# Fate of 13 C labelled root and shoot residues in soil and anecic earthworm casts: A mesocosm experiment

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

1  **Fate of $^{13}$C labelled root and shoot residues in soil and anecic earthworm casts: a**

2  **mesocosm experiment**

3  A.Vidal [a], K. Quenea [a,*], M. Alexis [a], T. T. Nguyen Tu [a], J. Mathieu [b], V. Vaury [b], S. Derenne [a]

4  [a]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  [b]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

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

12

## Abstract

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

## Keywords

38    Casts

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 $CO_2$ 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 $CO_2$ 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

4

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 $^{13}C$ labelled and deposited onto the soil surface.

## 2. Materials and Methods

### 2.1. Soil characteristics and mesocosm experiment

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

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

### 2.2. Earthworms and root-shoot

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

150 weight of 4.5 g. They were pre-conditioned during one week in the soil used for the

151 experiment. At the beginning of the experiment, 6 earthworms per container were deposited

152 on the top of three of the mesocosms. Mean living mass was 26.8 ± 0.5 g per container

153 (around 48 ind./$m^2$ and 224 g fresh wt/$m^2$). This low density was chosen in accordance to

154 Fründ et al. (2010) review, showing that the typical earthworm density (all species

155 concerned) in temperate pastures was 300-1000 ind./$m^2$ (50-100 g fresh wt/$m^2$). Thus, as

156 *L.terrestris* is a territorial earthworm, these choices favored their survival in conditions closest

157 to reality. By the end of the experiment, the soil volume extracted during the year of sampling

158 represents approximatively 6 % of the initial soil volume. Thus, it is assumed that earthworms

159 were not limited in their movement along the experiment.

160 Plants of Italian Ryegrass (*Lolium multiflorum* Lam.) were artificially labelled with $^{13}$C at the

161 Atomic Energy and Alternative Energies Commission (CEA) in Cadarache (France). In order

162 to obtain homogeneous material, plants were grown under a controlled and constant $^{13}CO_2$

163 enriched atmosphere. Immediately after $^{13}$C labelling, fresh roots and shoots were separated,

164 dried and chopped. They were chopped during 40 seconds with a laboratory blender (*Waring*

165 *Commercial*) in order to obtain residues of few millimeters. The mean δ$^{13}$C values were 1324

166 ‰ (± 43) and 1632 ‰ (± 16) for the roots and shoots, respectively (**Table 2**). Few hours after

167 earthworms had penetrated the soil, roots and shoots were similarly applied on the soil

168 surface. Thus, the impact of the chemical composition of roots vs shoots was tested,

169 excluding any influence of their location in the soil. Two mesocosms each contained 250 g of

170 shoots, two additional contained 250 g of roots and the latest two were used as control.

171 Curry and Schmidt (2007) reviewed that *L.terrestris* could ingest 17 mg of dry matter/g fresh

172 wt/day. However, the quantity of litter ingested can increase when the quality of litter

173 decreases. Thus, 250 g of litter (i.e. around 25 mg of dry matter/g fresh wt/day) was chosen

174 as a good compromise to favor earthworm survival.

175 *2.3. Sampling and analyses*

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 $\delta^{13}C$ analyses*

188  Organic C ($C_{org}$) and $\delta^{13}C$ 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 $\delta^{13}C$ relative to the PDB standard (**Equation**

200  **1**):

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

202    Where $R$ is the $^{13}C/^{12}C$ 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    $$C_{lab} (\%) = [(\delta_s - \delta_c) / (\delta_l - \delta_c)] * 100 \qquad (2)$$

206    Where $\delta_s$ is the $\delta^{13}C$ value of soil samples or of casts with labelled litter, $\delta_c$ is the $\delta^{13}C$ value

207    of the control soil sample or control casts without litter, $\delta_l$ is the $\delta^{13}C$ value of the labelled

208    litter.

209    In order to overcome the initial variability of the soil organic C measured in the different mes-

210    ocosms, all the organic C contents were normalized using equation **3**:

211    $$RC_{org\ x} = C_x / C_0 \qquad (3)$$

212    Where $RC_{org\ x}$ 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 α = 0.05.

**3. Results**

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

*3.1. Carbon derived from root and shoot residues*

*3.1.1. Soil samples*

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

252   ence of $C_{lab}$ between mesocosms in the 40-60 cm soil layer during the year of experiment

253   (**Fig.2B**). However, at 54 weeks $C_{lab}$ reached 0.2 % in Root-E and Shoot-E whereas it re-

254   mained lower than 0.05 % without earthworms.

*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.

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

*3.2.1. Soil samples*

264   Due to the very low litter C incorporation for the 40-60 cm soil layer, the following of this sec-

265   tion is focused on the 0-20 cm layer. In mesocosms containing shoots, with or without earth-

266   worms, organic C content tended to be lower than the organic C content before the experi-

267   ment ($RC_{org\ x} \leq 1$), for all time steps (**Fig.4 and Table S1**). On the contrary, in mesocosms

268   containing roots, with or without earthworms, organic C content tended to be higher than at

269   the beginning of the experiment ($RC_{org\ x} \geq 1$). $RC_{org}$ reached 1.2 after 54 weeks in Root-NE.

270   $RC_{org}$ was significantly different for Root-E and Shoot-E after 24 weeks (1.1 *vs* 0.85).This

271   observation was also made between Root-NE and Shoot-NE but to a lesser extent. For ex-

272   ample, after 24 weeks, $RC_{org}$ was 1.1 and 0.89 for Root-NE and Shoot-NE, respectively

273   (**Fig.4**).

