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Abstract

Earthworms are known to have a major impact on organic matter dynamics in soils. The precise dynamics of carbon incorporation and/or decomposition in soil under the influence of earthworms still need to be investigated. In a mesocosm experiment, the fate of Ryegrass root and shoot litter was monitored in the soil, in the presence and absence of anecic earthworms *Lumbricus terrestris* L. Residues were ^{13}C labelled and deposited onto the soil surface. Incorporation of ^{13}C in surface casts and in the 0-20 and 40-60 cm soil layers was monitored 1, 2, 4, 8, 24 and 54 weeks after adding labelled litter. Organic carbon content and $\delta^{13}C$ values were obtained for all samples, allowing the determination of the percentage of carbon derived from labelled litter (C_{lab}). Roots and shoots were incorporated in the 0-20 cm soil layer during the year of experiment, C_{lab} reaching 11.4 % of the soil organic carbon after 54 weeks. On the contrary, no significant contribution from labelled residues was observed in the 40-60 cm layer. Roots decomposed at a slower rate compared to shoots. Litter incorporation was observed in casts from the very first weeks of experiment (C_{lab} from 34.8 to 51.4 % after 2 weeks). In the soil, a significant effect of earthworms on the C_{lab} was detected after 24 weeks. Earthworms accelerated root and shoot decomposition in the soil. They also enhanced, in the presence of shoot residues, the decomposition of the organic matter originally present in the soil. However, after one year, earthworms smoothed the difference between residue types in casts and to a lesser extent in soil, revealing their capacity to enhance the decomposition of both roots and shoots.

Keywords

Organic matter dynamics

Carbon

Isotope labelling

Anecic earthworms

Casts

1. Introduction

Soil organic matter (SOM) is (1) a source of nutrients for soil organisms, (2) a key determinant of soil fertility, structuration and evolution and (3) a potential sink of carbon (C) in a context of CO_2 concentration increase in the atmosphere (Jobbágy and Jackson, 2000). SOM has been widely studied but the process of its incorporation and dynamics in soils remains unclear due to its complexity (Stockmann et al., 2013). The main source of SOM is plant residues, which encompass plant above-ground parts (dead leaves and shoots) deposited on the soil surface and below-ground parts (dead roots and rhizodeposits) within the soil (Kögel-Knabner, 2002). Residues can either be mineralized, releasing CO_2 to the atmosphere, or incorporated into soil in the form of organic compounds. After incorporation, these compounds can be mineralized, transferred in deeper layers and/or stored in soil. Residue decomposition and incorporation into soil depend on numerous abiotic and biotic factors. Abiotic factors mainly correspond to soil climate (temperature and moisture) and physico-chemical characteristics (texture and clay mineralogy). These factors have a critical influence on organo-mineral interactions, impacting organic matter decomposition and accumulation in soil. Biotic factors encompass residue quality, activity of soil fauna and microorganisms (Cortez, 1998; Oades, 1988). Soil microorganisms, which comprise, among others, bacteria and fungi, mediate the processes of organic compound mineralization and transformation in soil. Thus, they have a direct implication on residue decomposition (Kuzyakov and Blagodatskaya, 2015). Under relatively constant climatic and edaphic conditions, abiotic factors often influence litter decomposition at large time and space scales, while biotic factors act at shorter time and space scales (Lavelle et al., 1993; Swift et al., 1979),

Residue quality (*i.e.* physico-chemical features including chemical composition, anatomical characteristics, etc.) influences their decomposition by macro and microorganisms (Lavelle et

al., 1993). While above-ground biomass has long been considered as the main source for SOM, roots are currently recognized as essential contributors to stable organic C. The relative contribution from root and shoot litter to SOM has been the subject of many studies during the last decades (Balesdent and Balabane, 1996; Comeau et al., 2013; Gale and Cambardella, 2000; Lu et al., 2003; Mambelli et al., 2011; Puget and Drinkwater, 2001; Rasse et al., 2005) and it is now recognized that roots decompose at a slower rate compared to shoots. However, the reasons explaining the slower decomposition of roots and its higher contribution to soil carbon pool remain debated. Are they driven by the chemical composition, physical protection and/or physico-chemical protection of roots in soils (Rasse et al., 2005)? The contribution from these 3 factors on C dynamics is particularly dependent on residue location in soil (Coppens et al., 2006a, b; Tahir et al., 2016). For example, Coppens et al. (2006a) found that, after 9 weeks of experiment, new organic C storage in soil was increased when rapeseed residues were deposited onto the soil surface compared to residues incorporated in the 0-10 cm layer. In order to investigate the impact of the chemical compositions of residues, the latter must be placed in the same conditions (Rasse et al., 2005). Thus, in the present study, both roots and shoots were deposited onto the soil surface.

Among biotic factors, earthworms play a key role in soil structuration, residue decomposition and C cycling (Blouin et al., 2013; Bossuyt et al., 2005). Earthworms feed on both mineral (soil) and organic matter (litter, humus and microorganism), in different proportion depending on their ecological category. For example, anecic earthworms, which represent the dominant earthworm biomass in temperate regions, feed on litter deposited on the soil surface and transfer it along vertical burrows which can reach 1-2 meters (Lee et al., 1985). During ingestion, residues are fragmented and the preexisting soil microstructures destroyed. Mineral and organic elements are mixed, complexed with mucus and released in the form of organo-mineral aggregates called casts (Lee, 1985; Six et al., 2004). This process creates nuclei for the formation of organo-mineral aggregates in soil. Thus, earthworms promote the

formation of biogenic macroaggregates (Bossuyt et al., 2005), which are generally more stable than non-biogenic aggregates (Six et al., 2004; Zangerlé et al., 2011). Earthworms are highly mobile in soil and induce heterogeneous resource distribution (Decaëns et al., 2010). As the ingested residues contain higher concentration of organic C than soil, they modify the incorporation and the stock of C in aggregates (Arai et al., 2013; Fonte et al., 2012, 2007; Hong et al., 2011) and along the soil profile (Jégou et al., 2000). The net impact of earthworms on soil C strongly varies depending on the studied spatial and time scale. During gut transit and in fresh casts, mineralization of organic C is accelerated. Indeed, in earthworm gut, the physical protections of the organic matter originally present in the soil are broken and plant residues are fragmented (Lavelle and Martin, 1992). Moreover, the presence of water and intestinal mucus creates conditions favorable to mineralization by the microorganisms present in the earthworm gut and fresh casts (Brown et al., 2000; Drake and Horn, 2007; Martin et al., 1987; Vidal et al., 2016a). At month or year scale, drying and ageing casts, lead to C stabilization (Brown et al., 2000; Lavelle and Martin, 1992; Martin, 1991). Residue decomposition rate by earthworms also depends on residue palatability (Cortez et al., 1989). For example, it has generally been observed that earthworms fed mainly on shoots rather than roots (Bouché and Kretzschmar, 1974; Curry and Schmidt, 2007). However, little is known about the incorporation and decomposition of root residues by earthworms in soil (Curry and Schmidt, 2007; Zangerlé et al., 2011).

