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A. Vidal, K. Quenea, M. Alexis, T.T. Nguyen Tu, J. Mathieu, V. Vaury, S. Derenne

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1 **Fate of <sup>13</sup>C labelled root and shoot residues in soil and anecic earthworm casts: a**  
2 **mesocosm experiment**

3 A.Vidal <sup>a</sup>, K. Quenea <sup>a,\*</sup>, M. Alexis <sup>a</sup>, T. T. Nguyen Tu <sup>a</sup>, J. Mathieu <sup>b</sup>, V. Vaury <sup>b</sup>, S. Derenne <sup>a</sup>

4 <sup>a</sup>UMR Milieux environnementaux, transferts et interactions dans les hydrosystèmes et les  
5 sols (METIS), UMR 7619, UPMC, CNRS, EPHE, 4 place Jussieu, F-75252 Paris, France

6 <sup>b</sup>UMR IEES-Paris, UMR 7618, UPMC, UPEC, CNRS, INRA, IRD, AgroParisTech, 4 place  
7 Jussieu, F-75252 Paris, France

8 \*Corresponding author:

9 +33 1 44 27 42 21

10 Email address: [katell.quenea@upmc.fr](mailto:katell.quenea@upmc.fr)

11 Address: UPMC, Tour 56-66, 4 place Jussieu, 75252 Paris, France

12

13 **Abstract**

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

33 **Keywords**

34 Organic matter dynamics

35 Carbon

36 Isotope labelling

37 Anecic earthworms

39 **1. Introduction**

40 Soil organic matter (SOM) is (1) a source of nutrients for soil organisms, (2) a key  
41 determinant of soil fertility, structuration and evolution and (3) a potential sink of carbon (C) in  
42 a context of CO<sub>2</sub> concentration increase in the atmosphere (Jobbágy and Jackson, 2000).  
43 SOM has been widely studied but the process of its incorporation and dynamics in soils  
44 remains unclear due to its complexity (Stockmann et al., 2013). The main source of SOM is  
45 plant residues, which encompass plant above-ground parts (dead leaves and shoots)  
46 deposited on the soil surface and below-ground parts (dead roots and rhizodeposits) within  
47 the soil (Kögel-Knabner, 2002). Residues can either be mineralized, releasing CO<sub>2</sub> to the  
48 atmosphere, or incorporated into soil in the form of organic compounds. After incorporation,  
49 these compounds can be mineralized, transferred in deeper layers and/or stored in soil.  
50 Residue decomposition and incorporation into soil depend on numerous abiotic and biotic  
51 factors. Abiotic factors mainly correspond to soil climate (temperature and moisture) and  
52 physico-chemical characteristics (texture and clay mineralogy). These factors have a critical  
53 influence on organo-mineral interactions, impacting organic matter decomposition and  
54 accumulation in soil. Biotic factors encompass residue quality, activity of soil fauna and  
55 microorganisms (Cortez, 1998; Oades, 1988). Soil microorganisms, which comprise, among  
56 others, bacteria and fungi, mediate the processes of organic compound mineralization and  
57 transformation in soil. Thus, they have a direct implication on residue decomposition  
58 (Kuzyakov and Blagodatskaya, 2015). Under relatively constant climatic and edaphic  
59 conditions, abiotic factors often influence litter decomposition at large time and space scales,  
60 while biotic factors act at shorter time and space scales (Lavelle et al., 1993; Swift et al.,  
61 1979),  
62 Residue quality (*i.e.* physico-chemical features including chemical composition, anatomical  
63 characteristics, etc.) influences their decomposition by macro and microorganisms (Lavelle et

