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# Effect of decomposition products produced in the presence or absence of epigeic earthworms and minerals on soil carbon stabilization

J. Barthod, M.F. Dignac, C. Rumpel

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1 **Effect of decomposition products produced in presence or absence of**  
2 **epigeic earthworms and minerals on soil carbon stabilization**

3 Barthod, J., Dignac, M.-F., Rumpel, C.

4 Sorbonne Université, INRA, CNRS, Institute for Ecology and Ecological Sciences (iEES  
5 Paris, UMR Sorbonne Université, CNRS, INRA, UPEC, IRD), Paris, France

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8

## 9 **Abstract**

10

11 Microbial use efficiency is thought to influence greatly organic carbon storage in soils  
12 through the formation of decomposition products and their stabilization as organo-mineral  
13 complexes. Earthworm activity may play a significant role in these processes. Of the three  
14 ecological earthworm groups only two (endogeic or anaecic species) are thought to be  
15 involved in carbon stabilization as they are living in mineral soil. The aim of this study was to  
16 investigate the role of decomposition products produced with and without minerals by epigeic  
17 earthworms living at the litter-soil interphase for carbon stabilization. We investigated the  
18 impact of eight different types of decomposition products on CO<sub>2</sub> emissions, microbial  
19 biomass and watersoluble C and mineral N and SOC pool allocation of an arenic cambisol  
20 during 79 days under laboratory conditions.

21 Our results indicated that the nature of decomposition products affected their impact on SOC  
22 mineralization and soil physico-chemical parameters. In general, the presence of epigeic  
23 earthworms during OM decomposition decreased CO<sub>2</sub> emissions after soil addition of  
24 decomposition products and increased microbial carbon use efficiency as compared to those  
25 produced without earthworms and in some cases also as compared to un-amended soil.  
26 Mineral-containing decomposition products increased CO<sub>2</sub> emissions after their addition to  
27 soil as compared to their mineral free counterparts. They also changed carbon allocation to  
28 physico-chemically protected pools, decreasing the contribution of particulate organic matter  
29 as compared to mineral free decomposition products. Extrapolation of the data showed that  
30 these short-term effects were not necessarily lasting in the long run, but they also indicated  
31 that microbial products produced in the presence of epigeic earthworms and minerals may  
32 increase SOC sequestration in the amended soil. We therefore conclude that the nature of  
33 decomposition products is crucial for their fate in soil and that in contrast to the general  
34 paradigm, epigeic earthworms could have an important role in SOC sequestration through the  
35 formation of material susceptible to be protected, when incorporated into mineral soil.

## 36 **Introduction**

37 It is generally accepted now that microbial activity is a major control of soil organic matter  
38 (SOM) formation (Schimel and Scheffer, 2012). In recent years, microbial decomposition  
39 products have been identified as main contributors to SOM (Miltner et al., 2012; Kallenbach  
40 et al., 2016), by favoring aggregation and also through its association with clay-sized minerals  
41 (Cotrufo et al., 2013). They are source of stabilized SOM, and may be composed of degraded  
42 plant litter as well as microbial-derived material (Rumpel et al., 2015; Barré et al., 2018). In  
43 this study, we addressed the fate of end products from decomposition processes, which we  
44 hypothesized to contain both, microbial residues and partly decomposed plant material.

45 In soils, earthworms, which are considered as key engineers, may affect decomposition  
46 processes. By mixing and simultaneous ingestion of fresh organic matter (OM) and minerals  
47 and their digestion and transformation in the earthworms' gut, they may not only lead to  
48 enhanced carbon mineralisation (Lubbers et al., 2015), but also to stabilization of SOM  
49 through the formation of new aggregates (Bossuyt *et al*, 2005; Shipitalo and Protz, 1988;  
50 Schrader and Zhang; 1997). These new aggregates might enhance the physical protection of  
51 carbon from microbial mineralization from years to decades (Brown et al, 2000). Recently,  
52 experimental evidence showed that earthworms may also enhance the transformation of fresh  
53 OM into microbial necromass (Angst et al., 2019) and affect OM transformations in presence  
54 of minerals through their impact on microbial biomass and its habitat (Barthod et al., 2020).  
55 Despite of their potential importance for the stabilization of SOM, the impact and fate of the  
56 various types of decomposition products on SOM dynamics is not well understood. Such  
57 knowledge may advance our process understanding of SOM turnover.

58 In this study we focused on decomposition products generated in a decomposition system  
59 with high OM concentrations in presence and absence of clay-sized minerals and epigeic  
60 earthworm species. Such conditions may occur at soil surfaces, where aboveground litter  
61 inputs are abundant (e.g. agricultural no-till systems, grassland and forest).

62 We investigated the effect of the addition of different types of decomposition products to a  
63 silty agricultural soil on carbon mineralization, microbial biomass and OC allocation to  
64 physicochemical SOM fractions. To this end we carried out a laboratory incubation with  
65 materials produced during a 6 months decomposition experiment transforming an OM  
66 mixture in presence or absence of epigeic earthworms and clay-sized minerals. We monitored  
67 CO<sub>2</sub> emissions after soil addition of decomposition products, studied their effect on microbial

68 biomass and determined SOC contribution to different SOM pools after the end of the  
69 experiment.

70 The general paradigm is that epigeic earthworm species ingest exclusively OM and do not  
71 contribute to organo-mineral complex formation. However, recent studies indicated that this  
72 species may be able to form these interactions (Barthod et al., 2020), and stabilize OM  
73 through the formation of aggregates and/or organo-mineral complexes containing microbial-  
74 derived material (Le Mer et al., 2020).

75 We thus hypothesized that epigeic earthworms are able to form organo-mineral complexes  
76 and that the addition of decomposition products containing minerals formed in the presence of  
77 earthworms decreases SOC mineralization.