*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

12

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    Schauermann, 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 $^{13}CO_2$ (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 $RC_{org\,x}$ results (**Fig. 3** and **5**). After a strong increase within the

360  first week of experiment, both $C_{lab}$ and $RC_{org}$ in casts remained stable during the four

361  following weeks. In this phase, earthworms have probably combined direct and indirect

362  incorporation of residues. During direct incorporation, residues are ingested and pass

363  through the earthworm gut (Shipitalo and Protz, 1989). Indirect incorporation, also called

364  'pre-oral litter decomposition', consists in a 'ploughing-in' of residues, *i. e.* casts are

365  deposited on residues which are not ingested, initiating microbial decomposition (Cortez and

366  Bouché, 1998). An increase in $C_{lab}$ and $RC_{org}$ was observed between 4 and 8 weeks. After

367  few months, earthworms are able to re-ingest their casts in which residues have been

368  partially decomposed by microorganisms (Swift et al., 1979; Jiménez et al., 1998; Brown et

369  al., 2000). Thus, between 4 and 8 weeks of experiment, the 'ploughing-in' and ingestion of

370  undecomposed labelled residues still present at the soil surface were probably associated to

371  the re-ingestion of previously labelled casts (from 1 to 4 weeks), increasing the $C_{lab}$. During

372  the last 6 months, $C_{lab}$ and $RC_{org}$ highly decreased, no more litter was present at the surface

373  of the mesocosms containing earthworms and litter, and surface casts were starting ageing

374  and drying. Thus, all the residues have been either incorporated in the soil and casts, or

375  mineralized. The decrease in $C_{lab}$ and $RC_{org}$ indicated that labelled residues incorporated in

376  casts sampled at 54 weeks have been partially mineralized. This decomposition was

377  enhanced by bacteria and fungi as observed on Cast-Shoot NanoSIMS images after 24

378  weeks of experiment (Vidal et al., 2016a).

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

380  A slight increase in $C_{lab}$ was observed in the 40-60 cm soil layer during the 54 weeks in the

381  presence of earthworms, although not statistically significant (**Fig.2B**). This was observed in

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). Jegou 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 ($RC_{org} \geq 1$)  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.

## 5. Conclusion

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

489 presence of earthworms, they further require field-based experiment, including other

490 earthworm ecological categories, to be generalized.

491 **Acknowledgements**

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$^{-1}$ |
| δ$^{13}$C | -28.1 ‰ |

498

499

22

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

501  deviation for the 13 samples.

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

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

532 **References**

533

534 Amador, J.A., Görres, J.H., 2007. Microbiological characterization of the structures built by
535       earthworms and ants in an agricultural field. Soil Biol. Biochem. 39, 2070–2077.
536 Arai, M., Tayasu, I., Komatsuzaki, M., Uchida, M., Shibata, Y., Kaneko, N., 2013. Changes in soil
537       aggregate carbon dynamics under no-tillage with respect to earthworm biomass revealed by
538       radiocarbon analysis. Soil Tillage Res. 126, 42–49.
539 Balesdent, J., Balabane, M., 1996. Major contribution of roots to soil carbon storage inferred from
540       maize cultivated soils. Soil Biol. Biochem. 28, 1261–1263.
541 Barois, I., Lavelle, P., 1986. Changes in respiration rate and some physicochemical properties of a
542       tropical soil during transit through *Pontoscolex corethrurus* (glossoscolecidae, oligochaeta).
543       Soil Biol. Biochem. 18, 539–541.
544 Beuch, S., Boelcke, B., Belau, L., 2000. Effect of the Organic Residues of Miscanthus × giganteus on
545       the Soil Organic Matter Level of Arable Soils. J. Agron. Crop Sci. 184, 111–120.
546 Bird, J., Torn, M., 2006. Fine Roots vs. Needles: A Comparison of 13C and 15N Dynamics in a
547       Ponderosa Pine Forest Soil. Biogeochemistry 79, 361–382.
548 Blair, N., Faulkner, R., Till, A., Sanchez, P., 2005. Decomposition of 13C and 15N labelled plant residue
549       materials in two different soil types and its impact on soil carbon, nitrogen, aggregate
550       stability, and aggregate formation. Aust J Soil Res 43, 873–886.
551 Blouin, M., Hodson, M.E., Delgado, E.A., Baker, G., Brussaard, L., Butt, K.R., Dai, J., Dendooven, L.,
552       Peres, G., Tondoh, J.. E., Cluzeau, D., Brun, J.-J., 2013. A review of earthworm impact on soil
553       function and ecosystem services. Eur. J. Soil Sci. 64, 161–182. doi:10.1111/ejss.12025
554 Bossuyt, H., Six, J., Hendrix, P.F., 2005. Protection of soil carbon by microaggregates within
555       earthworm casts. Soil Biol. Biochem. 37, 251–258.
556 Bouché, M., Kretzschmar, A., 1974. Fonction des lombriciens; II: Recherches méthodologiques pour
557       l'analyse du sol ingéré (étude du peuplement de la station RCP-165/PBI). Rev Ecol Biol Sol 11,
558       127–139.
559 Brown, G., 1995. How do earthworms affect microfloral and faunal community diversity? Plant Soil
560       170, 209–231.
561 Brown, G.G., Barois, I., Lavelle, P., 2000. Regulation of soil organic matter dynamics and microbial
562       activityin the drilosphere and the role of interactionswith other edaphic functional domains.
563       Eur. J. Soil Biol. 36, 177–198.
564 Brüggemann, N., Gessler, A., Kayler, Z., Keel, S.G., Badeck, F., Barthel, M., Boeckx, P., Buchmann, N.,
565       Brugnoli, E., Esperschütz, J., Gavrichkova, O., Ghashghaie, J., Gomez-Casanovas, N., Keitel, C.,
566       Knohl, A., Kuptz, D., Palacio, S., Salmon, Y., Uchida, Y., Bahn, M., 2011. Carbon allocation and
567       carbon isotope fluxes in the plant-soil-atmosphere continuum: a review. Biogeosciences 8,
568       3457–3489.
569 Comeau, L.-P., Lemke, R.L., Knight, J.D., Bedard-Haughn, A., 2013. Carbon input from 13C-labeled
570       crops in four soil organic matter fractions. Biol. Fertil. Soils 49, 1179–1188.
571 Coppens, F., Garnier, P., De Gryze, S., Merckx, R., Recous, S., 2006a. Soil moisture, carbon and
572       nitrogen dynamics following incorporation and surface application of labelled crop residues
573       in soil columns. Eur. J. Soil Sci. 57, 894–905.

