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

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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}C labelled and deposited onto the soil sur-
19 face. Incorporation of ^{13}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_{lab}). Roots and shoots were incorporated in the 0-20 cm
23 soil layer during the year of experiment, C_{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_{lab} from 34.8 to 51.4 %
27 after 2 weeks). In the soil, a significant effect of earthworms on the C_{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 CO₂ 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 CO₂ 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 ¹³C 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 ± 0.5 g per container
153 (around 48 ind./m² and 224 g fresh wt/m²). 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./m² (50-100 g fresh wt/m²). 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 ¹³C 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 ¹³CO₂
163 enriched atmosphere. Immediately after ¹³C 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 ‰ (± 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

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_{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 μL 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_{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 δ_s is the $\delta^{13}\text{C}$ value of soil samples or of casts with labelled litter, δ_c is the $\delta^{13}\text{C}$ value
207 of the control soil sample or control casts without litter, δ_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 8th 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 8th 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 ($RC_{org} >> 1$) (**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**).

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 %,
307 respectively. 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}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_{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_{lab} and C_{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_{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}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_{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}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}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_{lab} and RC_{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}C labelled cellulose input in a
445 soil during 161 days. They showed that, after one month, respiration of unlabelled CO_2
446 reached a maximum, revealing a priming effect. A continuous and slow decrease in
447 unlabelled 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, RC_{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 C_{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 RC_{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 C_{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

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}
$\delta^{13}\text{C}$	-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 (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)

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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533

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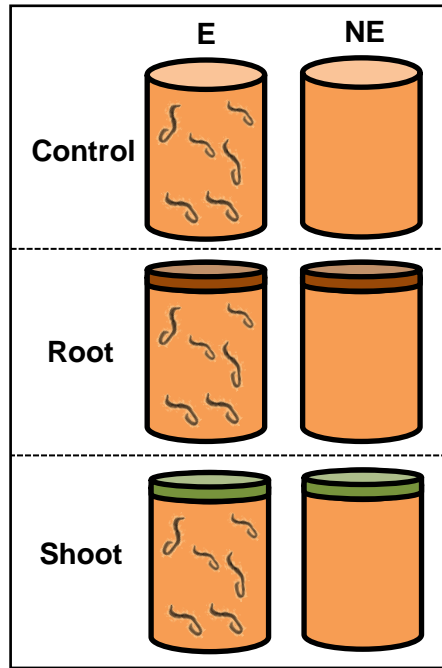