64 al., 1993). While above-ground biomass has long been considered as the main source for  
65 SOM, roots are currently recognized as essential contributors to stable organic C. The  
66 relative contribution from root and shoot litter to SOM has been the subject of many studies  
67 during the last decades (Balesdent and Balabane, 1996; Comeau et al., 2013; Gale and  
68 Cambardella, 2000; Lu et al., 2003; Mambelli et al., 2011; Puget and Drinkwater, 2001;  
69 Rasse et al., 2005) and it is now recognized that roots decompose at a slower rate compared  
70 to shoots. However, the reasons explaining the slower decomposition of roots and its higher  
71 contribution to soil carbon pool remain debated. Are they driven by the chemical composition,  
72 physical protection and/or physico-chemical protection of roots in soils (Rasse et al., 2005)?  
73 The contribution from these 3 factors on C dynamics is particularly dependent on residue  
74 location in soil (Coppens et al., 2006a, b; Tahir et al., 2016). For example, Coppens et al.  
75 (2006a) found that, after 9 weeks of experiment, new organic C storage in soil was increased  
76 when rapeseed residues were deposited onto the soil surface compared to residues  
77 incorporated in the 0-10 cm layer. In order to investigate the impact of the chemical  
78 compositions of residues, the latter must be placed in the same conditions (Rasse et al.,  
79 2005). Thus, in the present study, both roots and shoots were deposited onto the soil  
80 surface.

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

91 formation of biogenic macroaggregates (Bossuyt et al., 2005), which are generally more  
92 stable than non-biogenic aggregates (Six et al., 2004; Zangerlé et al., 2011). Earthworms are  
93 highly mobile in soil and induce heterogeneous resource distribution (Decaëns et al., 2010).  
94 As the ingested residues contain higher concentration of organic C than soil, they modify the  
95 incorporation and the stock of C in aggregates (Arai et al., 2013; Fonte et al., 2012, 2007;  
96 Hong et al., 2011) and along the soil profile (Jégou et al., 2000). The net impact of  
97 earthworms on soil C strongly varies depending on the studied spatial and time scale. During  
98 gut transit and in fresh casts, mineralization of organic C is accelerated. Indeed, in  
99 earthworm gut, the physical protections of the organic matter originally present in the soil are  
100 broken and plant residues are fragmented (Lavelle and Martin, 1992). Moreover, the  
101 presence of water and intestinal mucus creates conditions favorable to mineralization by the  
102 microorganisms present in the earthworm gut and fresh casts (Brown et al., 2000; Drake and  
103 Horn, 2007; Martin et al., 1987; Vidal et al., 2016a). At month or year scale, drying and  
104 ageing casts, lead to C stabilization (Brown et al., 2000; Lavelle and Martin, 1992; Martin,  
105 1991). Residue decomposition rate by earthworms also depends on residue palatability  
106 (Cortez et al., 1989). For example, it has generally been observed that earthworms fed  
107 mainly on shoots rather than roots (Bouché and Kretzschmar, 1974; Curry and Schmidt,  
108 2007). However, little is known about the incorporation and decomposition of root residues  
109 by earthworms in soil (Curry and Schmidt, 2007; Zangerlé et al., 2011).

110 Artificial isotopic labelling of residues allows precise monitoring of the residues introduced (at  
111 a given date and place) and distinguishing the added organic matter from that initially present  
112 in the soil (Brüggemann et al., 2011; Comeau et al., 2013; Fahey et al., 2013; Klumpp et al.,  
113 2007; Thompson, 1996). Studies using artificial labelling have focused on **(1)** the impact of  
114 residue quality (roots vs. shoots or plant species) on organic C incorporation and  
115 decomposition into soil (Blair et al., 2005; Comeau et al., 2013; Mambelli et al., 2011; Rubino  
116 et al., 2010; Williams et al., 2006) or **(2)** the influence of earthworms on shoot decomposition  
117 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 <sup>13</sup>C labelled and deposited onto the soil surface.

## 124 **2. Materials and Methods**

### 125 *2.1. Soil characteristics and mesocosm experiment*

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

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

### 147 *2.2. Earthworms and root-shoot*

148 The common anecic earthworm *Lumbricus terrestris* L. was used. Earthworms were provided  
149 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  $26.8 \pm 0.5$  g per container  
153 (around 48 ind./m<sup>2</sup> and 224 g fresh wt/m<sup>2</sup>). 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<sup>2</sup> (50-100 g fresh wt/m<sup>2</sup>). 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 <sup>13</sup>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 <sup>13</sup>CO<sub>2</sub>  
163 enriched atmosphere. Immediately after <sup>13</sup>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  $\delta^{13}\text{C}$  values were 1324  
166 ‰ ( $\pm 43$ ) and 1632 ‰ ( $\pm 16$ ) 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