574  Coppens, F., Merckx, R., Recous, S., 2006b. Impact of crop residue location on carbon and nitrogen
575      distribution in soil and in water-stable aggregates. Eur. J. Soil Sci. 57, 570–582.
576  Coq, S., Barthès, B.G., Oliver, R., Rabary, B., Blanchart, E., 2007. Earthworm activity affects soil
577      aggregation and organic matter dynamics according to the quality and localization of crop
578      residues—An experimental study (Madagascar). Soil Biol. Biochem. 39, 2119–2128.
579  Cortez, J., 1998. Field decomposition of leaf litters: relationships between decomposition rates and
580      soil moisture, soil temperature and earthworm activity. Soil Biol. Biochem. 30, 783–793.
581  Cortez, J., Bouché, M.B., 1998. Field decomposition of leaf litters: earthworm–microorganism
582      interactions —the ploughing-in effect. Soil Biol. Biochem. 30, 795–804.
583  Cortez, J., Bouche, M.B., 1992. Do earthworms eat living roots? Soil Biol. Biochem. 24, 913–915.
584  Cortez, J., Hameed, R., Bouché, M.B., 1989. C and N transfer in soil with or without earthworms fed
585      with 14C- and 15N-labelled wheat straw. Soil Biol. Biochem. 21, 491–497.
586  Curry, J.P., Schmidt, O., 2007. The feeding ecology of earthworms – A review. Pedobiologia 50, 463–
587      477.
588  Daniel, O., 1991. Leaf-litter consumption and assimilation by juveniles of *Lumbricus terrestris* L.
589      (Oligochaeta, Lumbricidae) under different environmental conditions. Biol. Fertil. Soils 12,
590      202–208.
591  Datta, R., Vranová, V., Pavelka, M., Rejšek, K., Formánek, P., 2014. Effect of soil sieving on respiration
592      induced by low-molecular-weight substrates. Int. Agrophysics 28, 119–124.
593  Decaëns, T., 2010. Macroecological patterns in soil communities. Glob. Ecol. Biogeogr. 19, 287–302.
594  Decaëns, T., Rangel, A.F., Asakawa, N., Thomas, R.J., 1999. Carbon and nitrogen dynamics in ageing
595      earthworm casts in grasslands of the eastern plains of Colombia. Biol. Fertil. Soils 30, 20–28.
596  Don, A., Steinberg, B., Schöning, I., Pritsch, K., Joschko, M., Gleixner, G., Schulze, E.-D., 2008. Organic
597      carbon sequestration in earthworm burrows. Soil Biol. Biochem. 40, 1803–1812.
598  Drake, H.L., Horn, M.A., 2007. As the worm turns: The earthworm gut as a transient habitat for soil
599      microbial biomes. Annu. Rev. Microbiol. 61, 169–189.
600  Fahey, T.J., Yavitt, J.B., Sherman, R.E., Maerz, J.C., Groffman, P.M., Fisk, M.C., Bohlen, P.J., 2013.
601      Earthworm effects on the incorporation of litter C and N into soil organic matter in a sugar
602      maple forest. Ecol. Appl. 23, 1185–1201.
603  Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., Rumpel, C., 2007. Stability of organic carbon in
604      deep soil layers controlled by fresh carbon supply. Nature 450, 277–280.
605  Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revaillot, S., Maron,
606      P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through
607      their priming effect. Soil Biol. Biochem. 43, 86–96.
608  Fonte, S., Quintero, D.C., Velásquez, E., Lavelle, P., 2012. Interactive effects of plants and earthworms
609      on the physical stabilization of soil organic matter in aggregates. Plant Soil 359, 205–214.
610  Fonte, S.J., Kong, A.Y.Y., van Kessel, C., Hendrix, P.F., Six, J., 2007. Influence of earthworm activity on
611      aggregate-associated carbon and nitrogen dynamics differs with agroecosystem
612      management. Soil Biol. Biochem. 39, 1014–1022.
613  Franzluebbers, A.J., Haney, R.L., Honeycutt, C.W., Schomberg, H.H., Hons, F.M., 2000. Flush of carbon
614      dioxide following rewetting of dried soil relates to active organic pools. Soil Sci. Soc. Am. J.
615      64, 613–623.
616  Frouz, J., Krištůfek, V., Livečková, M., van Loo, D., Jacobs, P., Van Hoorebeke, L., 2011. Microbial
617      properties of soil aggregates created by earthworms and other factors: spherical and
618      prismatic soil aggregates from unreclaimed post-mining sites. Folia Microbiol. (Praha) 56, 36–
619      43.
620  Fründ, H.-C., Butt, K., Capowiez, Y., Eisenhauer, N., Emmerling, C., Ernst, G., Potthoff, M., Schädler,
621      M., Schrader, S., 2010. Using earthworms as model organisms in the laboratory:
622      Recommendations for experimental implementations. Pedobiologia 53, 119–125.
623  Gaillard, V., Chenu, C., Recous, S., Richard, G., 1999. Carbon, nitrogen and microbial gradients
624      induced by plant residues decomposing in soil. Eur. J. Soil Sci. 50, 567–578.