Fig.1

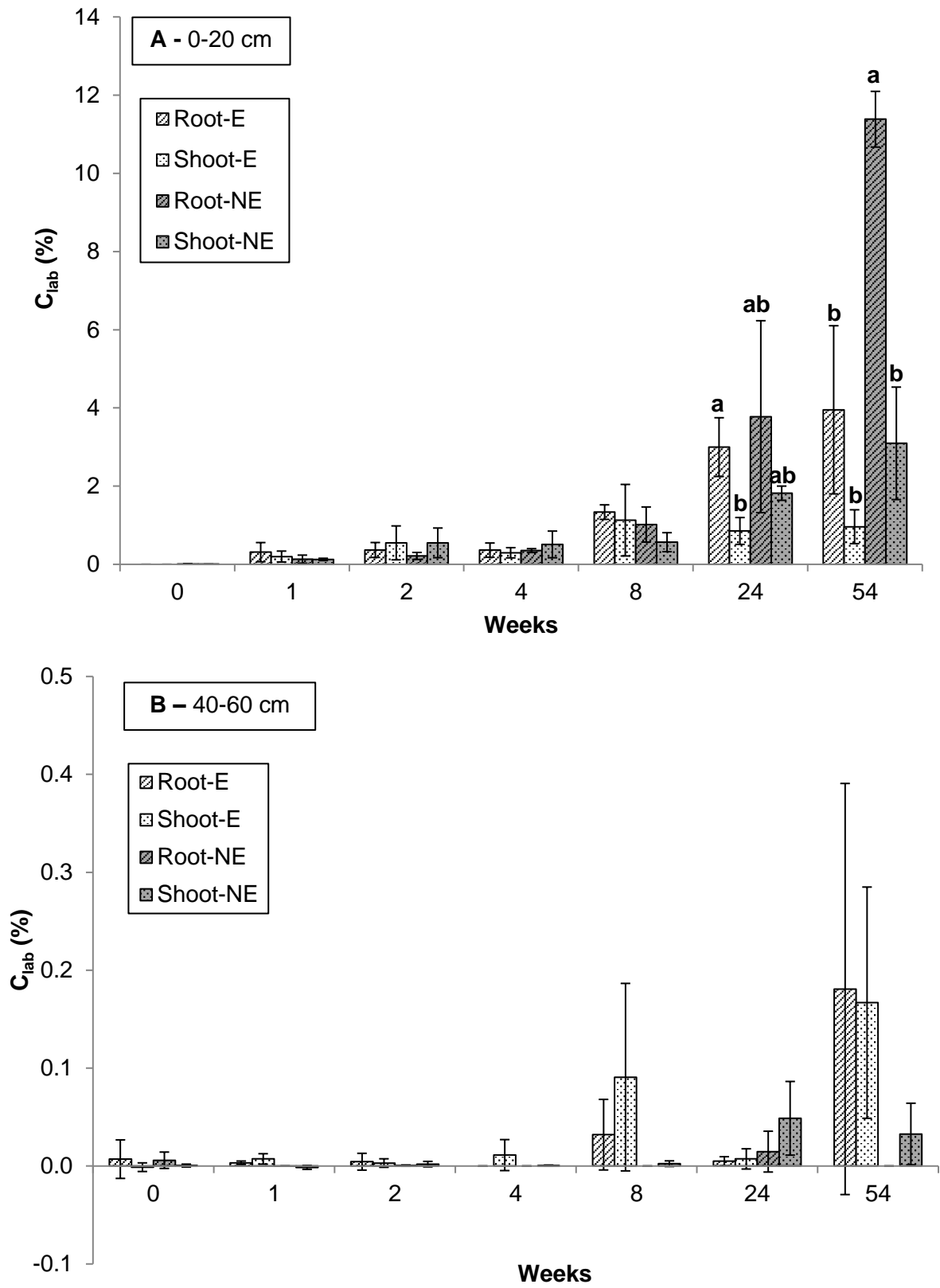
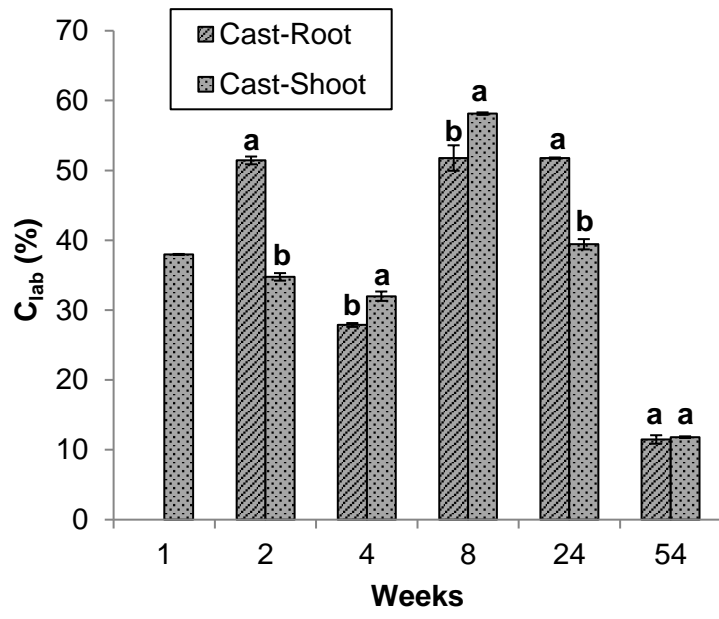