Artificial isotopic labelling of residues allows precise monitoring of the residues introduced (at a given date and place) and distinguishing the added organic matter from that initially present in the soil (Brüggemann et al., 2011; Comeau et al., 2013; Fahey et al., 2013; Klumpp et al., 2007; Thompson, 1996). Studies using artificial labelling have focused on (**1**) the impact of residue quality (roots *vs.* shoots or plant species) on organic C incorporation and decomposition into soil (Blair et al., 2005; Comeau et al., 2013; Mambelli et al., 2011; Rubino et al., 2010; Williams et al., 2006) or (**2**) the influence of earthworms on shoot decomposition and incorporation into soil and the consequences on soil C storage (Fahey et al., 2013;

118 Fonte et al., 2007; Stromberger et al., 2012). The above mentioned studies have proven that,
119 taken separately, these two biotic factors (earthworm and residue quality) have a crucial
120 impact on soil C cycling. However, is the diverging fate of root and shoot residues in soil
121 modified in the presence of earthworms? In this study, a one year mesocosm experiment
122 was set up to monitor the fate of root and shoot residues in the presence and absence of
123 earthworms. Residues were artificially ^{13}C labelled and deposited onto the soil surface.

2. Materials and Methods

2.1. Soil characteristics and mesocosm experiment

The surface layer of a soil was collected on a permanent grassland, largely dominated by Ryegrass, in the North of France (Aux-Marais, Oise). The mean annual precipitation and temperature in the region were 650 mm and 11°C, repectively. The soil collected is a loamy-sand soil with characteristics presented in **Table 1**. The experiment was performed in six PVC containers (length - 80 cm, diameter - 40 cm) filled with ca. 75 L of soil. It took place in a greenhouse, under controlled conditions (air conditioning), with natural light and the soil was kept at approximately 13°C and 23 % of humidity. The soil was homogenized and sieved at 4 mm in order to obtain homogenous columns of soil. Before starting the experiment, the soil containers were pre-incubated during 6 months in the same conditions as during the experiment. This allowed passing the bacterial flush induced by drying-rewetting of soil (Van Gestel et al., 1993; Franzluebbers et al., 2000) and/or soil physical disturbance during sieving (Datta et al., 2014). The soil macrofauna was first removed manually during sieving and remaining earthworms were extracted from the soil using an electrical device during pre-incubation, as described in Weyers et al. (2008). Each container was weekly vaporized with water (1L/week) and seedlings or mosses in mesocosms were eliminated.

The six treatments were identified as (1) Root-E for the mesocosm with root residues and earthworms (2) Root-NE for the mesocosm with root residues and without earthworms (3) Shoot-E, for the mesocosm with shoot and earthworms (4) Shoot-NE for the mesocosm with shoot and without earthworms (5) Control-E, for the mesocosm without plant residues and with earthworms and (6) Control-NE for the mesocosm without plant residues and without earthworms (**Fig.1**).

2.2. Earthworms and root-shoot

The common anecic earthworm *Lumbricus terrestris* L. was used. Earthworms were provided by the SARL Lombri'carraz (France) and were received at adult stage, with an average

150 weight of 4.5 g. They were pre-conditioned during one week in the soil used for the
151 experiment. At the beginning of the experiment, 6 earthworms per container were deposited
152 on the top of three of the mesocosms. Mean living mass was 26.8 ± 0.5 g per container
153 (around 48 ind./m^2 and 224 g fresh wt/m^2). This low density was chosen in accordance to
154 Fründ et al. (2010) review, showing that the typical earthworm density (all species
155 concerned) in temperate pastures was 300-1000 ind./m^2 (50-100 g fresh wt/m^2). Thus, as
156 *L.terrestris* is a territorial earthworm, these choices favored their survival in conditions closest
157 to reality. By the end of the experiment, the soil volume extracted during the year of sampling
158 represents approximatively 6 % of the initial soil volume. Thus, it is assumed that earthworms
159 were not limited in their movement along the experiment.

160 Plants of Italian Ryegrass (*Lolium multiflorum* Lam.) were artificially labelled with ^{13}C at the
161 Atomic Energy and Alternative Energies Commission (CEA) in Cadarache (France). In order
162 to obtain homogeneous material, plants were grown under a controlled and constant $^{13}CO_2$
163 enriched atmosphere. Immediately after ^{13}C labelling, fresh roots and shoots were separated,
164 dried and chopped. They were chopped during 40 seconds with a laboratory blender (*Waring*
165 *Commercial*) in order to obtain residues of few millimeters. The mean $\delta^{13}C$ values were 1324
166 ‰ (± 43) and 1632 ‰ (± 16) for the roots and shoots, respectively (**Table 2**). Few hours after
167 earthworms had penetrated the soil, roots and shoots were similarly applied on the soil
168 surface. Thus, the impact of the chemical composition of roots vs shoots was tested,
169 excluding any influence of their location in the soil. Two mesocosms each contained 250 g of
170 shoots, two additional contained 250 g of roots and the latest two were used as control.
171 Curry and Schmidt (2007) reviewed that *L.terrestris* could ingest 17 mg of dry matter/g fresh
172 wt/day. However, the quantity of litter ingested can increase when the quality of litter
173 decreases. Thus, 250 g of litter (i.e. around 25 mg of dry matter/g fresh wt/day) was chosen
174 as a good compromise to favor earthworm survival.

175 *2.3. Sampling and analyses*

Samples were collected at seven time steps: before and 1, 2, 4, 8, 24 and 54 weeks after the addition of roots or shoots and earthworms. At each time step and for each mesocosm, three replicates of soil samples were collected at 0-20 and 40-60 cm with an aluminum auger (height: 100 cm, diameter: 2.5 cm), after putting aside plant residues. Few grams of 3-4 earthworm cast fragments were collected on the soil surface in the mesocosms containing earthworms in order to obtain one composite sample for each time step. During the first 6 months of experiment, most casts were fresh when collected. Casts collected after 54 weeks were starting ageing and drying. The casts collected in the mesocosms Root-E, Shoot-E and Control-E will be identified as Cast-Root, Cast-Shoot and Cast-Control, respectively. All samples were freeze-dried and ground. After 54 weeks, roots and shoots remaining at the soil surface (i.e. for Root-NE and Shoot-NE) were collected and weighted.

2.4. Organic carbon and $\delta^{13}C$ analyses

Organic C (C_{org}) and $\delta^{13}C$ were measured on all soil replicates with Elemental Analysis – Isotope Ratio Mass Spectrometry (EA-IRMS) *Vario pyro cube – Micromass Isoprime*. The standard gas was calibrated in relation to the international standard (PeeDee Belemnite-PDB). For the casts, the analysis was repeated three times. All samples were decarbonated before the analysis, using the method developed by Harris et al. (2001). Briefly, subsamples of soil (~40 mg) and casts (~15 mg) were placed in open silver capsules (8*5 mm). Samples were placed in a microtiter plate and 15 µL of ultra-pure water were added. They were placed in a vacuum desiccator containing a 100 mL beaker of HCl (12 M). Samples were exposed to HCl vapor during 6 hours and then placed under vacuum, without HCl, to remove HCl potentially trapped in the sample. Samples were then transferred to an oven at 40°C for 15 hours.