78 The aim of the study was to evaluate:

- 79 • how contrasting decomposition products produced in the presence or absence of  
80 minerals and earthworms affect microbial activity and carbon use efficiency after soil  
81 application.
- 82 • how decomposition products affect soil physicochemical parameters, carbon pool  
83 allocation and soil nitrogen
- 84 • if decomposition products of epigeic earthworms contribute to SOC stabilisation

85

## 86 **Material and Methods**

### 87 *Decomposition products*

88 Decomposition products were obtained during a 6 months decomposition experiment carried  
89 out in a model system inspired from composting or vermicomposting systems (Barthod et al.,  
90 2016). To this end, fresh organic materials composed of lettuces, apples, residual maize, spent  
91 coffee ground and pieces of cardboard (2x3cm) were decomposed in the presence or absence  
92 of epigeic earthworms (*Eisenia andrei* and *foetida*) and different types and quantities of  
93 minerals during 196 days. We used lettuces and apples, products of organic agriculture, which  
94 were obtained from a general store, spent coffee ground from a public cafeteria, and maize  
95 leafs and stems from a field in Grignon (France). Moreover, we used montmorillonite (a 2:1  
96 clay), kaolinite (a 1:1 clay) and goethite, an iron oxide, which were purchased from Sigma-  
97 Aldrich in the powder form.

98 Eight decomposition products were produced in presence or absence of epigeic earthworms  
99 and 4 different mineral treatments: (i) decomposition products without minerals (control); (ii)  
100 decomposition products produced in the presence of 15% montmorillonite (15M); (iii)  
101 decomposition products produced in the presence of 30% montmorillonite (30M) and (iv)  
102 decomposition products produced with a mixture of 15% kaolinite and 15% goethite (KG).  
103 These materials were chosen because of their higher stability against decomposition as  
104 compared to decomposition products with pure kaolinite, goetite, and montmorillonite/goetite  
105 mixtures (Barthod et al., 2020). The main characteristics of these materials are summarized in  
106 Table 1. Detailed description of the formation and composition of the decomposition products  
107 can be found in Barthod et al. (2020) and Le Mer et al. (2020). Briefly, these studies showed  
108 that decomposition products formed in the presence of earthworms had higher aromatic  
109 carbon contribution, contained more mineral-associated of OM and showed higher thermal  
110 stability than those produced without earthworm activity.

#### 111 *Soil sampling and preparation*

112 The soil was collected from an agricultural plot near Crespières (Yvelines, France, 571° 053E  
113 131° 535N). It is classified as an arenic cambisol (Noirot-Cosson *et al*, 2017) with a pH of 6.6,  
114 cropped with wheat and its characteristics are presented in Table 2. The upper layer of the soil  
115 (0-10 cm) was sampled before seeding in December 2015. After sampling, the soil was air-  
116 dried, sieved at 2 mm and plant residues and mesofauna were removed. The soil pF was  
117 determined in the laboratory: the soil humidity was 26.6% at pF 2.5.

#### 118 *Incubation experiment*

119 We carried out an incubation experiment with soils amended with the different decomposition  
120 products under controlled laboratory conditions. Before incubation, the soil was mixed with  
121 the decomposition products and re-humidified at pF 2.5 during one week. Briefly, 23 g soil  
122 (dry weight) were amended with decomposition products and placed in 1L jar at 20°C in the  
123 dark during 7 days (pre-incubation) plus 79 days (incubation). For amended soils, the  
124 application rate of organic material was 20 mg g<sup>-1</sup> of soil (dry weight). Three replicates of the  
125 following treatments were incubated: two control treatments including (i) soil without  
126 amendment, and (ii) soil amended with decomposition products produced with (EW) and  
127 without earthworms (No EW). Other treatments included (iii) soil amended with  
128 decomposition product 15M produced with (EW) and without earthworms (No EW), (iv) soil  
129 amended with decomposition product 30M produced with (EW) and without earthworms (No

130 EW), (v) soil amended with decomposition product KG produced with (EW) and without  
131 earthworms (EW). The incubated soil was collected at days 2, 15, 30 and 79 for further  
132 analysis.

### 133 *Physico-chemical fractionation*

134 After the end of the experiment, soils were fractionated by combining physical and chemical  
135 methods, in order to isolate five fractions, which were shown to represent carbon pools used  
136 in soil organic carbon models (Zimmerman *et al*, 2007; Poeplau *et al*, 2013): two slowly  
137 decomposable carbon pools -silt and clay fraction (S+C) and stable aggregates fraction  
138 (S+A)-, two labile carbon pools -water soluble soil organic matter (WSOC) and particulate  
139 organic matter (POM)- and one recalcitrant carbon pool -oxidation resistant (rSOC).

140 Briefly, 15g of dried soil were submerged in 80 mL of deionised water and treated  
141 ultrasonically (energy output: 22j mL<sup>-1</sup>) to disperse labile macroaggregates. Afterwards, the  
142 soil suspension was wet-sieved by adding additional deionized water on a 63 µm sieve to  
143 separate the sand fraction. The suspension containing the silt and clay (S+C) fractions was  
144 introduced into a pressure filtration system, which allowed for the separation of water-soluble  
145 SOC. The sand fraction > 63 µm was freeze-dried and separated into particulate organic  
146 matter (POM) and sand and aggregate (S+A) fractions by using a sodium polytungstate  
147 solution with a density of 1.8 g cm<sup>-3</sup>. The recalcitrant fraction (rSOC) was quantified after  
148 sodium hypochlorite oxidation of the S+C fraction (1 g).

### 149 *Elemental content and WSOC and NO<sub>3</sub> and NH<sub>4</sub>*

150 OC and N contents of bulk soils and fractions were measured using a CHN auto-analyser  
151 (CHN NA 1500, Carlo Erba). We considered all carbon as being organic as the soils pH was  
152 6.6 and does not contain carbonates. Water-soluble SOC (WSOC) contents were measured  
153 using a total organic carbon analyzer (TOC 5050A, Shimadzu). NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> contents were  
154 determined in 1M KCl extracts (1: 5 w/v), as they may allow to assess differences in the SOM  
155 mineralization activity by soil micro-organisms. The extracts were filtered then analyzed by  
156 colorimetry on a continuous flow analyzer (Skalar, The Netherlands).