625 Gale, W.J., Cambardella, C.A., 2000. Carbon Dynamics of Surface Residue– and Root-derived Organic
626         Matter under Simulated No-till1Reference to trade names and companies is made for
627         information purposes only and does not imply endorsement by the USDA. Soil Sci Soc Am J
628         64, 190–195.
629 Gleixner, G., Czimczik, C.J., Kramer, C., Lühker, B., Schmidt, M.W.I., 2001. 1.15 - Plant Compounds and
630         Their Turnover and Stabilization as Soil Organic Matter, in: Schulze, E.-D., Heimann, M.,
631         Harrison, S., Holland, E., Lloyd, J., Prentice, I.C., Schimel, D. (Eds.), Global Biogeochemical
632         Cycles in the Climate System. Academic Press, San Diego, pp. 201–215.
633 Harris, D., Horwáth, W.R., van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to
634         total organic carbon or Carbon-13 isotopic analysis. Soil Sci Soc Am J 65, 1853–1856.
635 Hong, H.N., Rumpel, C., Henry des Tureaux, T., Bardoux, G., Billou, D., Tran Duc, T., Jouquet, P., 2011.
636         How do earthworms influence organic matter quantity and quality in tropical soils? Soil Biol.
637         Biochem. 43, 223–230.
638 Hopkins, D.W., Marinari, S., Tilston, E.L., Halpin, C., 2005. *Lumbricus terrestris* counteract the effects
639         of modified lignin biosynthesis on the decomposition of tobacco plant residues. Soil Biol.
640         Biochem. 37, 1141–1144.
641 Jégou, D., Cluzeau, D., Balesdent, J., Tréhen, P., 1998. Effects of four ecological categories of
642         earthworms on carbon transfer in soil. Appl. Soil Ecol. 9, 249–255.
643 Jégou, D., Cluzeau, D., Hallaire, V., Balesdent, J., Tréhen, P., 2000. Burrowing activity of the
644         earthworms *Lumbricus terrestris* and *Aporrectodea giardi* and consequences on C transfers in
645         soil. Eur. J. Soil Biol. 36, 27–34.
646 Jiménez, J.J., Moreno, G.A., Decaëns, T., Lavelle, P., Fisher, J.M., Thomas, J.R., 1998. Earthworm
647         communities in native savannas and man-made pastures of the Eastern Plains of Colombia.
648         Biol. Fertil. Soils 28, 101–110.
649 Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil carbon and its relation to climate
650         and vegetation. Ecol. Appl. 10, 423–436.
651 Kammer, A., Hagedorn, F., 2011. Mineralisation, leaching and stabilisation of $^{13}$C-labelled leaf and
652         twig litter in a beech forest soil. Biogeosciences 8, 2195–2208.
653 Klumpp, K., Soussana, J.F., Falcimagne, R., 2007. Long-term steady state $^{13}$C labelling to investigate
654         soil carbon turnover in grasslands. Biogeosciences 4, 385–394.
655 Kögel-Knabner, I., 2002. The macromolecular organic composition of plant and microbial residues as
656         inputs to soil organic matter. Soil Biol. Biochem. 34, 139–162.
657 Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. Soil Biol.
658         Biochem. 42, 1363–1371.
659 Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept & review.
660         Soil Biol. Biochem. 83, 184–199.
661 Lavelle, P., Martin, A., 1992. Small-scale and large-scale effects of endogeic earthworms on soil
662         organic matter dynamics in soils of the humid tropics. Soil Biol. Biochem. 24, 1491–1498.
663 Lavelle, P., Blanchart, E., Martin, A., Martin, S., Spain, A., 1993. A Hierarchical model for
664         decomposition in terrestrial ecosystems: application to soils of the humid tropics. Biotropica
665         25, 130–150.
666 Lavelle, P., Lattaud, C., Trigo, D., Barois, I., 1995. Mutualism and biodiversity in soils, in: The
667         Significance and Regulation of Soil Biodiversity. Springer, pp. 23–33.
668 Lee, K.E., 1985. Earthworms: their ecology and relationships with soils and land use. Academic Press,
669         Sydney ; Orlando.
670 Lu, Y., Watanabe, A., Kimura, M., 2003. Carbon dynamics of rhizodeposits, root- and shoot-residues
671         in a rice soil. Soil Biol. Biochem. 35, 1223–1230.
672 Machinet, G.E., Bertrand, I., Barrière, Y., Chabbert, B., Recous, S., 2011. Impact of plant cell wall
673         network on biodegradation in soil: role of lignin composition and phenolic acids in roots from
674         16 maize genotypes. Soil Biol. Biochem. 43, 1544–1552.