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

#### 187 2.4. Organic carbon and $\delta^{13}\text{C}$ analyses

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

199 Isotopic composition of samples is expressed in  $\delta^{13}\text{C}$  relative to the PDB standard (**Equation**  
200 **1**):

$$201 \quad \delta^{13}\text{C} (\text{‰}) = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] * 1000 \quad (1)$$

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

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

$$205 \quad C_{\text{lab}} (\%) = [(\delta_s - \delta_c) / (\delta_l - \delta_c)] * 100 \quad (2)$$

206 Where  $\delta_s$  is the  $\delta^{13}\text{C}$  value of soil samples or of casts with labelled litter,  $\delta_c$  is the  $\delta^{13}\text{C}$  value  
207 of the control soil sample or control casts without litter,  $\delta_l$  is the  $\delta^{13}\text{C}$  value of the labelled  
208 litter.

209 In order to overcome the initial variability of the soil organic C measured in the different meso-  
210 ecosms, all the organic C contents were normalized using equation 3:

$$211 \quad \text{RC}_{\text{org } x} = C_x / C_0 \quad (3)$$

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

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

## 221 2.5. Statistical analysis

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

## 226 3. Results

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

### 239 3.1. Carbon derived from root and shoot residues

#### 240 3.1.1. Soil samples

241 The C derived from labelled litter ( $C_{lab}$ ) increased continuously in the 0-20 cm soil layer along  
242 the year of experiment, reaching between 0.96 and 11.4 % after 54 weeks, for Shoot-E and  
243 Root-NE, respectively (**Fig.2A**). From the beginning of the experiment to the 8<sup>th</sup> week, there  
244 were no significant differences between mesocosms, with a mean  $C_{lab}$  of 0.40 %. After 24  
245 weeks,  $C_{lab}$  was significantly lower for Shoot-E (0.85 %) than Root-E (3.00 %). After 54  
246 weeks,  $C_{lab}$  was significantly higher in Root-NE (11.4 %) than in the other mesocosms. Thus,  
247 two general trends could be observed after 24 and 54 weeks: (1)  $C_{lab}$  in Root-NE and Shoot-  
248 NE tended to be higher than Root-E and Shoot-E, respectively and (2)  $C_{lab}$  in Root-E and NE  
249 tended to be higher than Shoot-E and NE, respectively. Contrary to other mesocosms,  $C_{lab}$  in  
250 Shoot-E barely varied from 8<sup>th</sup> week until the end of experiment, with values of 1.13, 0.85 and  
251 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 ( $RC_{org} \leq 1$ ), 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 ( $RC_{org} \geq 1$ ).  $RC_{org}$  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 ( $RC_{org} \gg 1$ ) (**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 24<sup>th</sup> 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**).

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

#### 329 4.2. *Effect of earthworms on residue fate*

#### 330 4.2.1. *Earthworms tended to accelerate residue incorporation and decomposition*

331 No more residues were observed at the soil surface after the year of experiment in the  
332 mesocosm containing earthworms compared to the one without earthworms. Thus, residues  
333 had probably been incorporated in soil or casts and/or mineralized. Although absolute  
334 quantification was not achieved, it can reasonably be suggested that earthworms accelerated  
335 decomposition of residues in soil. Thus, part of the  $^{13}\text{C}$  was probably removed from the  
336 system in the form of  $^{13}\text{CO}_2$  (microbial and/or earthworm respiration). Indeed, from 6 months  
337 of experimentation, the  $C_{\text{lab}}$  in soil tended to be lower in the presence of earthworms, for both  
338 root and shoot residues (**Fig.2A**). This can be explained by the direct impact of earthworms  
339 which assimilate and mineralize part of the labelled organic C during the ingestion of  
340 residues, as suggested by Daniel (1991). Moreover, decomposition and mineralization are  
341 governed by microorganisms acting directly on particulate residues, or on soluble  
342 compounds in the soil matrix (Gaillard et al., 1999). In the presence of earthworms, this  
343 phenomenon can be amplified as the ingestion of residues by earthworms generally favors  
344 microbial activity (Amador and Görres, 2007; Brown, 1995; Frouz et al., 2011; Parle, 1963;  
345 Vidal et al., 2016a).

