
	Nature × Localization	1	22.61	22.61	1.98	0.17
	Fraction × Nature × Localization	1	3.05	3.05	0.27	0.61
	Residuals	22	250.96	11.41	-	-
Microbial biomass	Fraction	1	30	30	0.016	0.90
	Nature	1	7	7	0.0038	0.95
	Localization	1	880	880	0.46	0.51
	Fraction × Nature	1	1	1	< 0.001	0.99
	Fraction × Localization	1	1205	1205	0.63	0.44
	Nature × Localization	1	5055	5055	2.62	0.12
	Fraction × Nature × Localization	1	1941	1941	1.01	0.33
	Residuals	24	46227	1926	-	-

Figure 1. Experimental setup. Effects of earthworm addition, presence and nature of added residues and localization of added residues were tested in mesocosms.