157

### 158 *Carbon mineralisation*

159 CO<sub>2</sub> emissions were monitored throughout the incubation with a MicroGC (Agilent, Santa  
160 Clara, USA). Jars were maintained closed between the measurements. When the CO<sub>2</sub>

161 concentration was above 20 000 ppm, jars were flushed during 20 min with synthetic air free  
 162 of CO<sub>2</sub> to avoid anaerobic conditions.

163

#### 164 *Microbial biomass*

165 Microbial biomass was determined on days 2, 15, 30 and 79 using the chloroform-fumigation  
 166 extraction method (Vance *et al.*, 1987). Briefly, 5 g of fresh soil were fumigated with CHCl<sub>3</sub> in  
 167 a desiccator during 16h, allowing for lysis of microbial cells. Fumigated and non-fumigated  
 168 samples were extracted using 0.034 M K<sub>2</sub>SO<sub>4</sub> (1:4 w/v) and the extract was filtered and  
 169 analyzed for DOC (see above). Microbial biomass carbon (MBC) was calculated using the  
 170 following equation:

171

$$172 \text{ MBC (mg C kg}^{-1}\text{)} = 2.22 * (\text{DOC fumigated} - \text{DOC non-fumigated}),$$

173

174 where MBC is the microbial biomass carbon, DOC is the organic carbon extracted from  
 175 fumigated and non-fumigated soil samples. The extraction efficiency was taken into account  
 176 by the constant 2.22 (Wu *et al.*, 1990).

177

#### 178 *Microbial carbon use efficiency (CUE)*

179 We estimated the microbial carbon use efficiency (CUE) based on the difference in CO<sub>2</sub>  
 180 mineralisation and microbial biomass carbon (MBC) of amended and unamended soils after  
 181 the incubation:

182

$$183 \text{ CUE} = (\text{MBC}_{\text{treatment}} - \text{MBC}_{\text{soil}}) / (\text{MBC}_{\text{soil}} + \text{C} - \text{CO}_2_{\text{treatment mineralised}}),$$

184

185 where CUE is the microbial carbon use efficiency, MBC<sub>treatment</sub> the microbial biomass  
 186 recorded at day 79 in all amended soils, MBC<sub>soil</sub> the microbial biomass recorded in the  
 187 unamended soil at day 79 and C-CO<sub>2 treatment mineralised</sub> the cumulative CO<sub>2</sub> mineralised from  
 188 amended soils at day 79.

189

#### 190 *Statistical analysis*



191 The CO<sub>2</sub> emitted after organic amendment application to soil was fitted to a first-order model  
 192 with two pools (Marquardt-Levenberg algorithm), using the following equations:

$$193 \quad C_{\text{mineralized}} = C_1(1 - e^{-k_1 t}) + C_2(1 - e^{-k_2 t}) \quad \text{equation 1}$$

194 where  $C_{\text{mineralized}}$  is the carbon mineralized as CO<sub>2</sub> (mg C-CO<sub>2</sub> g<sup>-1</sup> soil) at the time t (day),  $C_1$   
 195 (mg C-CO<sub>2</sub> g<sup>-1</sup> soil) is the labile/fast mineralisable carbon pool, associated with the rate  
 196 constant  $k_1$  (day<sup>-1</sup>) and  $C_2$  (mg C-CO<sub>2</sub> g<sup>-1</sup> soil) is the slow mineralisable carbon pool,  
 197 associated with the rate constant  $k_2$  (day<sup>-1</sup>).

198

199 All reported data are the arithmetic means of three replicates. A Kruskal-Wallis test and a  
 200 Dunn posthoc test were performed to assess the significance of differences among treatments.  
 201 Differences between decomposition products produced in the presence of earthworm and  
 202 without earthworms were tested using the Mann and Whitney test. Differences were declared  
 203 significant at the 0.05 level. A principal component analysis (PCA) was carried out to  
 204 differentiate the treatments based on the physical and chemical characteristics of organic  
 205 materials.

206

207 Statistical analyses were carried out using Excel and the R 3.12 software for Windows  
 208 (Agricolae and FactoMineR package) (R Core Team, 2020).

209

## 210 **Results**

### 211 *Physico-chemical soil parameters after incubation*

212 After 79 days of incubation, the carbon content of the un-amended control soil remained  
 213 unchanged, whereas the addition of organic materials to soil increased their total SOC and  
 214 total N contents, with total SOC contents ranging between 11 and 16 mg g<sup>-1</sup> (Table 4). SOC  
 215 contents and C/N ratios of amended soils showed significant differences when the  
 216 decomposition products were produced in the presence of a kaolinit/goethite mixture (KG).  
 217 For this treatment, the lowest values were recorded for the two parameter and significant  
 218 differences were noted with regards to the control treatments amended with mineral free  
 219 decomposition products. No differences were noted between EW and no-EW treatments.

220 Strong effects of the nature of the decomposition products were noted for soluble carbon and  
 221 nitrogen compounds. WSOC contents ranged from 15.2 to 49.2 µg g<sup>-1</sup> soil at the end of the  
 222 incubation, whereas NO<sub>3</sub> and NH<sub>4</sub> showed lower contents ranging from 10 to 33 µg g<sup>-1</sup> for  
 223 NO<sub>3</sub> and 5 and 27 for NH<sub>4</sub> (Table 4). Decomposition products containing minerals induced

224 significant differences mainly for KG. While the decomposition products produced in the  
225 presence of earthworms (EW treatments) induced lower WSOC contents in most cases as  
226 compared to no EW treatments after 79 days, higher  $\text{NH}_4$  contents could be observed.