#### 346 4.2.2. *Casts – a key role in residue decomposition*

347 Cast and soil samples presented different trends of organic C incorporation and  
348 decomposition (fig. 2.A vs. 3 and fig 4 vs. 5). As expected, the  $C_{\text{lab}}$  and  $C_{\text{org}}$  are higher in  
349 casts compared to soil. The organic C content of casts was 1.3 to 3.6 higher compared to the  
350 soil. Similarly, Mariani et al. (2007) observed that the C content in anecic casts collected in  
351 burrows of a Colombian pasture, was twice higher compared to bulk soil. This can be  
352 explained by the feeding behavior of earthworms, especially anecic ones, which incorporate  
353 fresh residues and/or organic-rich compounds in casts (Decaëns et al., 1999; Frouz et al.,  
354 2011; Hong et al., 2011; Zangerlé et al., 2011; Zhang et al., 2003). The maximum value for  
355  $C_{\text{lab}}$  (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  $RC_{org}$  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

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

#### 396 4.3. *Effect of earthworms on the fate of root vs. shoot*

##### 397 4.3.1. *Earthworms minimized the diverging fate of root and shoot residues*

398 In soil samples, the difference in  $C_{\text{lab}}$  and  $\text{RC}_{\text{org}}$  between root and shoot mesocosms with  
399 earthworms, after 54 weeks, was weaker than in the absence of earthworms (**Fig.2A and 3**),  
400 underlying their ability to enhance root decomposition, as mentioned by Cortez and Bouche  
401 (1992). Hopkins et al. (2005) have compared decomposition of natural tobacco roots and  
402 modified tobacco roots with altered lignin structure and composition (*i.e.* less resistant to  
403 decomposition). They found that earthworms reduced the difference between both residue  
404 types for C mineralization. Earthworms can enhance the decomposition of recalcitrant  
405 residues and smooth the difference between residues of various qualities, probably by  
406 facilitating the access of plant residues to microorganisms. Indeed, a mutualistic relation is  
407 maintained between bacteria and earthworms in their gut, which enabled the decomposition  
408 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

436 studies (Kuzyakov, 2010). Indeed, microorganisms depend on the energy present in fresh  
437 residues to decompose the recalcitrant organic matter present in the soil (Fontaine et al.,  
438 2011, 2007). For example, Lu et al. (2003) observed that the simple presence of fresh  
439 residues, either root or shoot, favored the degradation of native soil organic matter after 8  
440 weeks of experiment, probably due to the stimulation of the microbial activity. The soil fauna,  
441 including earthworms, was also mentioned as inducing a priming effect in soil by Lavelle et  
442 al. (1995). More recently, Coq et al. (2007) showed that total soil C had decreased after 5  
443 months of incubation of rice or soybean shoot residues, in the presence of endogeic  
444 earthworms. Fontaine et al. (2011) measured the effect of a  $^{13}\text{C}$  labelled cellulose input in a  
445 soil during 161 days. They showed that, after one month, respiration of unlabelled  $\text{CO}_2$   
446 reached a maximum, revealing a priming effect. A continuous and slow decrease in  
447 unlabelled  $\text{CO}_2$  respiration was then observed until the end of the experiment. This decrease  
448 in priming effect is in agreement with the present results showing that, after one year,  $\text{RC}_{\text{org}}$   
449 was around 1 for Shoot-E and Shoot-NE. The suggested priming effect observed for the  
450 mesocosm containing shoots was not observed for the one containing roots. Indeed, during  
451 the first 6 months of experiment, the  $\text{C}_{\text{org}}$  tended to be higher in mesocosm containing roots  
452 than at the beginning of the experiment ( $\text{RC}_{\text{org}} \geq 1$ ) Thus, in the present study, the priming  
453 effect induced by root residues seems to be lower compared to that of shoot residues during  
454 the first six months of experiment. This priming might also be compensated by the higher  
455 preservation of root-derived carbon coupled to reduced activation of microbial activity  
456 compared to shoot residues (Shi et al., 2013). This process is smoothed after one year;  
457 supporting comment from Rasse et al. (2005) that the preferential shoot priming effect on  
458 SOM mineralization cannot explain the higher root-derived C in soil, as observed after one  
459 year on **Fig.2A**. It has to be noted that the increase in  $\text{RC}_{\text{org}}$  observed for Control-NE after 24  
460 and 54 weeks can be explained by the development of mosses in this mesocosm from 24  
461 weeks until the end of the experiment, potentially increasing the  $\text{C}_{\text{org}}$  concentration in the 0-  
462 20 cm soil layer.