Isotopic composition of samples is expressed in $\delta^{13}C$ relative to the PDB standard (**Equation 1**):

$$\delta^{13}C\ (‰) = [(R_{sample} - R_{standard}) / R_{standard}] * 1000 \qquad \textbf{(1)}$$

Where R is the $^{13}C/^{12}C$ ratio of the sample or the standard.

The proportion of C derived from the labelled litter in the soil or casts (C_{lab}), was expressed in percentage by applying equation **2**:

$$C_{lab}\ (\%) = [(\delta_s - \delta_c) / (\delta_l - \delta_c)] * 100 \quad \textbf{(2)}$$

Where δ_s is the $\delta^{13}C$ value of soil samples or of casts with labelled litter, δ_c is the $\delta^{13}C$ value of the control soil sample or control casts without litter, δ_l is the $\delta^{13}C$ value of the labelled litter.

In order to overcome the initial variability of the soil organic C measured in the different mesocosms, all the organic C contents were normalized using equation **3**:

$$RC_{org\ x} = C_x / C_0 \quad \textbf{(3)}$$

Where $RC_{org\ x}$ represents the trend in organic C content ratio of C after x weeks, C_x is the organic C content after x weeks of experiment and C_0 is the soil organic C content before adding earthworms and residues, for the corresponding mesocosm. Thus, values lower or higher than one express a decrease or increase in organic C content in comparison to the beginning of the experiment, respectively.

A similar formula was applied for the casts, where C_x is the organic C content in cast and C_0 is the organic C content in the soil before adding earthworms and residues, for the corresponding mesocosm. Thus, values higher than one indicate that the organic C content is higher in the casts compared to the initial soil.

2.5. Statistical analysis

Statistical analyses were realized using the R statistical software. As data were not normally distributed (Shapiro-Wilk test), samples from different mesocosms were compared using the non-parametric Kruskal-Wallis test followed by a pairwise comparison of groups from the package '*RcmdrPlugin.coin*'. For all analyses, statistical significance was set as $\alpha = 0.05$.

3. Results

Observation of mesocosms during the year of experiment gave first indications on the incorporation of the labelled residues. After 24 weeks of experiment, no more shoot residues were observed in the Shoot-E mesocosm whereas, in the Root-E mesocosm, root residues were still observed on the soil surface, occupying less than 10 % of the surface. After one year of experiment, no more residues were observed at the soil surface in the mesocosm containing earthworms. On the contrary, in the absence of earthworms, after one year, some remaining residues were still visible at the soil surface, for either root or shoot residues. They were roughly separated from the soil and weighted. They accounted for ca. 30 % of the initial weight. After one week, casts were observed in Shoot-E. However, no casts were identified until two weeks in the mesocosm with root residues. In spite of regular removal of seedling and mosses during the experiment, some mosses developed in the Control-NE mesocosm, from 24 weeks until the end of the experiment.

3.1. Carbon derived from root and shoot residues

3.1.1. Soil samples

The C derived from labelled litter (C_{lab}) increased continuously in the 0-20 cm soil layer along the year of experiment, reaching between 0.96 and 11.4 % after 54 weeks, for Shoot-E and Root-NE, respectively (**Fig.2A**). From the beginning of the experiment to the 8th week, there were no significant differences between mesocosms, with a mean C_{lab} of 0.40 %. After 24 weeks, C_{lab} was significantly lower for Shoot-E (0.85 %) than Root-E (3.00 %). After 54 weeks, C_{lab} was significantly higher in Root-NE (11.4 %) than in the other mesocosms. Thus, two general trends could be observed after 24 and 54 weeks: (1) C_{lab} in Root-NE and Shoot-NE tended to be higher than Root-E and Shoot-E, respectively and (2) C_{lab} in Root-E and NE tended to be higher than Shoot-E and NE, respectively. Contrary to other mesocosms, C_{lab} in Shoot-E barely varied from 8th week until the end of experiment, with values of 1.13, 0.85 and 0.96 % after 8, 24 and 54 weeks, respectively. There was no statistically significant differ-

252 ence of C_{lab} between mesocosms in the 40-60 cm soil layer during the year of experiment
253 (**Fig.2B**). However, at 54 weeks C_{lab} reached 0.2 % in Root-E and Shoot-E whereas it re-
254 mained lower than 0.05 % without earthworms.

255 *3.1.2. Earthworm casts*

256 For all the time steps, C_{lab} measured in casts was higher than C_{lab} in soil, with a mean C_{lab} of
257 37 % with respect to 1.3 % for soils, all time steps and mesocosms included (**Fig.3**). C_{lab} in
258 Cast-Shoot reached 34.9 % in average during the first month of experiment. C_{lab} reached a
259 maximum after 8 weeks for shoots (58.1 %) and after 8 and 24 weeks for roots (51.8 %). C_{lab}
260 then decreased during the last six months of experiment, reaching around 12 % for both
261 Cast-Root and Cast-Shoot after 54 weeks.

262 *3.2. Organic carbon content in soil and cast samples*

263 *3.2.1. Soil samples*

264 Due to the very low litter C incorporation for the 40-60 cm soil layer, the following of this sec-
265 tion is focused on the 0-20 cm layer. In mesocosms containing shoots, with or without earth-
266 worms, organic C content tended to be lower than the organic C content before the experi-
267 ment ($RC_{org\ x} \leq 1$), for all time steps (**Fig.4 and Table S1**). On the contrary, in mesocosms
268 containing roots, with or without earthworms, organic C content tended to be higher than at
269 the beginning of the experiment ($RC_{org\ x} \geq 1$). RC_{org} reached 1.2 after 54 weeks in Root-NE.
270 RC_{org} was significantly different for Root-E and Shoot-E after 24 weeks (1.1 *vs* 0.85).This
271 observation was also made between Root-NE and Shoot-NE but to a lesser extent. For ex-
272 ample, after 24 weeks, RC_{org} was 1.1 and 0.89 for Root-NE and Shoot-NE, respectively
273 (**Fig.4**).

274 *3.2.2. Earthworm casts*

275 The organic C measured in casts was always higher than the soil organic C measured before
276 the experiment ($RC_{org} >> 1$) (**Fig.5**). For Cast-Control, the RC_{org} was stable for all the time

277 steps, with a mean value of 1.3. Both Cast-Root and Cast-Shoot, RC_{org} remained stable at
278 ca. 1.8, during the first month of experiment before increasing to their maximum at 8 weeks
279 and subsequently decreasing until the end of the experiment. When Cast-Root and Cast-
280 Shoot are compared, their RC_{org} are similar during the first month and it becomes higher for
281 Cast-Root after 8 (3.6 *vs* 2.7) and 24 (2.8 *vs* 2.3) weeks of experiments before reaching
282 again similar values after 54 weeks (1.5 and 1.6).