### 227 *Microbial biomass*

228 The amount of microbial biomass ranged between 0.06 and 0.57  $\text{mg g}^{-1}$  soil (Fig. 1).  
229 Microbial biomass in un-amended soil remained unchanged during the incubation experiment.  
230 Soils amended with decomposition products showed a similar microbial biomass dynamics,  
231 which reached a maximum at day 15 for all treatments, except for the mineral-free control  
232 treatment without earthworms. In the latter treatment the maximum was reached at day 2 (Fig.  
233 1). At days 30 and 79, microbial biomass decreased in all treatments and was at the end of  
234 incubation no longer different from the control in the case of KG treatments (Fig. 1). The  
235  $\text{C}_{\text{mic}}/\text{C}_{\text{org}}$  ratio ranged between 0.6 and 1.3 % after 79 days of incubation (Table 4). It was  
236 slightly above 1 % for all treatments except those amended with KG and un-amended soil.

### 237 *Carbon mineralisation*

238 Cumulative carbon mineralisation is presented in Fig. 3. Highest carbon mineralization was  
239 recorded for soils amended with decomposition products produced without earthworms (no  
240 EW). Most carbon was mineralised for the treatment 30M (no EW), amounting to 80.9  $\text{mg}$   
241  $\text{CO}_2\text{-C g}^{-1}$  soil after 79 days. In the other no EW treatments, mineralised carbon decreased in  
242 the order: 30M < KG < 15M < control (Fig. 3, No EW).

243 In contrast, soils amended with decomposition products produced in the presence of  
244 earthworms showed lower or similar carbon emissions compared to un-amended soil (Fig 3,  
245 EW) and the carbon emissions followed the order: soil=control < 15M < 30M < KG.

246 In order to describe the dynamics of carbon mineralization, carbon mineralization data were  
247 fitted with a two pool model. According to this model, the labile carbon pool (C1) was higher  
248 in amended soils than in un-amended soils. Moreover, it was lower in EW treatments as  
249 compared to no EW treatments. The degradation rates associated with this pool ( $k_1$ ) showed  
250 the opposite behavior (no EW treatments < EW treatments). For instance, the application of  
251 30M (no EW) to soil led to the highest labile carbon pool (32.7  $\text{mg g}^{-1}$  soil) while its  
252 degradation rate was the lowest (0.124  $\text{day}^{-1}$ ). The second model pool (C2) represents the  
253 slowly mineralisable carbon fraction. This fraction increased when in amended soil, except

254 for 15M (EW) treatments. The degradation rate  $k_2$  related to this carbon pool was lower than  
255  $k_1$  and ranged from 0.002 to 0.007 day<sup>-1</sup>.

256 We extrapolated carbon mineralization in all soils for a period of 2 years (Fig. 3). Amended  
257 soils with lower CO<sub>2</sub> emissions than the un-amended soil during the first 79 days, showed  
258 higher C mineralization over the 2 year period. Moreover, the application of decomposition  
259 products produced with montmorillonite and without earthworms (30M no EW) seemed to  
260 increase carbon release while this was not the case for the 30M EW treatments. For the 15M  
261 treatments, the EW decomposition products reduced CO<sub>2</sub> emissions as compared to un-  
262 amended soil. This treatment was thus the only one, which might reduce C release from soil  
263 in the longer term. In contrast, addition of decomposition products produced with KG and  
264 earthworms increased SOC mineralization with regards to the un-amended control.

265

#### 266 *Microbial carbon use efficiency (CUE)*

267 The microbial carbon use efficiency (CUE) ranged between 0 and 3.37 at the end of the  
268 incubation experiment (Table 4). It was calculated based on the microbial biomass recorded in  
269 un-amended soils and may therefore represent microbial use of added substrates. CUE was  
270 highest in soils amended with decomposition products produced without minerals and 0 in  
271 both KG treatments. Generally, CUE was higher in EW treatments as compared to no EW  
272 treatments.

273

#### 274 *Soil organic carbon distribution in soil fractions*

275 The SOC distribution in physico-chemical fractions is shown in Fig 4. Most SOC was found  
276 in the silt and clay fraction (S+C), representing more than 45% of the total carbon. The  
277 particulate organic matter (POM) fractions were the second largest fraction, comprising 20-  
278 33% of total SOC, except for soil, which was amended with decomposition products  
279 produced without minerals. In these two treatments, the relative proportions of carbon were  
280 higher in POM than in (S+C) fractions and those of the recalcitrant fraction (rSOC)  
281 significantly decreased.

282 For mineral-containing decomposition products, the SOC in stable aggregates (SA)  
283 significantly increased by 5-10%, compared to un-amended soil, with the application of KG  
284 amendments. Increasing amounts of minerals (15% to 30%) led to higher relative contribution

285 of SOC associated to the S+C fraction and reduced that of the POM fraction, up to a  
286 proportion similar to un-amended soil. Finally, the DOC fractions showed the lowest  
287 contribution to SOC for all treatments, comprising less than 2%, except for the 15M no EW  
288 treatment, where its contribution was 4%.

289 EW treatments showed similar SOC distribution than no EW treatments in POM and (S+C)  
290 fractions of amended soil. While the contribution of DOC remained similar or increased  
291 compared to that of un-amended soils in 'no EW' treatments, the addition of EW  
292 decomposition products decreased its contribution to SOC

## 293 **Discussion**

### 294 *Effect of decomposition products on SOC mineralization, CUE and microbial biomass carbon*

295 The nature of decomposition products and in particular their production in presence or  
296 absence of earthworms affected SOC mineralization. Generally, the addition of no EW  
297 decomposition products induced increased SOC mineralization. This is in agreement with  
298 studies on compost and vermicompost addition to a similar soil type (Ribeiro *et al.*, 2010) and  
299 may be explained by priming effects induced by the addition of labile carbon (Fontaine *et al.*,  
300 2003) through exogenous OM input (Peltre *et al.*, 2012, Bernal *et al.*, 1998). Indeed,  
301 decomposition products produced in the presence of earthworms may contain be less readily  
302 decomposable OM as compared to the ones produced without earthworms due to lower  
303 amounts of WSOC, higher aromatic C contribution (Barthod *et al.*, 2020) and increased  
304 stabilization through organo-mineral associations (Le Mer *et al.*, 2020). These observations  
305 suggest that decomposition products produced in the presence of earthworms are not a very  
306 efficient energy sources (Ngo *et al.*, 2012) to sustain catabolic processes (respiration) but that  
307 they may be efficiently used for anabolic processes (microbial growth). This is supported by  
308 the observation of higher microbial carbon use efficiency (CUE) in EW than no EW  
309 treatment.