675  Mambelli, S., Bird, J.A., Gleixner, G., Dawson, T.E., Torn, M.S., 2011. Relative contribution of foliar
676      and fine root pine litter to the molecular composition of soil organic matter after in situ
677      degradation. Org. Geochem. 42, 1099–1108.
678  Mariani, L., Jiménez, J.J., Asakawa, N., Thomas, R.J., Decaëns, T., 2007. What happens to earthworm
679      casts in the soil? A field study of carbon and nitrogen dynamics in Neotropical savannahs. Soil
680      Biol. Biochem. 39, 757–767.
681  Martin, A., 1991. Short- and long-term effects of the endogeic earthworm Millsonia anomala
682      (Omodeo) (Megascolecidæ, Oligochæta) of tropical savannas, on soil organic matter. Biol.
683      Fertil. Soils 11, 234–238.
684  Martin, A., Cortez, J., Barois, I., Lavelle, P., 1987. Les mucus intestinaux de ver de terre moteur de
685      leurs interactions avec la microflore. Rev Ecol Biol Sol 24, 549–558.
686  Nguyen Tu, T.T., Egasse, C., Zeller, B., Bardoux, G., Biron, P., Ponge, J.-F., David, B., Derenne, S., 2011.
687      Early degradation of plant alkanes in soils: A litterbag experiment using $^{13}$C-labelled leaves.
688      Soil Biol. Biochem. 43, 2222–2228.
689  Oades, J.M., 1988. The retention of organic matter in soils. Biogeochemistry 5, 35–70.
690  Parle, J.N., 1963. A Microbiological Study of Earthworm Casts. J. Gen. Microbiol. 31, 13–22.
691  Puget, P., Drinkwater, L.E., 2001. Short-Term Dynamics of Root- and Shoot-Derived Carbon from a
692      Leguminous Green Manure. Soil Sci Soc Am J 65, 771–779.
693  Rasse, D., Rumpel, C., Dignac, M.-F., 2005. Is soil carbon mostly root carbon? Mechanisms for a
694      specific stabilisation. Plant Soil 269, 341–356.
695  Rubino, M., Dungait, J.A.J., Evershed, R.P., Bertolini, T., De Angelis, P., D'Onofrio, A., Lagomarsino, A.,
696      Lubritto, C., Merola, A., Terrasi, F., Cotrufo, M.F., 2010. Carbon input belowground is the
697      major C flux contributing to leaf litter mass loss: Evidences from a $^{13}$C labelled-leaf litter
698      experiment. Soil Biol. Biochem. 42, 1009–1016.
699  Scheu, S., Schauermann, J., 1994. Decomposition of roots and twigs: Effects of wood type (beech and
700      ash), diameter, site of exposure and macrofauna exclusion. Plant Soil 163, 13–24.
701  Shi, A., Penfold, C., Marschner, P., 2013. Decomposition of roots and shoots of perennial grasses and
702      annual barley—separately or in two residue mixes. Biol. Fertil. Soils 49, 673–680.
703  Shipitalo, M.J., Protz, R., 1989. Chemistry and micromorphology of aggregation in earthworm casts.
704      Geoderma 45, 357–374.
705  Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between
706      (micro)aggregates, soil biota, and soil organic matter dynamics. Adv. Soil Struct. Res. 79, 7–
707      31.
708  Steffens, C., Helfrich, M., Joergensen, R.G., Eissfeller, V., Flessa, H., 2015. Translocation of 13C-
709      labeled leaf or root litter carbon of beech (Fagus sylvatica L.) and ash (Fraxinus excelsior L.)
710      during decomposition – A laboratory incubation experiment. Soil Biol. Biochem. 83, 125–137.
711  Stockmann, U., Adams, M.A., Crawford, J.W., Field, D.J., Henakaarchchi, N., Jenkins, M., Minasny, B.,
712      McBratney, A.B., Courcelles, V. de R. de, Singh, K., Wheeler, I., Abbott, L., Angers, D.A.,
713      Baldock, J., Bird, M., Brookes, P.C., Chenu, C., Jastrow, J.D., Lal, R., Lehmann, J., O'Donnell,
714      A.G., Parton, W.J., Whitehead, D., Zimmermann, M., 2013. The knowns, known unknowns
715      and unknowns of sequestration of soil organic carbon. Agric. Ecosyst. Environ. 164, 80–99.
716  Stromberger, M.E., Keith, A.M., Schmidt, O., 2012. Distinct microbial and faunal communities and
717      translocated carbon in Lumbricus terrestris drilospheres. Soil Biol. Biochem. 46, 155–162.
718  Swift, M.J., Heal, O.W., Anderson, J.M., 1979. Decomposition in terrestrial ecosystems. Univ of
719      California Press.
720  Tahir, M.M., Recous, S., Aita, C., Schmatz, R., Pilecco, G.E., Giacomini, S.J., 2016. In situ roots
721      decompose faster than shoots left on the soil surface under subtropical no-till conditions.
722      Biol. Fertil. Soils 52, 853–865.
723  Taylor, B.R., Parkinson, D., Parsons, W.F., 1989. Nitrogen and lignin content as predictors of litter
724      decay rates: a microcosm test. Ecology 70, 97–104.
725  Thompson, R.B., 1996. Pulse-labelling a cover crop with $^{13}$C to follow its decomposition in soil under
726      field conditions. Plant Soil 180, 49–55.