283 **4. Discussion**

284 The proportion of C derived from the labelled litter into the 0-20 cm soil layer after one year
285 of experiment (C_{lab} between 0.96 and 11.4 % depending on the mesocosm, (**Fig.2A**)) is
286 consistent with previous studies (Kammer and Hagedorn, 2011; Rubino et al., 2010).
287 Residues placed on the soil surface have either been (1) totally mineralized, (2) left intact at
288 the soil surface and/or (3) transferred to the soil either in form of soluble compounds or as
289 undecomposed fragmented pieces (Cortez and Bouché, 1998; Rubino et al., 2010). Thus,
290 the ^{13}C signal measured in soil could either derived from the residues themselves (soluble or
291 small fragments) or from microorganisms that have degraded the residues. No significant
292 differences are observed between the mesocosms until the 24th week of experiment. The
293 absence of significant earthworm impact during the first 8 weeks can be explained by the
294 experimental conditions. Indeed, the low density of earthworms and the localized sampling to
295 preserve soil structure in mesocosms, accounted for the great variability of data and the late
296 significant impact of earthworms. Differences induced by root or shoot residues were not
297 detected until 6 months. This could be explained by the experimental conditions. Indeed, as
298 high volumes of soil were used in the present study, an important quantity of labelled C was
299 needed to reveal a significant difference between samples.

300 4.1. *Root vs. shoot effect*

301 For the last two time periods, the C_{lab} of mesocosms containing roots was higher than those
302 with shoots, although the difference was not systematically statistically significant (**Fig 2.A**).

The higher contribution to SOM of root compared to shoot residues was also supported by the soil RC_{org} results after 54 weeks in Root-NE and Shoot-NE (1.20 and 1.05, respectively). These results are coherent with William et al. (2006) who found, after having incubated roots and shoots of ryegrass in soil for 10 months, a C contribution of 5.5 % and 1.5 %, respectively. Two explanations can be put forward to explain the higher root-derived C input in soil. First, roots could be more transferred from residues into soil (as dissolved OM or small fragments), compared to shoots. However, this explanation is very unlikely, considering the characteristics of roots compared to shoots, as detailed in the second explanation. Second, root-derived C decomposed at a slower rate than shoot-derived C, as evidenced in numerous studies (Beuch et al., 2000; Gale and Cambardella, 2000; Scheu and Schauermann, 1994; Shi et al., 2013; Steffens et al., 2015). Indeed, the higher mineralization of shoots compared to roots can be explained by (**1**) the higher water soluble C concentration in shoots (Beuch et al., 2000; Bird and Torn, 2006; Shi et al., 2013), which is rapidly transferred to soil and decomposed by microorganisms (Shi et al., 2013); (**2**) the higher chemical recalcitrance of roots due to higher concentration of resistant molecules such as lignin, tannins and suberins (Gleixner et al., 2001; Kögel-Knabner, 2002; Rasse et al., 2005). The higher relative abundance of lignin-derived compounds in the roots used in the present study compared to shoots, was confirmed by Vidal et al. (2016b) using molecular characterization and (**3**) The lower C/N ratio of shoots vs. roots (15 *vs.* 31 for the litter used in this study – **Table 2**), which is generally related to an increased rate of litter decomposition (e.g. Nguyen Tu et al., 2011; Taylor et al., 1989; Witkamp, 1966).Thus, the present results highlight the importance of the chemical composition on residue decomposition. It can be suggested that the difference between root and shoot degradation is mainly driven by soluble carbon content at short term (first weeks of experiments), whereas, the chemical recalcitrance of roots probably becomes the dominant factor at longer terms, as suggested in the literature (e.g.Trinsoutrot et al., 2000; Machinet et al., 2011).

4.2. Effect of earthworms on residue fate

4.2.1. Earthworms tended to accelerate residue incorporation and decomposition

No more residues were observed at the soil surface after the year of experiment in the mesocosm containing earthworms compared to the one without earthworms. Thus, residues had probably been incorporated in soil or casts and/or mineralized. Although absolute quantification was not achieved, it can reasonably be suggested that earthworms accelerated decomposition of residues in soil. Thus, part of the ^{13}C was probably removed from the system in the form of $^{13}CO_2$ (microbial and/or earthworm respiration). Indeed, from 6 months of experimentation, the C_{lab} in soil tended to be lower in the presence of earthworms, for both root and shoot residues (**Fig.2A**). This can be explained by the direct impact of earthworms which assimilate and mineralize part of the labelled organic C during the ingestion of residues, as suggested by Daniel (1991). Moreover, decomposition and mineralization are governed by microorganisms acting directly on particulate residues, or on soluble compounds in the soil matrix (Gaillard et al., 1999). In the presence of earthworms, this phenomenon can be amplified as the ingestion of residues by earthworms generally favors microbial activity (Amador and Görres, 2007; Brown, 1995; Frouz et al., 2011; Parle, 1963; Vidal et al., 2016a).

4.2.2. Casts – a key role in residue decomposition

Cast and soil samples presented different trends of organic C incorporation and decomposition (fig. 2.A *vs.* 3 and fig 4 *vs.* 5). As expected, the C_{lab} and C_{org} are higher in casts compared to soil. The organic C content of casts was 1.3 to 3.6 higher compared to the soil. Similarly, Mariani et al. (2007) observed that the C content in anecic casts collected in burrows of a Colombian pasture, was twice higher compared to bulk soil. This can be explained by the feeding behavior of earthworms, especially anecic ones, which incorporate fresh residues and/or organic-rich compounds in casts (Decaëns et al., 1999; Frouz et al., 2011; Hong et al., 2011; Zangerlé et al., 2011; Zhang et al., 2003). The maximum value for C_{lab} (58.1% in Cast-Shoot, after 8 weeks of incubation) is in agreement with a previous study

356 which reported that half of the C in *L.terrestris* casts was derived from labelled litter after 246
357 days of experiment, adding labelled litter every day (Jégou et al., 1998).

358 A chronology can be proposed, explaining residue incorporation by earthworms in casts
359 (Figure 6), based on C_{lab} and $RC_{org\ x}$ results (**Fig. 3** and **5**). After a strong increase within the
360 first week of experiment, both C_{lab} and RC_{org} in casts remained stable during the four
361 following weeks. In this phase, earthworms have probably combined direct and indirect
362 incorporation of residues. During direct incorporation, residues are ingested and pass
363 through the earthworm gut (Shipitalo and Protz, 1989). Indirect incorporation, also called
364 'pre-oral litter decomposition', consists in a 'ploughing-in' of residues, *i. e.* casts are
365 deposited on residues which are not ingested, initiating microbial decomposition (Cortez and
366 Bouché, 1998). An increase in C_{lab} and RC_{org} was observed between 4 and 8 weeks. After
367 few months, earthworms are able to re-ingest their casts in which residues have been
368 partially decomposed by microorganisms (Swift et al., 1979; Jiménez et al., 1998; Brown et
369 al., 2000). Thus, between 4 and 8 weeks of experiment, the 'ploughing-in' and ingestion of
370 undecomposed labelled residues still present at the soil surface were probably associated to
371 the re-ingestion of previously labelled casts (from 1 to 4 weeks), increasing the C_{lab}. During
372 the last 6 months, C_{lab} and RC_{org} highly decreased, no more litter was present at the surface
373 of the mesocosms containing earthworms and litter, and surface casts were starting ageing
374 and drying. Thus, all the residues have been either incorporated in the soil and casts, or
375 mineralized. The decrease in C_{lab} and RC_{org} indicated that labelled residues incorporated in
376 casts sampled at 54 weeks have been partially mineralized. This decomposition was
377 enhanced by bacteria and fungi as observed on Cast-Shoot NanoSIMS images after 24
378 weeks of experiment (Vidal et al., 2016a).