310 In the presence of minerals, CUE was thought to control the formation of organo-mineral  
311 interactions and therefore the formation of stabilized SOC (Cotrufo *et al.*, 2013). This is in  
312 agreement with the observation that decomposition products, which emit high amounts of  
313 CO<sub>2</sub> during their formation, are characterized by lower CO<sub>2</sub> emissions after their addition to  
314 soil (Fig. 5). It was interesting to note that despite similar microbial biomass contribution to  
315 SOC (C<sub>mic</sub>/C<sub>org</sub>), CUE of the added substrates during the incubation was strongly dependent  
316 on the amendment type. CUE was considerably decreased in soils amended with substrates

317 produced without earthworms, suggesting that earthworm presence enhances SOC  
318 stabilization through enhancing CUE after their soil addition. However, when decomposition  
319 products contained minerals, their CUE was lower than for pure organic substrates. CUE also  
320 depended on the type of mineral and could be 0 in the case of KG. These results are  
321 somewhat contradictory to the common observation that minerals stabilize organic carbon  
322 against mineralization as mineral-containing decomposition products increased CO<sub>2</sub>  
323 emissions after soil addition in comparison to their mineral-free controls (Fig. 5).

324 *Effect of decomposition products produced with minerals and earthworms on soil*  
325 *physicochemical parameters, SOC distribution and soil nitrogen forms*

326 Relative to the un-amended soil, SOC was increased in the short term by organic amendment  
327 application, as already observed by Ngo et al (2012). Our results showed that the type of  
328 decomposition product affected significantly the OC distribution in soil fractions. No EW  
329 products are characterized by higher pH, OC, NH<sub>4</sub><sup>+</sup> and WSOC contents as compared to EW  
330 decomposition products. Moreover, they contain higher amounts of labile C most probably in  
331 form of easily available carbohydrate compounds (Barthod et al., 2020) than EW  
332 decomposition products.

333 The addition of these materials increased WSOC contents as compared to EW treatments,  
334 which might be positive for the improvement of soil fertility, since WSOC contains easily  
335 available energy sources (Christensen, 2001), which could favor microbial activity. However,  
336 the application of mineral-containing decomposition products, especially those formed in the  
337 presence of montmorillonite, might change the amount and composition of WSOC. The SOC  
338 content of mineral-associated and stable aggregate fractions of amended soils were also  
339 affected in 15M EW and KG EW treatments. Indeed, the SOC contribution of these fractions  
340 was increased with regards to the mineral-free controls and soil amended with no EW  
341 decomposition products, indicating that SOC in soils receiving these kinds of decomposition  
342 products are less susceptible to mineralization. This was supported by extrapolation of CO<sub>2</sub>  
343 emissions during 2 years (Fig. 3).

344 In addition to SOC storage, we investigated if the nature of the decomposition products has an  
345 impact on the biogeochemical cycling of nitrogen. According to our results, total soil nitrogen  
346 after incubation was increased by the addition of decomposition products regardless their  
347 nature (Table 4). Similar results have previously been reported (Bustamante *et al*, 2010). The  
348 nature of decomposition products affected available N forms. In particular, the addition of

349 EW decomposition products increased  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations of amended soils (Table  
350 4). This could be due to direct input of available N forms, which might not be used by the soil  
351 microbial biomass for anabolism due to the absence of easily available SOC compounds.  
352 However, the easily available nitrogen of the decomposition products may be used by plants  
353 and affect their growth, which was found to be enhanced after addition of mineral-containing  
354 decomposition products (Vidal et al., 2020).

355 *Microbial products and their interaction with minerals and epigeic earthworms: an efficient*  
356 *strategy to increase soil carbon storage?*

357 While the importance of endogeic and anaecic earthworm species for SOM stabilization  
358 through their cast forming activity has been known for long (Shapiro et al., 1989), epigeic  
359 earthworms used in our study were thought to contribute little to SOM stabilisation as they  
360 are assumed to produce mineral-free casts (Huang et al., 2020). The results from our study  
361 suggest that epigeic earthworms during OM decomposition in presence of minerals may favor  
362 the formation of material, capable of slowing down SOC mineralization and concomitantly  
363 increase CUE after soil addition. The beneficial activity of earthworms for stabilizing  
364 microbial necromass has recently been highlighted (Angst et al., 2019). For epigenic  
365 earthworm species, this process may be important in no till agricultural, grassland and forest  
366 soils at the litter-soil interphase and should be considered when assessing carbon stabilization  
367 potential of these (agro-)ecosystem types. As this ecological earthworm group is frequently  
368 used for organic waste transformation into soil amendment (vermicomposts), the results of  
369 our study could thus also be valuable in the context of ecotechnological applications as for  
370 example the development of low emission organic fertilizers (Calabi Floody et al., 2018;  
371 Barthod et al., 2018).

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**Table 1** Main characteristics of the decomposition products produced with (EW) and without (No EW) earthworms used in this experiment (Data from Barthod et al. (2020)). Small different letters indicate statistical differences among organic amendments.