727     Trigo, D., Lavelle, P., 1993. Changes in respiration rate and some physicochemical properties of soil
728           during gut transit through Allolobophora molleri (Lumbricidae, Oligochaeta). Biol. Fertil. Soils
729           15, 185–188.
730     Trinsoutrot, I., Recous, S., Bentz, B., Linères, M., Chèneby, D., Nicolardot, B., 2000. Biochemical
731           Quality of Crop Residues and Carbon and Nitrogen Mineralization Kinetics under Nonlimiting
732           Nitrogen Conditions 64, 918–926.
733     Van Gestel, M., Merckx, R., Vlassak, K., 1993. Microbial biomass responses to soil drying and
734           rewetting: The fate of fast- and slow-growing microorganisms in soils from different climates.
735           Soil Biol. Biochem. 25, 109–123.
736     Vidal, A., Remusat, L., Watteau, F., Derenne, S., Quenea, K., 2016a. Incorporation of 13C labelled
737           shoot residues in Lumbricus terrestris casts: A combination of transmission electron
738           microscopy and nanoscale secondary ion mass spectrometry. Soil Biol. Biochem. 93, 8–16.
739     Vidal, A., Quenea, K., Alexis, M., Derenne, S., 2016b. Molecular fate of root and shoot litter on
740           incorporation and decomposition in earthworm casts. Org. Geochem. 101, 1–10.
741     Weyers, S., Schomberg, H., Hendrix, P., Spokas, K., Endale, D., 2008. Construction of an electrical
742           device for sampling earthworm populations in the field. Appl. Eng. Agric. 24, 391–397.
743     Williams, M., Myrold, D., Bottomley, P., 2006. Distribution and fate of 13C-labeled root and straw
744           residues from ryegrass and crimson clover in soil under western Oregon field conditions. Biol.
745           Fertil. Soils 42, 523–531.
746     Witkamp, M., 1966. Decomposition of leaf litter in relation to environment, microflora, and microbial
747           respiration. Ecology 47, 194–201.
748     Zangerlé, A., Pando, A., Lavelle, P., 2011. Do earthworms and roots cooperate to build soil
749           macroaggregates? A microcosm experiment. Geoderma 167–168, 303–309.
750     Zhang, X., Wang, J., Xie, H., Wang, J., Zech, W., 2003. Comparison of organic compounds in the
751           particle-size fractions of earthworm casts and surrounding soil in humid Laos. Appl. Soil Ecol.
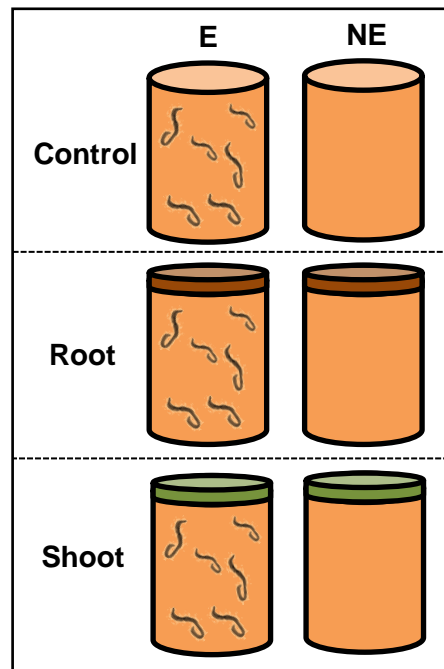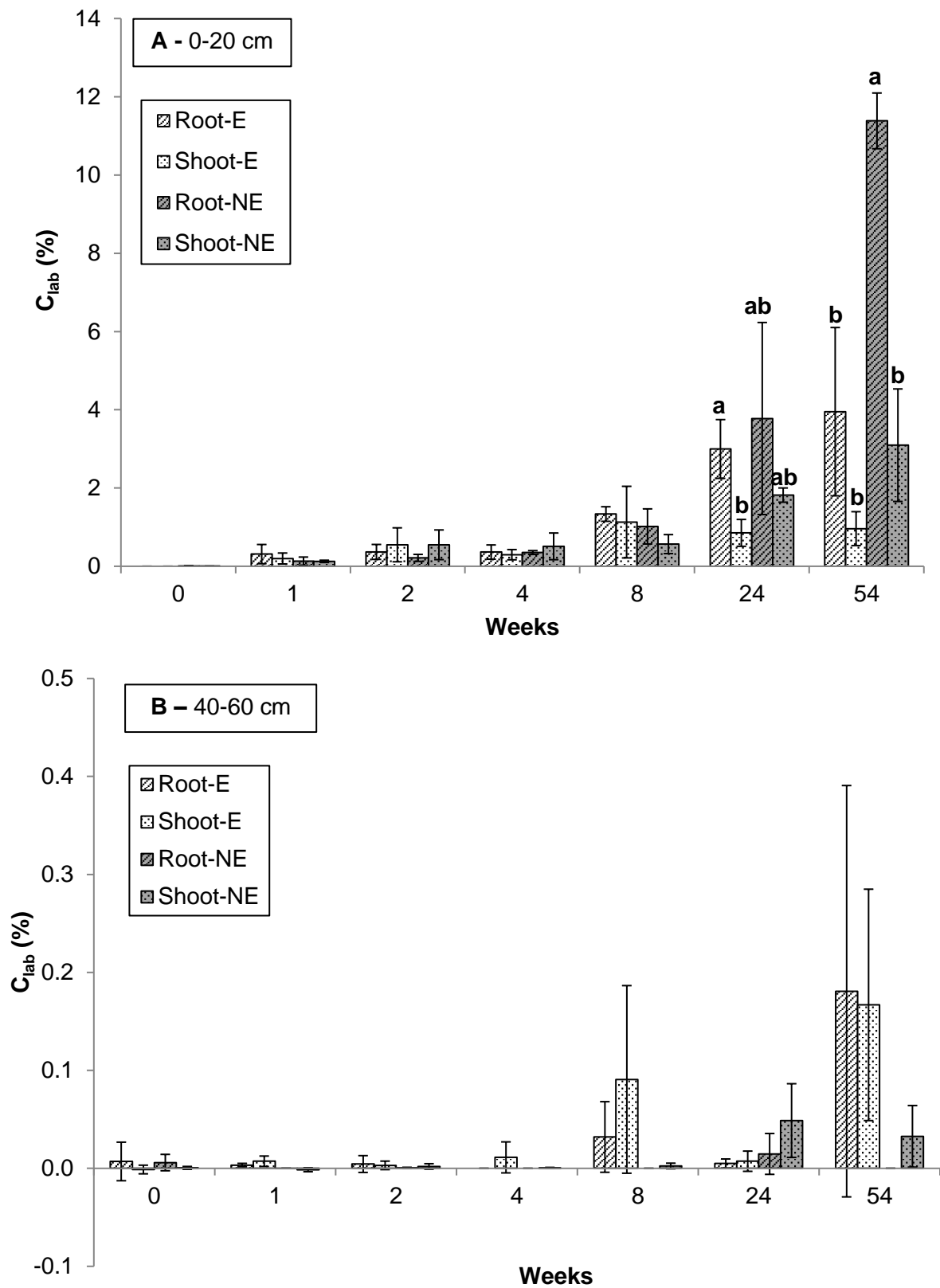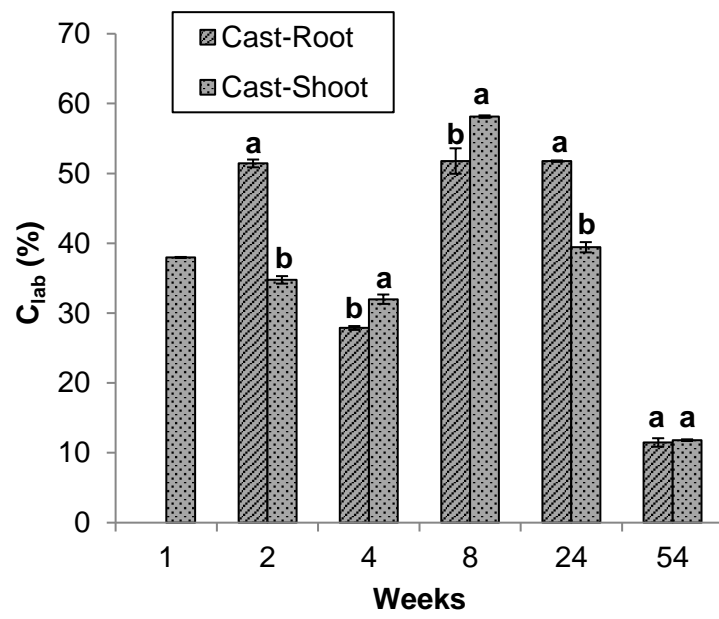752           23, 147–153.
753