## 463        **5. Conclusion**

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

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

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496

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

<b>Soil characteristics</b>	<b>Values</b>
Clay (< 2 µm)	189 g.kg <sup>-1</sup>
Loam (2-50 µm)	248 g.kg <sup>-1</sup>
Sand (50-2000 µm)	563 g.kg <sup>-1</sup>
Total carbonates (CaCO <sub>3</sub> )	19.0 g.kg <sup>-1</sup>
CEC Metson	9.90 cmol.kg <sup>-1</sup>
Organic Carbon	12.1 g.kg <sup>-1</sup>
Nitrogen	1.30 g.kg <sup>-1</sup>
δ <sup>13</sup> C	-28.1 ‰

498

499

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

	$\delta^{13}\text{C}$ (‰)	C ( $\text{g.kg}^{-1}$ )	N ( $\text{g.kg}^{-1}$ )	C:N
<b>Root</b>	1324 (42)	402 (40)	13.1 (1.2)	30.7 (3.1)
<b>Shoot</b>	1632 (16)	408 (6.3)	27.7 (2.3)	14.8 (1.1)

502

503

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

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

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

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

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

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

531

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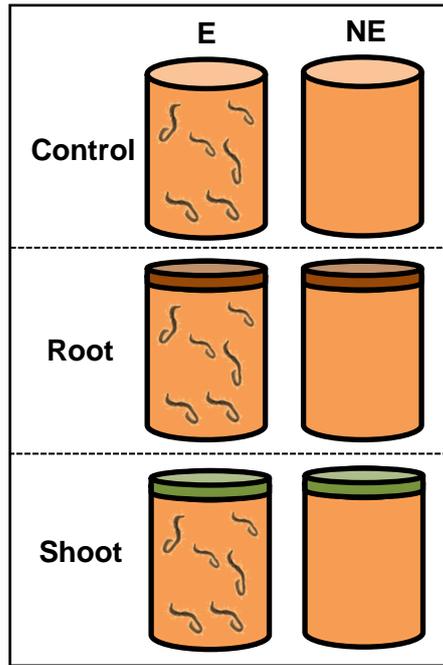