379 *4.2.3. A slight effect in the 40-60 cm soil layer*

380 A slight increase in C_{lab} was observed in the 40-60 cm soil layer during the 54 weeks in the
381 presence of earthworms, although not statistically significant (**Fig.2B**). This was observed in

other studies which found that below 2 or 5 cm, labelled litter incorporation was not significant, at least for short time scales (Jégou et al., 1998; Kammer and Hagedorn, 2011; Rubino et al., 2010), mainly due to the limited migration of soluble C in deeper soil layers (Thompson, 1996). However, the burrows of *L. terrestris* are considered as permanent structures that can reach deep soil layers (>1 m) (Jégou et al., 2000; Don et al., 2008). Thus, earthworms may have contributed to the incorporation of labelled organic compounds into the 40-60 cm soil layer directly, by covering their burrow walls with mucus and belowground casts, both enriched in ^{13}C (Jégou et al., 2000) or to a lesser extent, indirectly, by creating preferential paths for labelled water soluble C circulation (Don et al., 2008). The C_{lab} increase was probably not significant because earthworm influence is localized on burrow walls and their periphery, and casts (Don et al., 2008). Jegou et al. (2000) showed in an experiment using ^{13}C labelled litter that below the 2 cm soil layer, the C litter enrichment in the soil surrounding burrows was barely detectable. Thus, the ^{13}C signal was probably diluted in the soil as earthworm burrows were not sampled separately.

4.3. Effect of earthworms on the fate of root vs. shoot

4.3.1. *Earthworms minimized the diverging fate of root and shoot residues*

In soil samples, the difference in C_{lab} and RC_{org} between root and shoot mesocosms with earthworms, after 54 weeks, was weaker than in the absence of earthworms (**Fig.2A and 3**), underlying their ability to enhance root decomposition, as mentioned by Cortez and Bouche (1992). Hopkins et al. (2005) have compared decomposition of natural tobacco roots and modified tobacco roots with altered lignin structure and composition (*i.e.* less resistant to decomposition). They found that earthworms reduced the difference between both residue types for C mineralization. Earthworms can enhance the decomposition of recalcitrant residues and smooth the difference between residues of various qualities, probably by facilitating the access of plant residues to microorganisms. Indeed, a mutualistic relation is maintained between bacteria and earthworms in their gut, which enabled the decomposition of highly complex molecules (Barois and Lavelle, 1986; Trigo and Lavelle, 1993).

409 The difference between root and shoot residues was also weak in casts. Cast-Root and
410 Cast-Shoot both exhibited similar evolutionary trends for C_{lab} and $RC_{org\ x}$ (**Figs. 3** and **5**).
411 However, the observation of the mesocosms at the different time steps revealed some
412 differences. After one week of experiment, no cast could be observed on the soil surface in
413 the mesocosm containing roots and the litter appeared unaffected. Moreover, after 24 weeks,
414 while shoots had totally disappeared from the soil surface in Shoot-E, roots were still visible
415 in Root-E. Thus, in our experimental conditions, dead roots in early stage of decomposition
416 seemed less palatable for earthworms than shoots, confirming observations of Zangerlé et
417 al. (2011). Coq et al. (2007) also observed that less casts were produced with woody twigs of
418 soybean compared with small straws of rice, underlining the importance of residue
419 palatability for earthworm ingestion. This can be partly explained by the high C/N ratio of
420 roots in comparison to shoots as it is known that earthworms consume preferentially organic
421 compounds with low C/N (Curry and Schmidt, 2007). However, after 2 weeks, the C_{lab}
422 increases rapidly in Cast-Root, underlying the capacity of earthworms to process roots
423 (Cortez and Bouche, 1992). After 54 weeks, no more litter was observable in both
424 mesocosms and the C_{lab} and RC_{org} were not significantly different for casts containing roots
425 or shoots (Figs 3 and 5). Thus, even though earthworms prefer feeding on shoots, they are
426 able to incorporate roots in casts, where the decomposition is activated at a comparable rate
427 to that observed with shoots.

428 *4.3.2. Fate of organic matter initially present in soil*

429 In the presence of earthworms, during the first 6 months, the $RC_{org\ x}$ is higher in Root-E than
430 in Shoot-E (**Table S1**), with a significant difference after 6 months of experiment (**Fig. 4**).
431 Moreover, the C_{org} content in Shoot-E is generally lower than that measured before the
432 experiment in the same mesocosm ($RC_{org\ x} \leq 1$). This difference likely reflects a priming effect
433 in this mesocosm, leading to a high mineralization of the organic matter originally present in
434 the soil. This is also observed to a lesser extent with shoots without earthworms. The
435 enhanced SOM mineralization in the presence of fresh residues has been reported in many

studies (Kuzyakov, 2010). Indeed, microorganisms depend on the energy present in fresh residues to decompose the recalcitrant organic matter present in the soil (Fontaine et al., 2011, 2007). For example, Lu et al. (2003) observed that the simple presence of fresh residues, either root or shoot, favored the degradation of native soil organic matter after 8 weeks of experiment, probably due to the stimulation of the microbial activity. The soil fauna, including earthworms, was also mentioned as inducing a priming effect in soil by Lavelle et al. (1995). More recently, Coq et al. (2007) showed that total soil C had decreased after 5 months of incubation of rice or soybean shoot residues, in the presence of endogeic earthworms. Fontaine et al. (2011) measured the effect of a ^{13}C labelled cellulose input in a soil during 161 days. They showed that, after one month, respiration of unlabelled CO_2 reached a maximum, revealing a priming effect. A continuous and slow decrease in unlabelled CO_2 respiration was then observed until the end of the experiment. This decrease in priming effect is in agreement with the present results showing that, after one year, RC_{org} was around 1 for Shoot-E and Shoot-NE. The suggested priming effect observed for the mesocosm containing shoots was not observed for the one containing roots. Indeed, during the first 6 months of experiment, the C_{org} tended to be higher in mesocosm containing roots than at the beginning of the experiment ($RC_{org} \geq 1$) Thus, in the present study, the priming effect induced by root residues seems to be lower compared to that of shoot residues during the first six months of experiment. This priming might also be compensated by the higher preservation of root-derived carbon coupled to reduced activation of microbial activity compared to shoot residues (Shi et al., 2013). This process is smoothed after one year; supporting comment from Rasse et al. (2005) that the preferential shoot priming effect on SOM mineralization cannot explain the higher root-derived C in soil, as observed after one year on **Fig.2A**. It has to be noted that the increase in RC_{org} observed for Control-NE after 24 and 54 weeks can be explained by the development of mosses in this mesocosm from 24 weeks until the end of the experiment, potentially increasing the C_{org} concentration in the 0-20 cm soil layer.