	Mineral contribution	pH*		OC		NO <sub>3</sub> -		NH <sub>4</sub> <sup>+</sup>		WSOC*	
		No EW	EW	□ g g <sup>-1</sup>		μg g <sup>-1</sup>		No EW	EW	No EW	EW
				No EW	EW	No EW	EW				
<b>Control</b>	No minerals	8.5 <sup>a</sup>	7.6 <sup>c</sup>	381.2 <sup>a</sup>	331.2 <sup>a</sup>	1.7 <sup>d</sup>	670.4 <sup>a</sup>	50.6 <sup>a</sup>	29.0 <sup>b</sup>	39.8 <sup>a</sup>	12.2 <sup>c</sup>
<b>15M</b>	15% Montmorillonite	8.1 <sup>c</sup>	7.8 <sup>d</sup>	244.5 <sup>bc</sup>	206.5 <sup>b</sup>	1.0 <sup>d</sup>	231.2 <sup>b</sup>	33.3 <sup>ab</sup>	27.5 <sup>bc</sup>	20.8 <sup>b</sup>	6.3 <sup>d</sup>
<b>30M</b>	30% Montmorillonite	8.2 <sup>c</sup>	7.4 <sup>c</sup>	179.9 <sup>c</sup>	152.5 <sup>c</sup>	0.2 <sup>e</sup>	206.7 <sup>b</sup>	18.0 <sup>cd</sup>	14.4 <sup>d</sup>	4.7 <sup>e</sup>	3.5 <sup>e</sup>
<b>KG</b>	15% Kaolinite/15% Goethite	8.8 <sup>a</sup>	8.3 <sup>b</sup>	204.5 <sup>b</sup>	151.8 <sup>c</sup>	3.9 <sup>c</sup>	20.5 <sup>c</sup>	48.9 <sup>a</sup>	17.7 <sup>d</sup>	24.1 <sup>a</sup>	14.5 <sup>c</sup>

\*data from Barthod et al. (2020)

**Table 2:** Characteristics of the soil used in this experiment.

<b>Characteristics</b>	<b>Values</b>
EC (dS m <sup>-1</sup> )	0.055
pH	6.6
Bulk density (g cm <sup>3</sup> )	1.29
N (mg g <sup>-1</sup> )	1.03
Organic carbon (mg g <sup>-1</sup> )	10.45
C:N	10.16
Clay g kg <sup>-1</sup>	159
Silt g kg <sup>-1</sup>	380
Sand g kg <sup>-1</sup>	452

**Table 3:** Minerals used during the production of decomposition products

<b>Minerals</b>	<b>Contribution</b>	<b>Treatment name</b>
	<i>% of initial OM</i>	
Montmorillonite	15	15M
Montmorillonite	30	30M
Kaolinite + Goetite	15 + 15	KG

**Table 4:** General parameters of soil amended with contrasting organic material after 79 days of incubation. Amendment type refers to decomposition products produced with and without mineral and/or earthworms. Data are presented as means of 3 replicates. Different letters indicate significant differences among mineral treatments; \*indicate significant differences between EW and no EW treatments

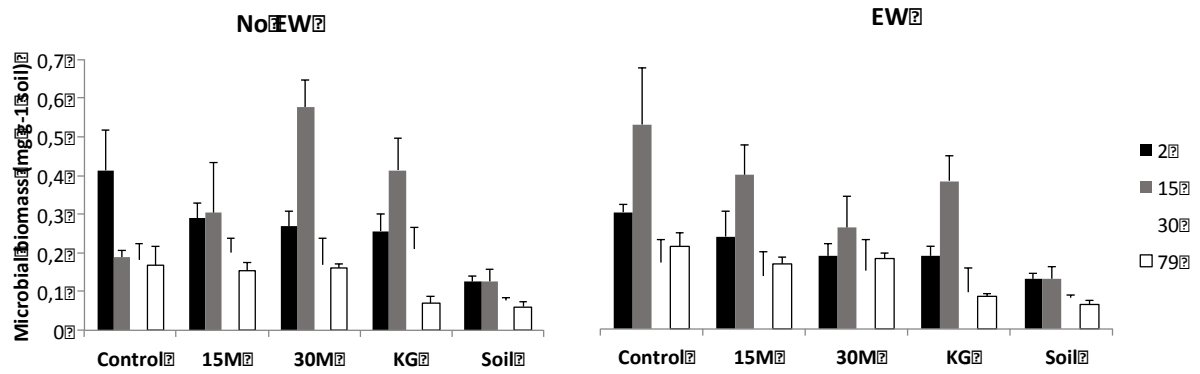
<b>Amendment type</b>	<b>C</b>	<b>N</b>	<b>C/N</b>	<b>DOC</b>	<b>NO3</b>	<b>NH4</b>	<b>Cmic/Corg</b>	<b>CUE</b>
	<i>mg g<sup>-1</sup></i>	<i>mg g<sup>-1</sup></i>		<i>μg g<sup>-1</sup></i>	<i>μg g<sup>-1</sup></i>	<i>μg g<sup>-1</sup></i>		
<b>soil</b>	10.3	1.0	10.1	15.4	12.2	5.3	0.6	
<b>Soil with decomposition products (no EW)</b>								
<b>Control</b>	15.7 <sup>a</sup>	1.6	9.6 <sup>a</sup>	49.2 <sup>a</sup>	10.1 <sup>a</sup>	5.4 <sup>a</sup>	1.0	1.86
<b>15M</b>	14.6 <sup>a</sup>	1.6	8.8 <sup>ab</sup>	33.8 <sup>ab</sup>	19.9 <sup>b</sup>	10.5 <sup>a</sup>	1.0	0.52
<b>30M</b>	13.7 <sup>ab</sup>	1.6	8.5 <sup>bc</sup>	27.7 <sup>bc</sup>	15.4 <sup>ab</sup>	17.0 <sup>ac</sup>	1.2	0.49
<b>KG</b>	11.6 <sup>b</sup>	1.6	7.3 <sup>c</sup>	33.3 <sup>bc</sup>	20.5 <sup>b</sup>	13.0 <sup>c</sup>	0.6	0.00
<b>Soil with decomposition products (EW)</b>								
<b>Control</b>	15.7 <sup>a</sup>	2.0	8.6 <sup>a</sup>	34.8 <sup>ab*</sup>	33.7 <sup>a*</sup>	7.7 <sup>a*</sup>	1.1	3.37
<b>15M</b>	14.3 <sup>ab</sup>	1.6	8.8 <sup>ab</sup>	36.8 <sup>bc*</sup>	26.3 <sup>ab</sup>	25.6 <sup>b*</sup>	1.2	0.94
<b>30M</b>	13.3 <sup>ab</sup>	1.5	8.5 <sup>ab</sup>	24.3 <sup>de*</sup>	25.5 <sup>b*</sup>	27.9 <sup>bc*</sup>	1.3	1.12
<b>KG</b>	12.5 <sup>b</sup>	1.5	8.3 <sup>b*</sup>	25.7 <sup>de*</sup>	22.1 <sup>bc</sup>	23.5 <sup>ac*</sup>	0.6	0.00