Fig.1

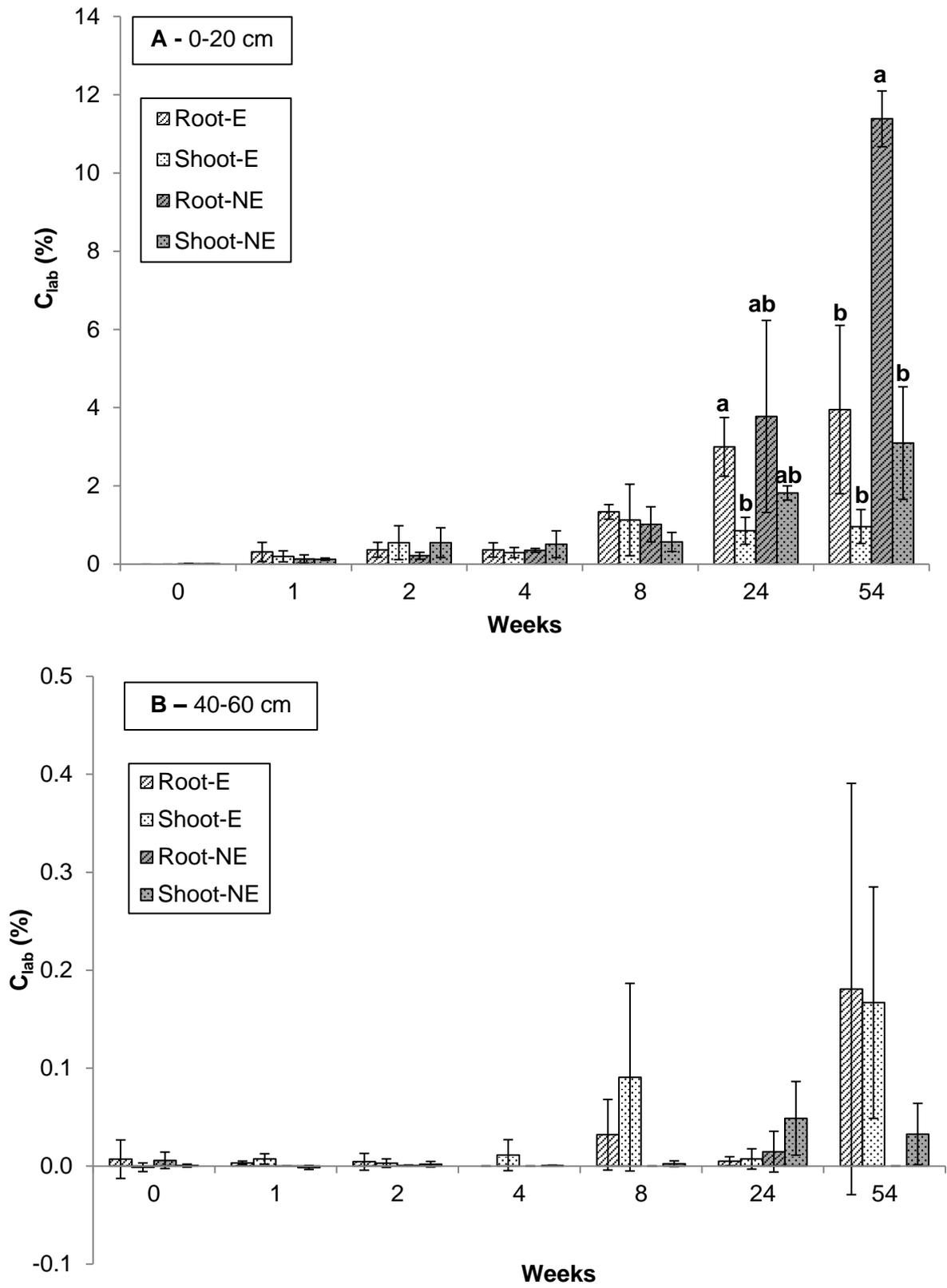
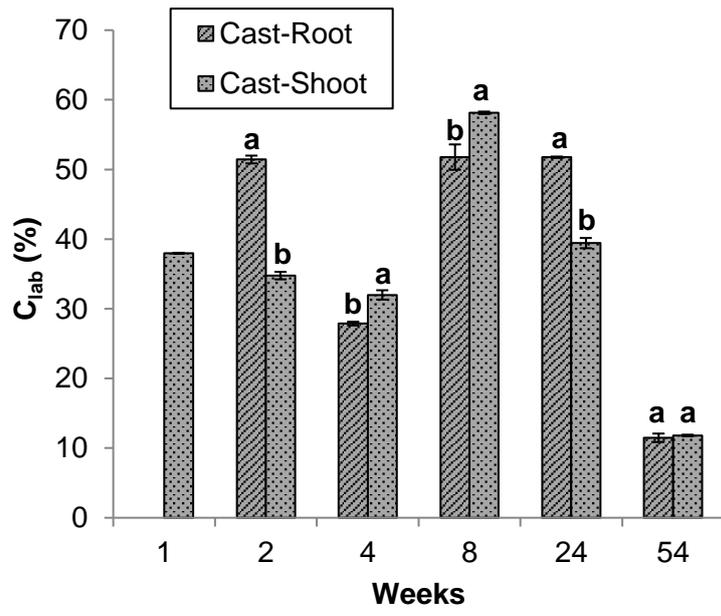