5. Conclusion

This mesocosm experiment allowed monitoring the fate of root and shoot residues in soil and earthworm casts. Residues deposited onto the soil surface were continuously incorporated in the 0-20 cm soil layer during the year of experiment. In agreement with previous studies, root residues incorporated in the 0-20 cm soil layer tended to decompose at a slower rate than shoot residues, underlining the essential role of litter chemical composition in the organic C fate. Earthworms tended to accelerate both root and shoot residue decomposition in soil. The activity of earthworms also induced a slight, but not statistically significant, transfer of C derived from residues in the deeper soil layer (40-60 cm), without any distinction between roots and shoots. In earthworm casts, root and shoot residues presented similar incorporation and decomposition of C_{org} trends. A chronology for cast formation and evolution was proposed, based on ^{13}C and organic C results. Three phases were identified: (**1**) the first month: mainly incorporation of fresh residues in casts, (**2**) until 6 months: re-ingestion of labelled casts and incorporation of fresh residues and finally (**3**) after 6 months: mainly microbial decomposition of residues in casts. This study provides new elements on the role of earthworms on incorporation and decomposition of organic C from roots and shoots into soil. Earthworms minimized the diverging fate of root and shoot residues, after the year of experiment, in both soil and cast samples. Thus, the presence of earthworms tended to enhance the decomposition of structures recognized as more chemically resistant. Moreover, during the first six months of experiment, the presence of earthworms and shoot residues enhanced the decomposition of soil organic C initially present in the soil. After one year, earthworms had accelerated the incorporation and decomposition of plant residues in the soil. However, after this period, the ^{13}C signal had not decreased in the soil and had only started to decrease in casts. Thus, longer term experiments are needed to investigate the stabilization of C-derived from root and shoot residues in the presence of earthworms. Moreover, while these results bring new light on the fate of root and shoot litter in the

presence of earthworms, they further require field-based experiment, including other earthworm ecological categories, to be generalized.

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497 **Table 1** – Bulk features of the soil used for the experiment

Soil characteristics	Values
Clay (< 2 µm)	189 $g.kg^{-1}$
Loam (2-50 µm)	248 $g.kg^{-1}$
Sand (50-2000 µm)	563 $g.kg^{-1}$
Total carbonates ($CaCO_3$)	19.0 $g.kg^{-1}$
CEC Metson	9.90 $cmol.kg^{-1}$
Organic Carbon	12.1 $g.kg^{-1}$
Nitrogen	1.30 $g.kg^{-1}$
$\delta^{13}C$	-28.1 ‰

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500 **Table 2** – Initial litter characterization (n=13). Numbers in parentheses indicate the standard

501 deviation for the 13 samples.

	δ^{13}C (‰)	**C** (*g.kg^{-1}*)	**N** (*g.kg^{-1}*)	**C:N**
Root	1324 (42)	402 (40)	13.1 (1.2)	30.7 (3.1)
Shoot	1632 (16)	408 (6.3)	27.7 (2.3)	14.8 (1.1)

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Fig. 1. Experimental design. E: with earthworms, NE: without earthworms

Fig. 2. Proportion of carbon derived from labelled litter (C_{lab}) measured in the soil samples at the **A.** 0-20 cm and **B.** 40-60 cm layer, for the seven time steps, in mesocosms with labelled residues (n=3). The scale is not the same for **fig.2A** and **B**. A significant difference between mesocosms was revealed after 24 weeks, thus the letters above histogram bars represent statistical results of Kruskal-Wallis test; different letters indicating significant difference between mesocosms, for a given time step. E: with earthworms, NE: without earthworms

Fig. 3. Proportion of carbon derived from labelled litter (C_{lab}) in casts sampled in mesocosms containing root (Cast-Root) or shoot residues (Cast-Shoot) (n=3). Letters above histogram bars represent statistical results of Kruskal-Wallis test, different letters indicating significant difference between mesocosms, for a given time step. No casts were identified until two weeks in the mesocosm with root residues, explaining the absence of data for Cast-Root after one week.

Fig. 4. Organic carbon content relative to the soil organic carbon content at the beginning of the experiment (RC_{org}) (n=3), for the 0-20 cm soil layer and after 24 and 54 weeks. The letters above histogram bars represent statistical results of Kruskal-Wallis test; different letters indicating significant difference between mesocosms, for a given time step.

Fig. 5. Organic carbon content relative to the soil organic carbon at the beginning of the experiment (RC_{org}) measured in casts sampled in the mesocosms containing no litter (Cast-Control), root (Cast-Root) or shoot residues (Cast-Shoot) (n=3). The letters above histogram bars represent statistical results of Kruskal-Wallis test; different letters indicating significant differences between mesocosms, for a given time step. No casts were identified until two weeks in the mesocosm with root residues, explaining the absence of data for Cast-Root after one week.

Fig. 6. Simplified representation of the proposed chronology explaining residue incorporation by earthworms within cast, in the present study. It has to be noted that most processes may be combined for a same time step. Only the suggested dominant processes are represented.

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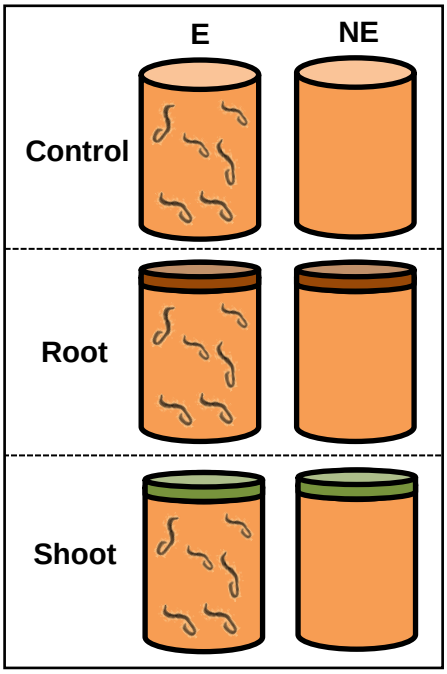