Fig.1
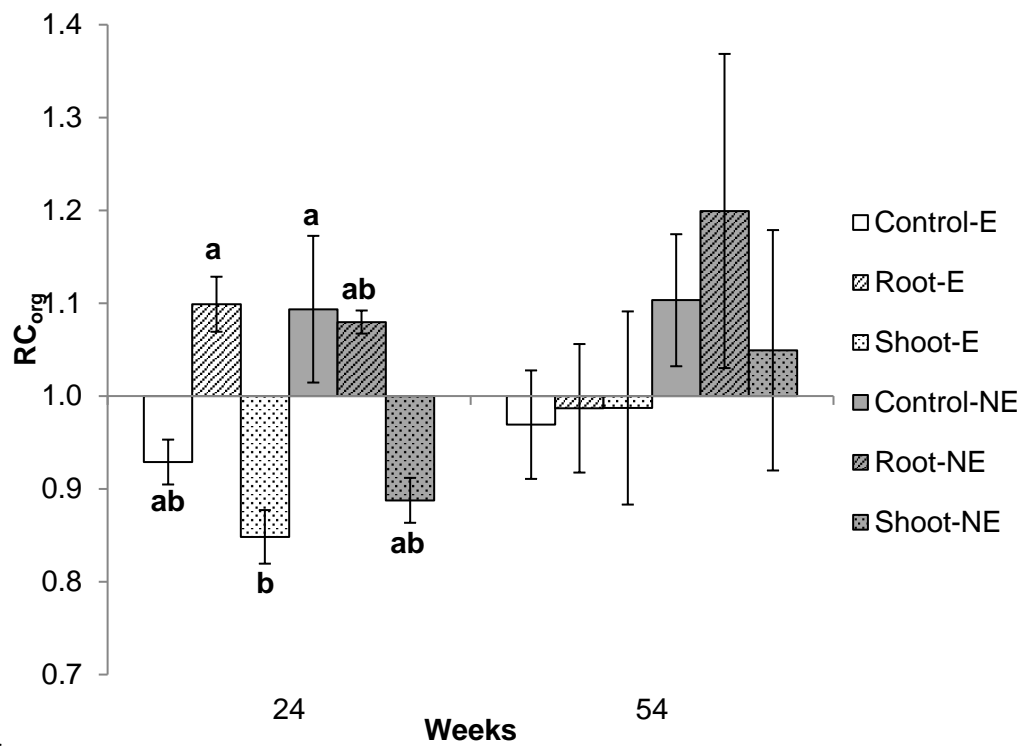
Fig. 2

Fig.4

The y-axis is labeled $RC_{org}$ and ranges from 0.7 to 1.4. The x-axis is labeled **Weeks** with groups at 24 and 54.