Fig. 2





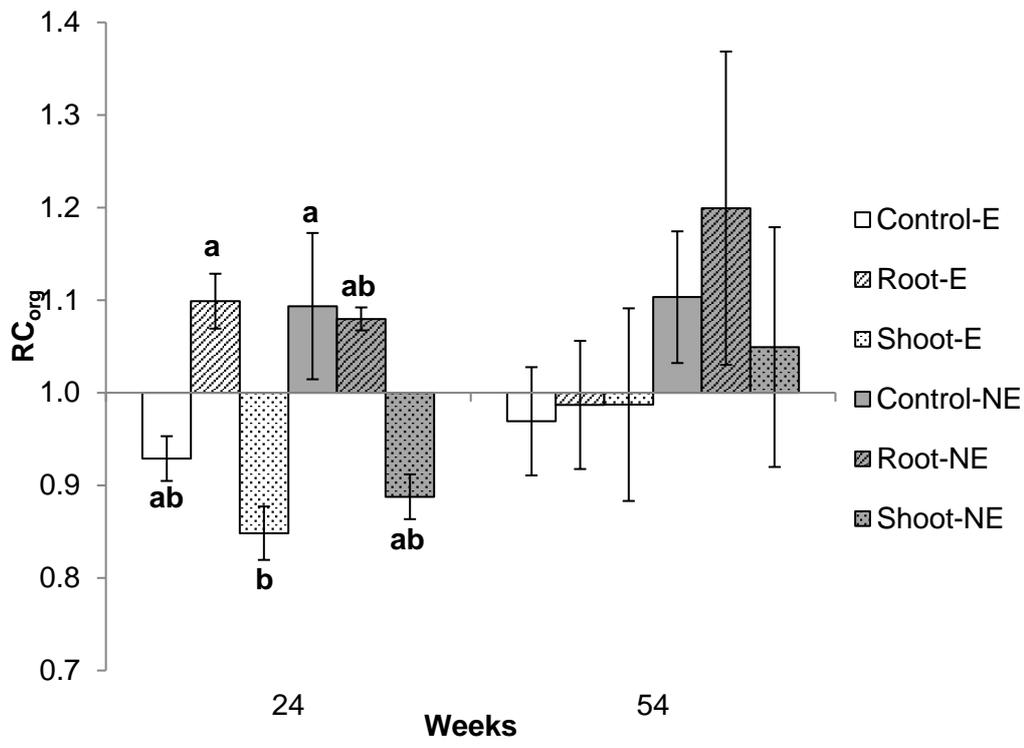


Fig.4

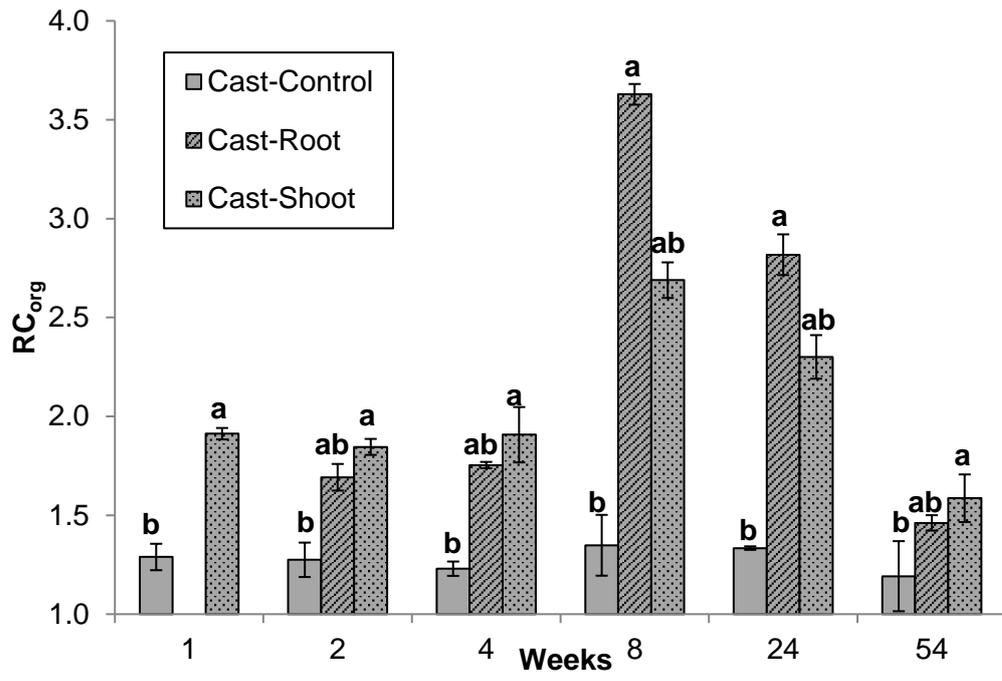


Fig.5

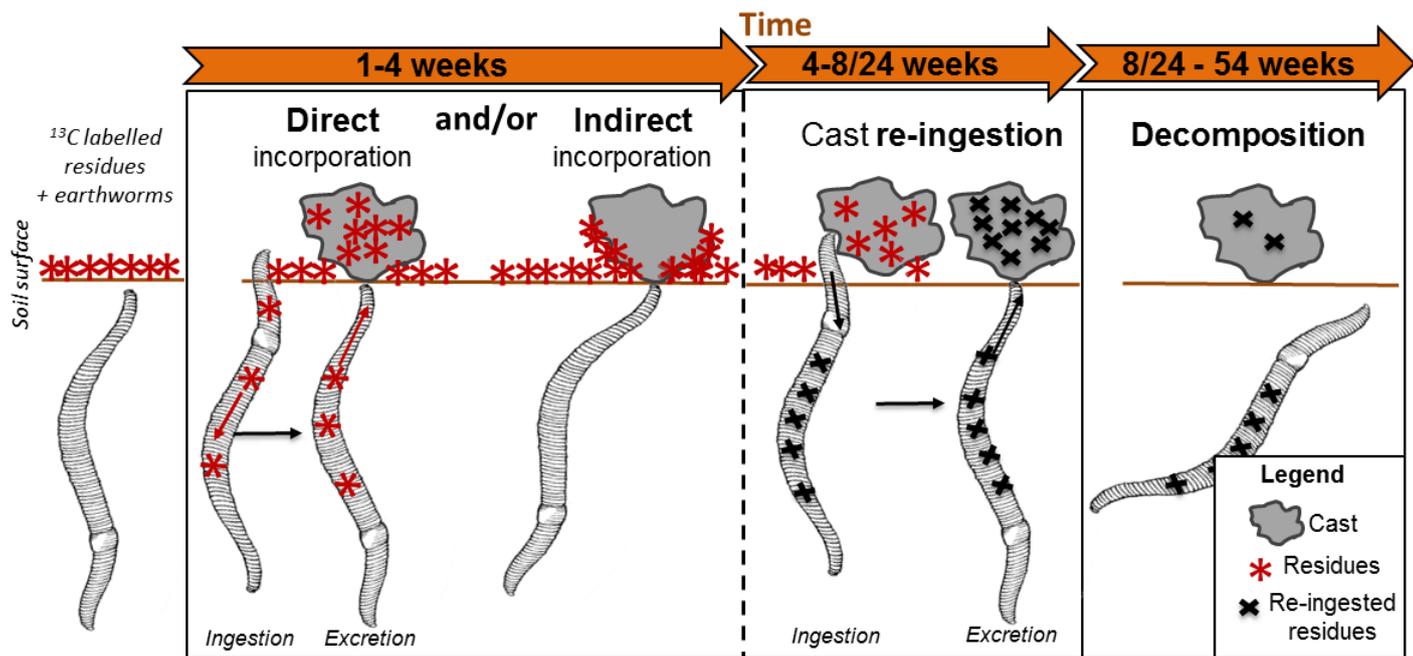


Fig.6

**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.

Week	Earthworms			No earthworms		
	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) <b>ab</b>	1.10 (0.08) <b>a</b>	0.98 (0.06) <b>ab</b>	0.98 (0.08) <b>ab</b>	1.03 (0.05) <b>ab</b>	0.85 (0.07) <b>b</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) <b>ab</b>	1.10 (0.03) <b>a</b>	0.85 (0.03) <b>b</b>	1.09 (0.08) <b>a</b>	1.08 (0.01) <b>ab</b>	0.89 (0.02) <b>ab</b>
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)