Fig. 2



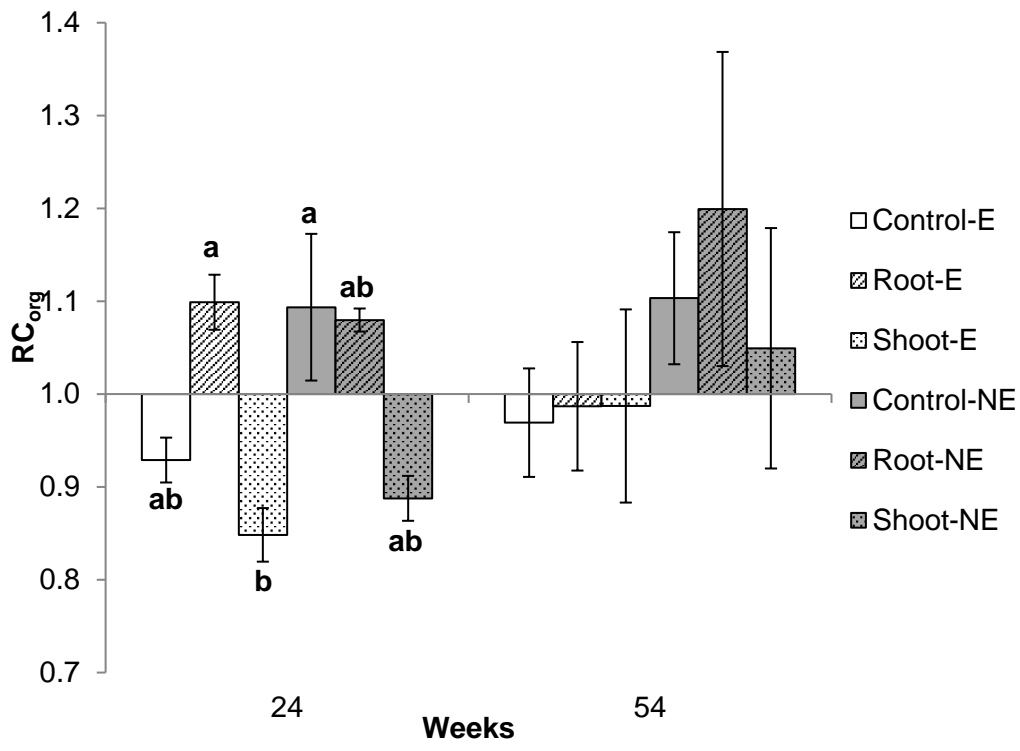


Fig.4

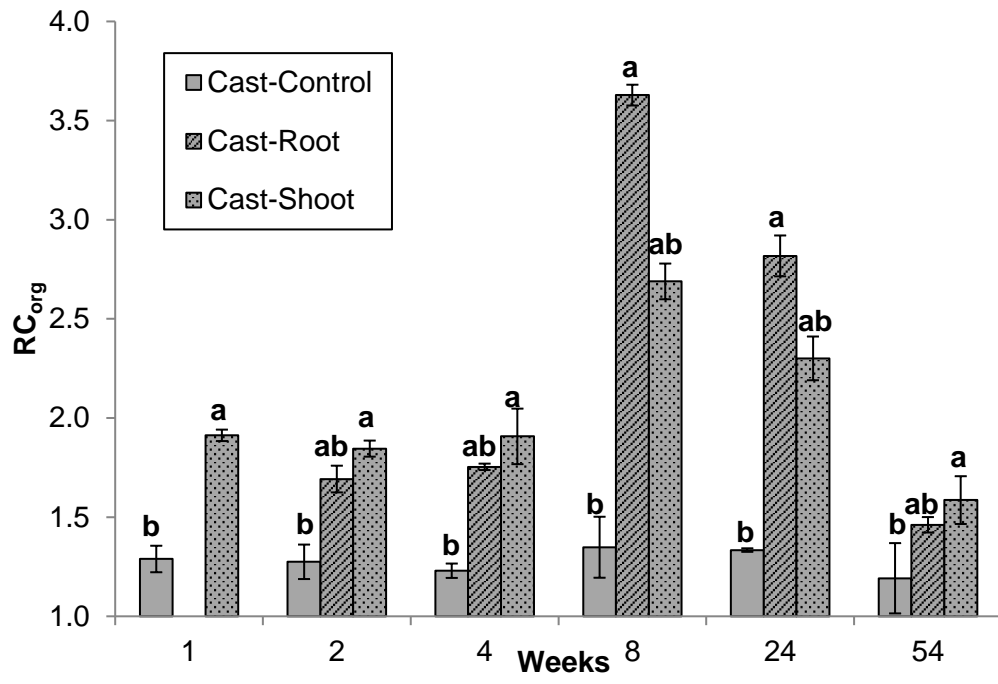


Fig.5

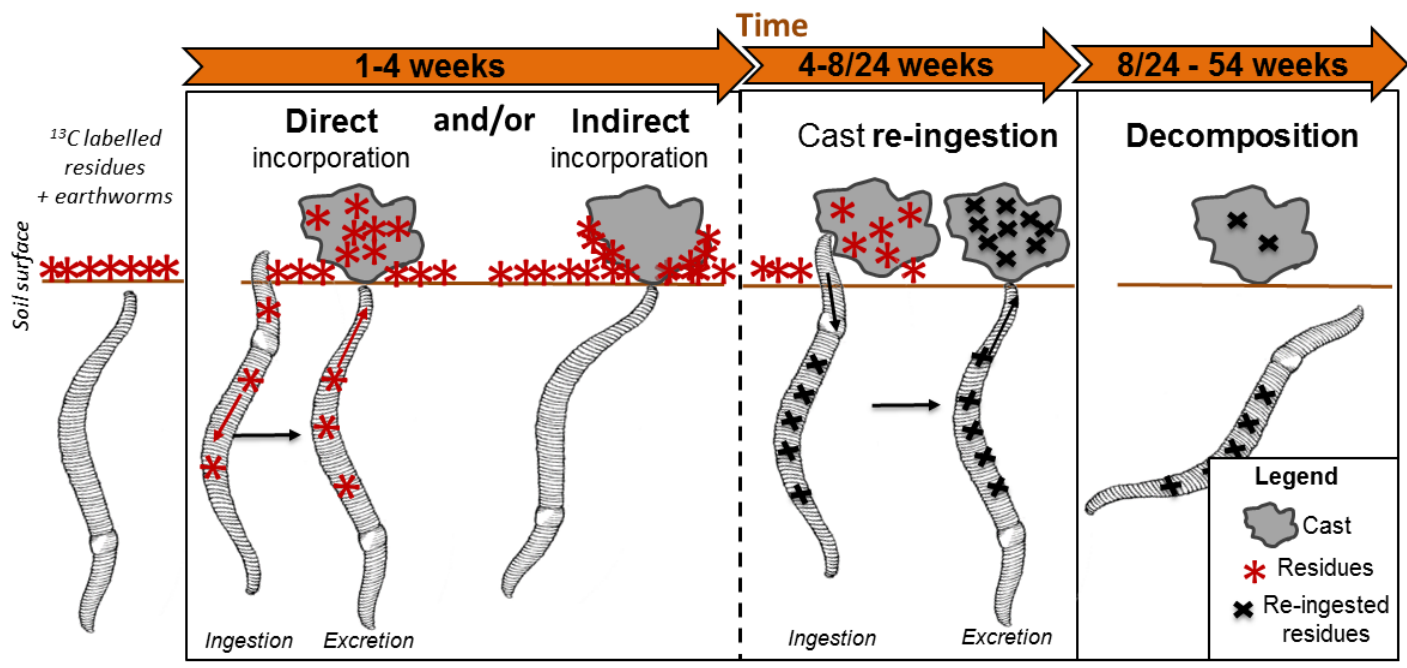


Fig.6

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) 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)