Legend:
- Control-E
- Root-E
- Shoot-E
- Control-NE
- Root-NE
- Shoot-NE
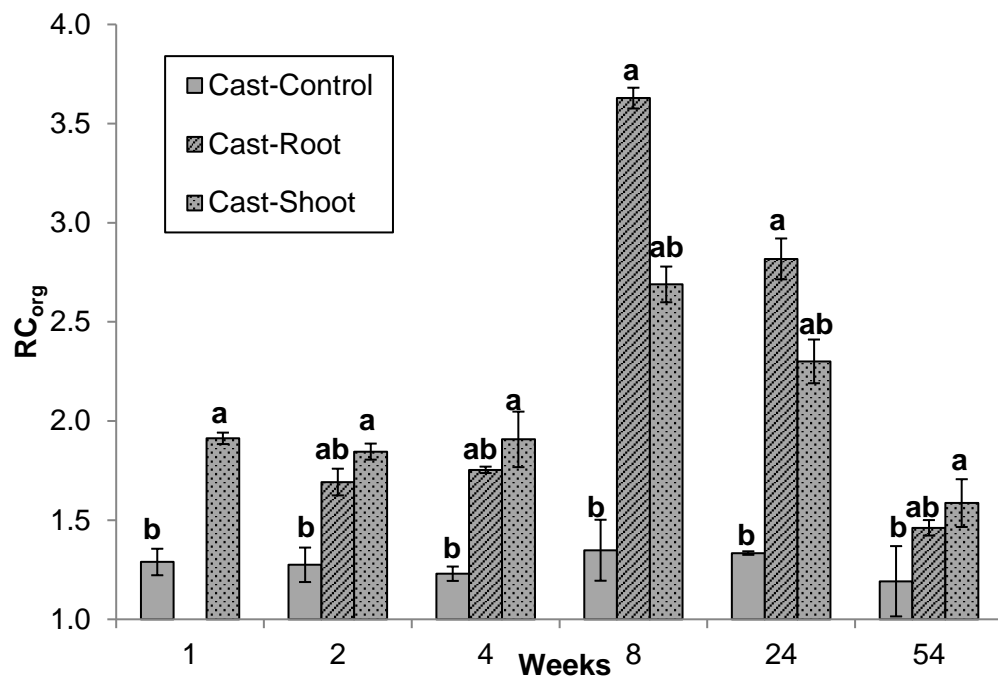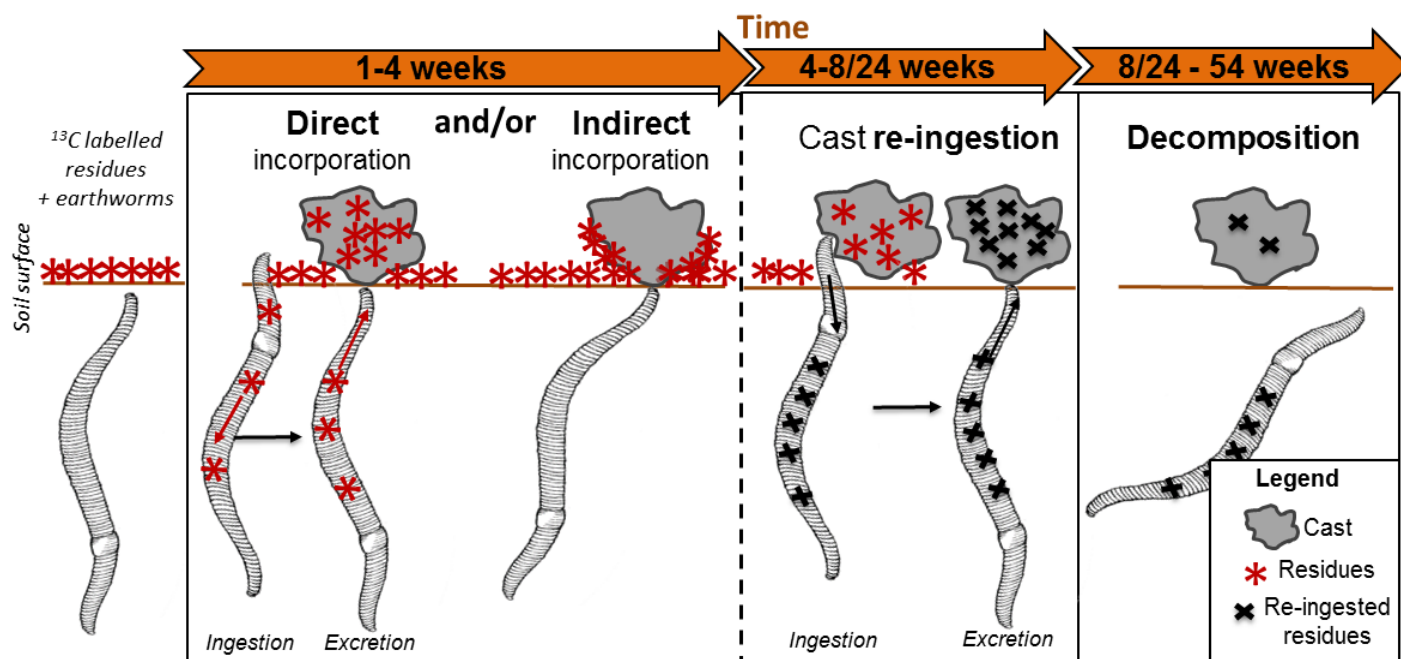
Fig.5

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) |