15M : Decomposition products produced in presence of 15% Montmorillonite ; 30M : Decomposition products produced in presence of 30% Montmorillonite ; KG : Decomposition products produced in presence of a Kaolinite 15%/Goetite 15% mixture ; control: decomposition products without minerals ; soil: un-amended soil before incubation

**Table 5:** Parameters of the 2 pool model used to predict C mineralization of the nine treatments (soils amended with decomposition products produced with and without earthworms and un-amended soil)

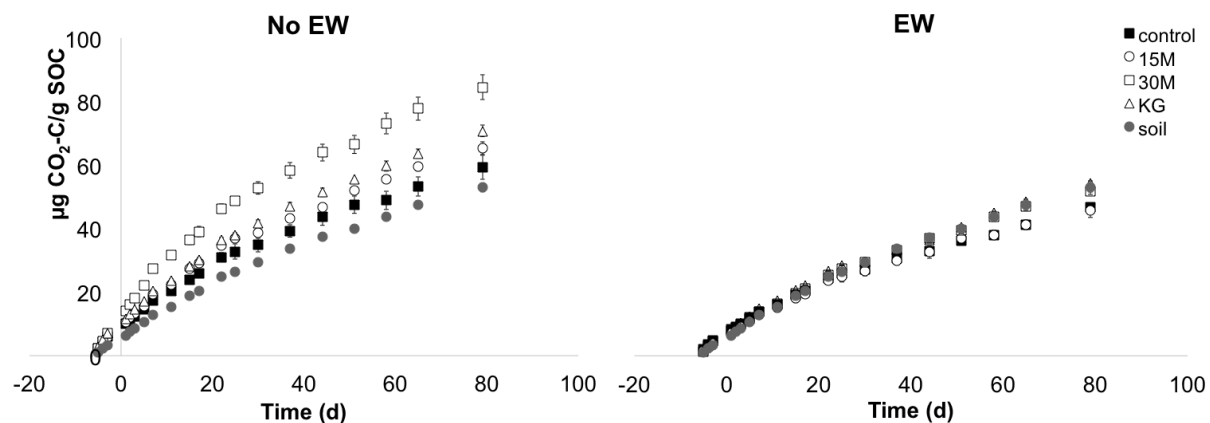
<b>Treatment</b>	<b>C1</b>	<b>K1</b>	<b>T1/2</b>	<b>C2</b>	<b>K2</b>	<b>T1/2</b>	<b>r<sup>2</sup></b>
<b>Control (EW)</b>	12.0	0.149	4.6	192.5	0.002	347	0.99
<b>Control (No EW)</b>	13.3	0.152	4.6	116.2	0.005	139	0.99
<b>15M (EW)</b>	10.6	0.157	4.4	87.7	0.006	115	0.99
<b>15M (No EW)</b>	17.4	0.139	5.0	183.3	0.003	231	0.99
<b>30M (EW)</b>	10.7	0.157	4.4	129.5	0.004	173	0.99
<b>30M (No EW)</b>	32.6	0.124	5.6	197.9	0.003	231	0.99
<b>KG (EW)</b>	11.7	0.162	4.3	192.8	0.003	231	0.99
<b>KG (No EW)</b>	14.6	0.163	4.2	125.5	0.007	99	0.99
<b>soil</b>	10.5	0.153	4.5	124.4	0.005	139	0.99

15M : Decomposition products produced in presence of 15% Montmorillonite ; 30M : Decomposition products produced in presence of 30% Montmorillonite ; KG : Decomposition products produced in presence of a Kaolinite 15%/Goetite 15% mixture



15M : Decomposition products produced in presence of 15% Montmorillonite ; 30M : Decomposition products produced in presence of 30% Montmorillonite ; KG : Decomposition products produced in presence of a Kaolinite 15%/Goetite 15% mixture