Fig.1

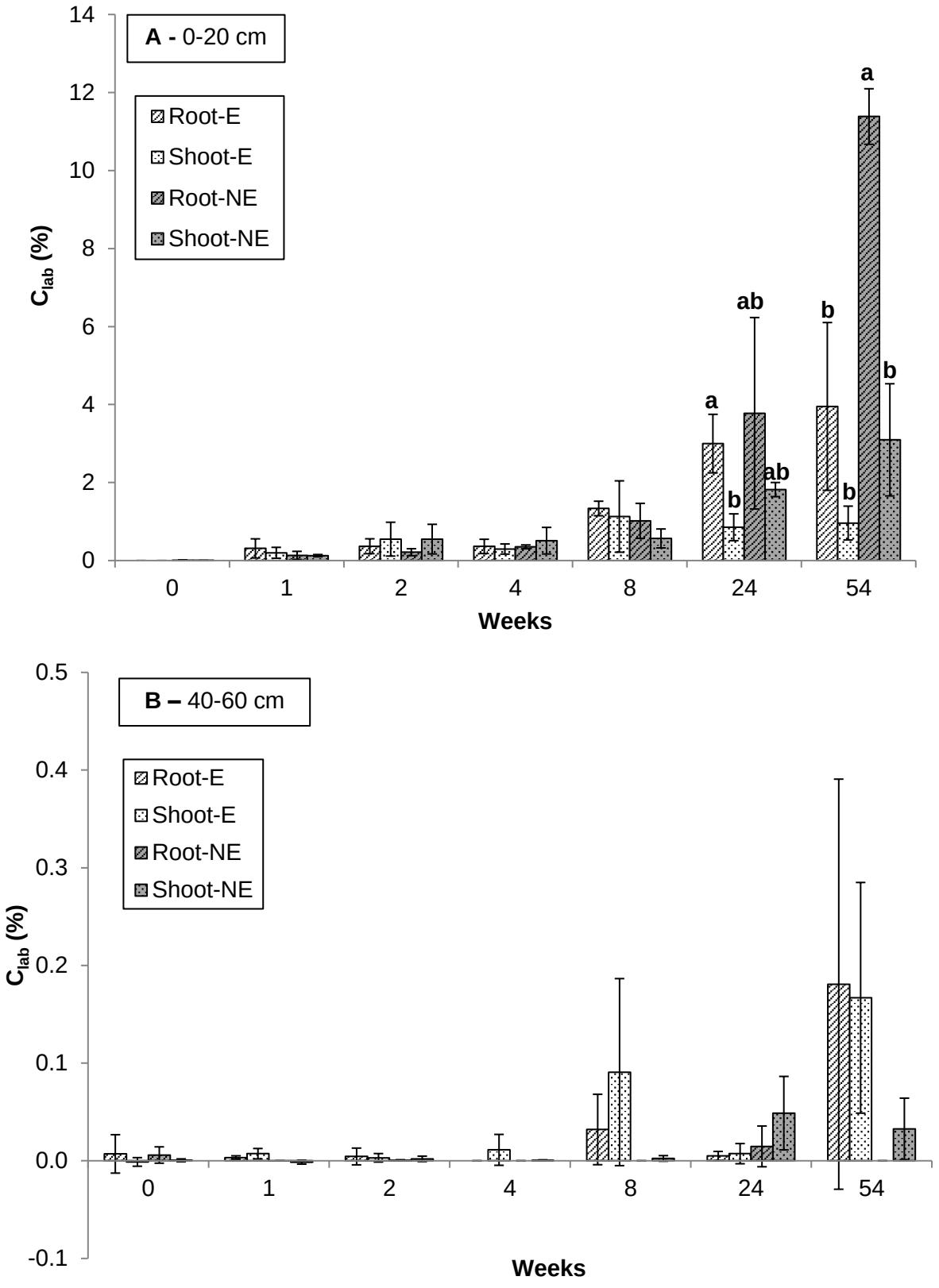
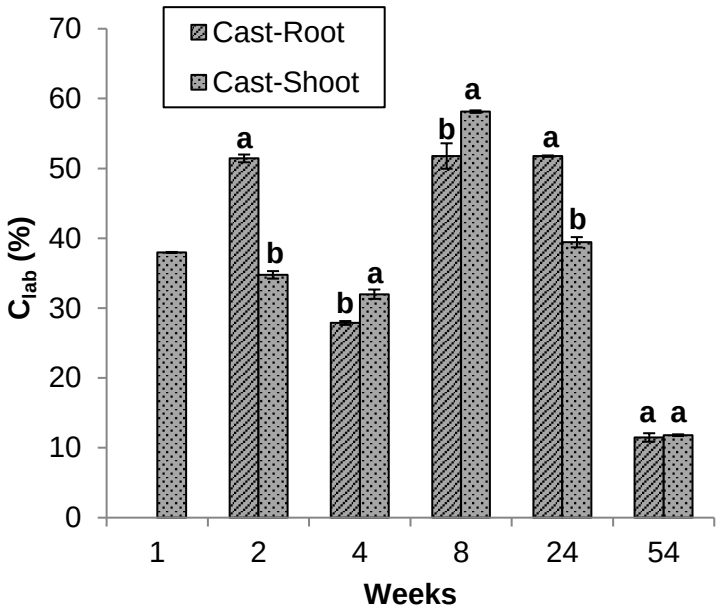

A - 0-20 cm
Root-E
Shoot-E
Root-NE
Shoot-NE
C_{lab} (%)
14
12
10
8
6
4
2
0
a
b
ab
ab
b
b
a
b
0
1
2
4
8
24
54
Weeks
B – 40-60 cm
Root-E
Shoot-E
Root-NE
Shoot-NE
C_{lab} (%)
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
0
1
2
4
8
24
54
Weeks

Fig. 2


Cast-Root
Cast-Shoot
70
60
50
40
30
20
10
0
C_{lab} (%)
a
b
b
a
b
a
a
b
a
a
1
2
4
8
24
54
Weeks

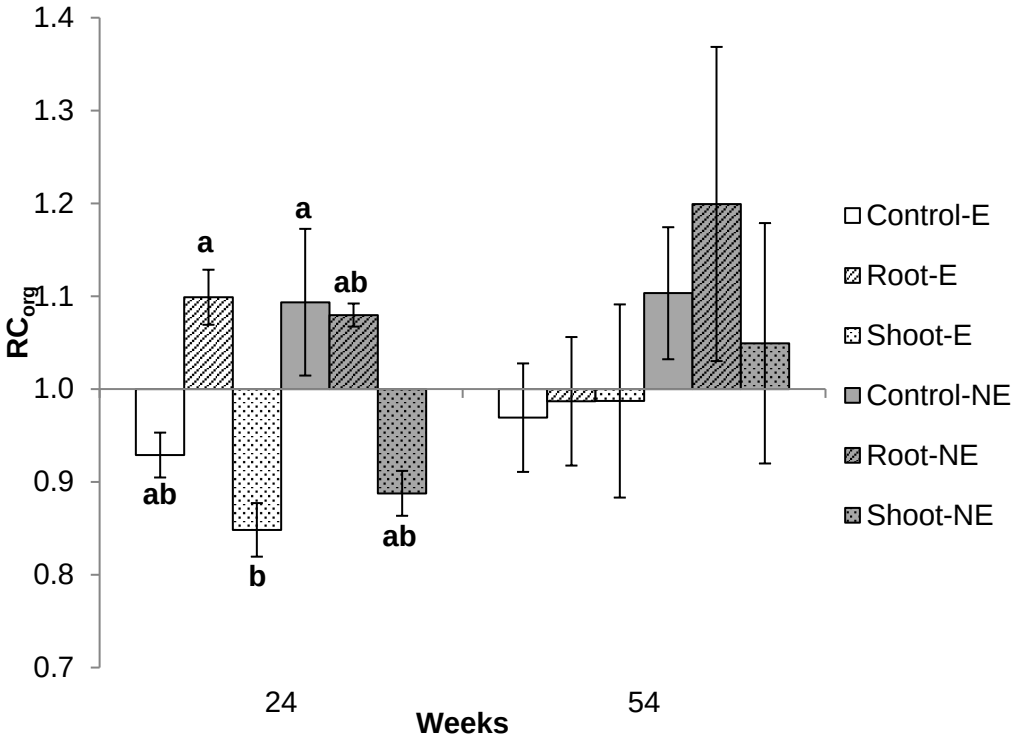

1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
RC org
a
ab
a
ab
ab
b
24
54
Weeks
Control-E
Root-E
Shoot-E
Control-NE
Root-NE
Shoot-NE

Fig.4

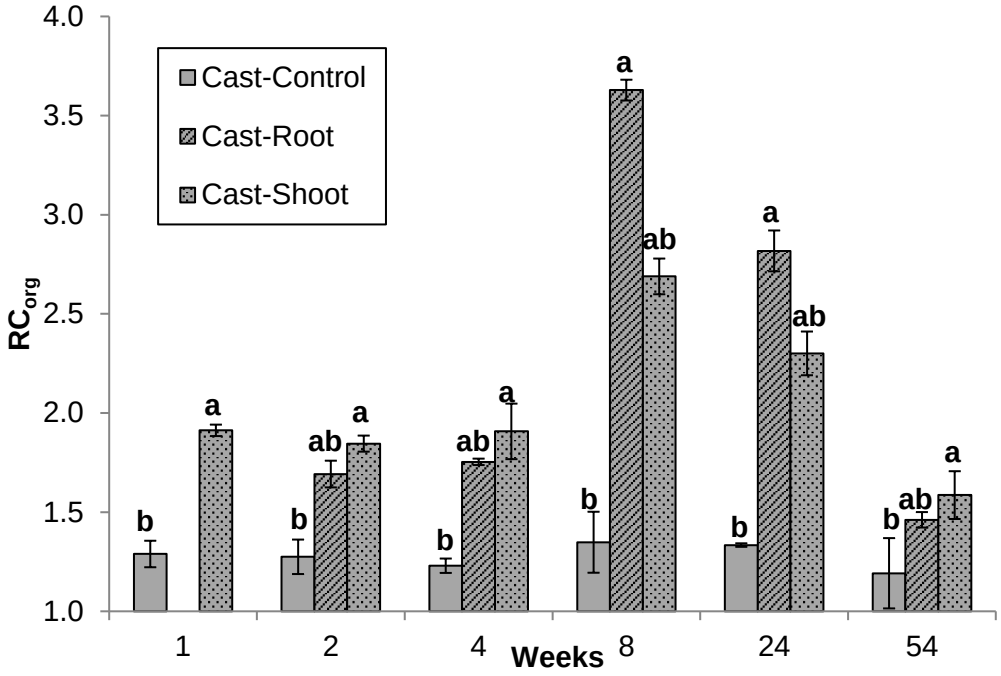

4.0
3.5
3.0
2.5
2.0
1.5
1.0
RCorg
Cast-Control
Cast-Root
Cast-Shoot
b
a
b
ab
a
b
ab
a
b
a
ab
b
a
ab
b
ab
a
1
2
4
8
24
54
Weeks

Fig.5

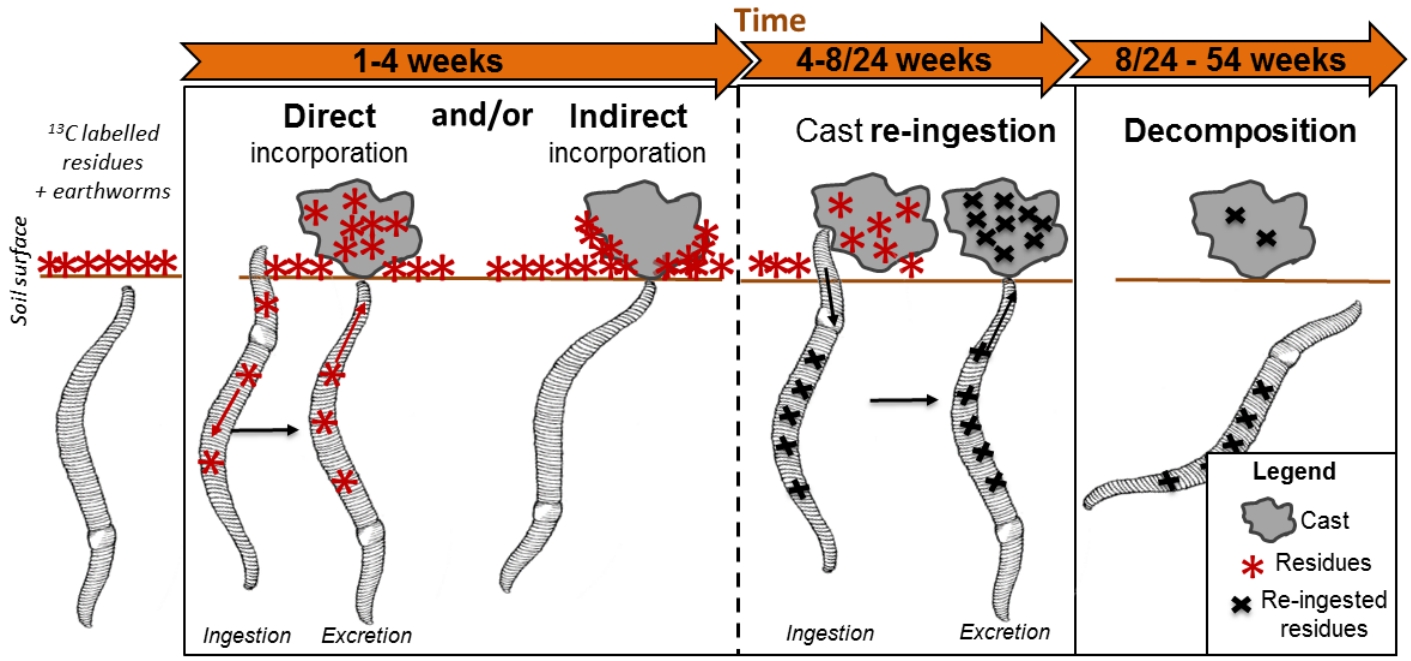

Time
1-4 weeks
4-8/24 weeks
8/24 - 54 weeks
^{13}C labelled residues + earthworms
Soil surface
Direct incorporation
and/or
Indirect incorporation
Cast re-ingestion
Decomposition
Ingestion
Excretion
Ingestion
Excretion
Legend
Cast
Residues
Re-ingested residues

Fig.6

Table S1. Organic carbon content relative to the soil organic carbon content at the beginning of the experiment (RC_{org}) (n=3). Numbers in parentheses indicate the standard deviation for the 3 samples. Bold letters represent statistical results of Kruskal-Wallis test, different letters indicating significant difference between the six mesocosms, for a given time step. Statistically significant differences were observed after 2 and 24 weeks of experiment.

	Earthworms			**No earthworms**		
Week	**Control**	**Root**	**Shoot**	**Control**	**Root**	**Shoot**
1	0.95 (0.03)	1.03 (0.11)	0.85 (0.08)	0.98 (0.07)	1.03 (0.06)	1.02 (0.02)
2	0.91 (0.03) **ab**	1.10 (0.08) **a**	0.98 (0.06) **ab**	0.98 (0.08) **ab**	1.03 (0.05) **ab**	0.85 (0.07) **b**
4	0.96 (0.02)	1.05 (0.03)	0.93 (0.05)	1.01 (0.10)	0.98 (0.07)	0.90 (0.02)
8	1.01 (0.06)	1.03 (0.03)	0.87 (0.13)	0.99 (0.07)	0.96 (0.06)	0.99 (0.04)
24	0.93 (0.02) **ab**	1.10 (0.03) **a**	0.85 (0.03) **b**	1.09 (0.08) **a**	1.08 (0.01) **ab**	0.89 (0.02) **ab**
54	0.97 (0.06)	0.99 (0.07)	0.99 (0.10)	1.10 (0.07)	1.20 (0.17)	1.05 (0.13)