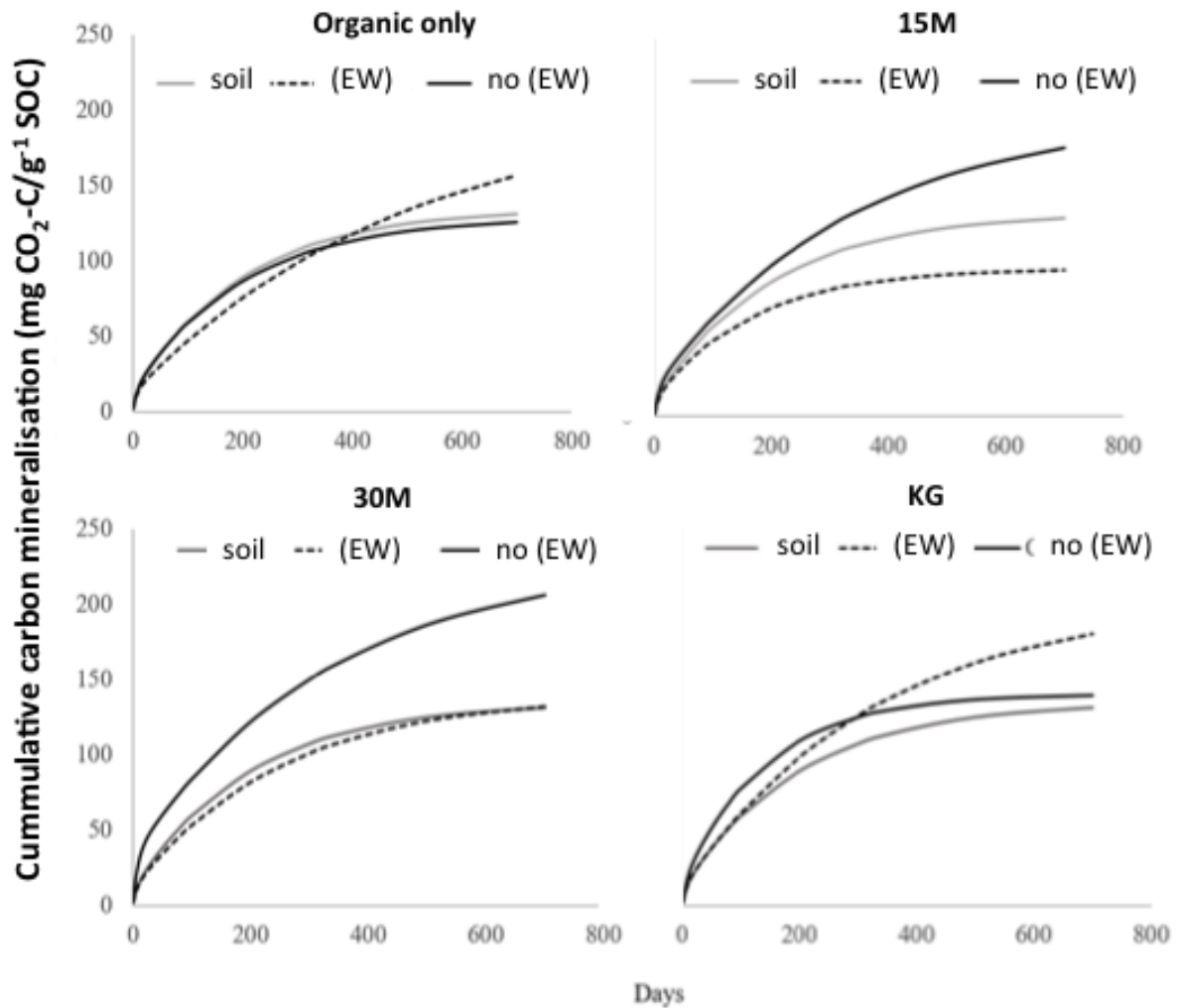
**Fig. 1:** Microbial biomass in soil amended with different types of decomposition products produced with and without earthworms at different times after the beginning of incubation.



Soil : un-amended soil ; 15M : Soil amended with decomposition products produced in presence of 15% Montmorillonite ; 30M : Soil amended with decomposition products produced in presence of 30% Montmorillonite ; KG : Soil amended with decomposition products produced in presence of a Kaolinite 15%/Goetite 15% mixture

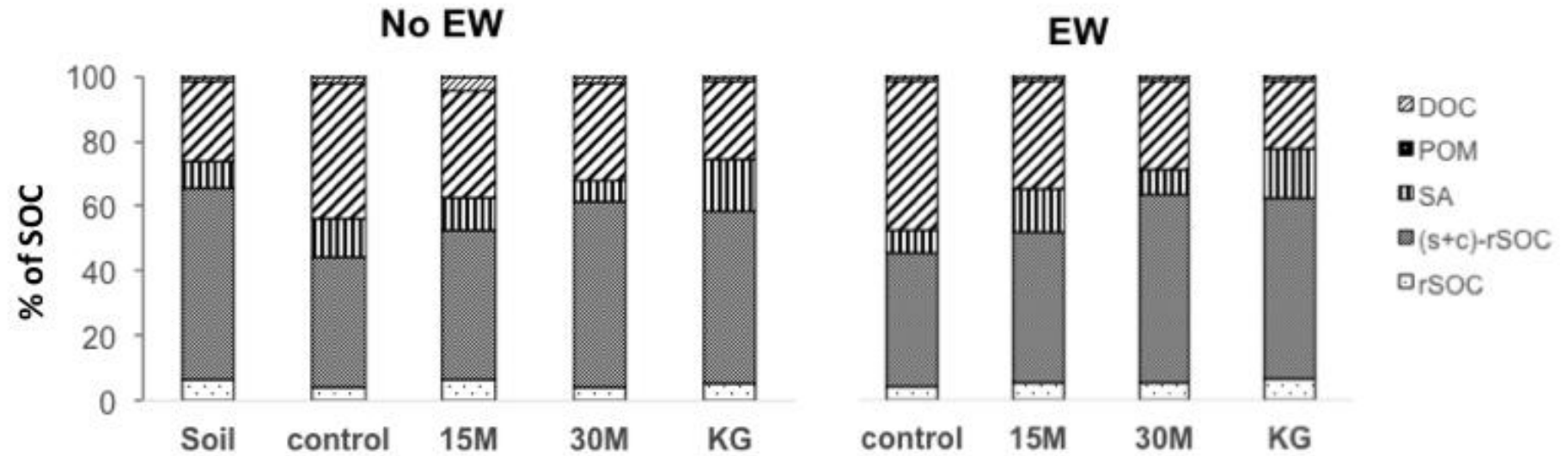
**Fig. 2:** Cumulative carbon mineralization in un-amended soil (soil) and soils amended with decomposition products produced in the presence (EW) and absence (no EW) of earthworms



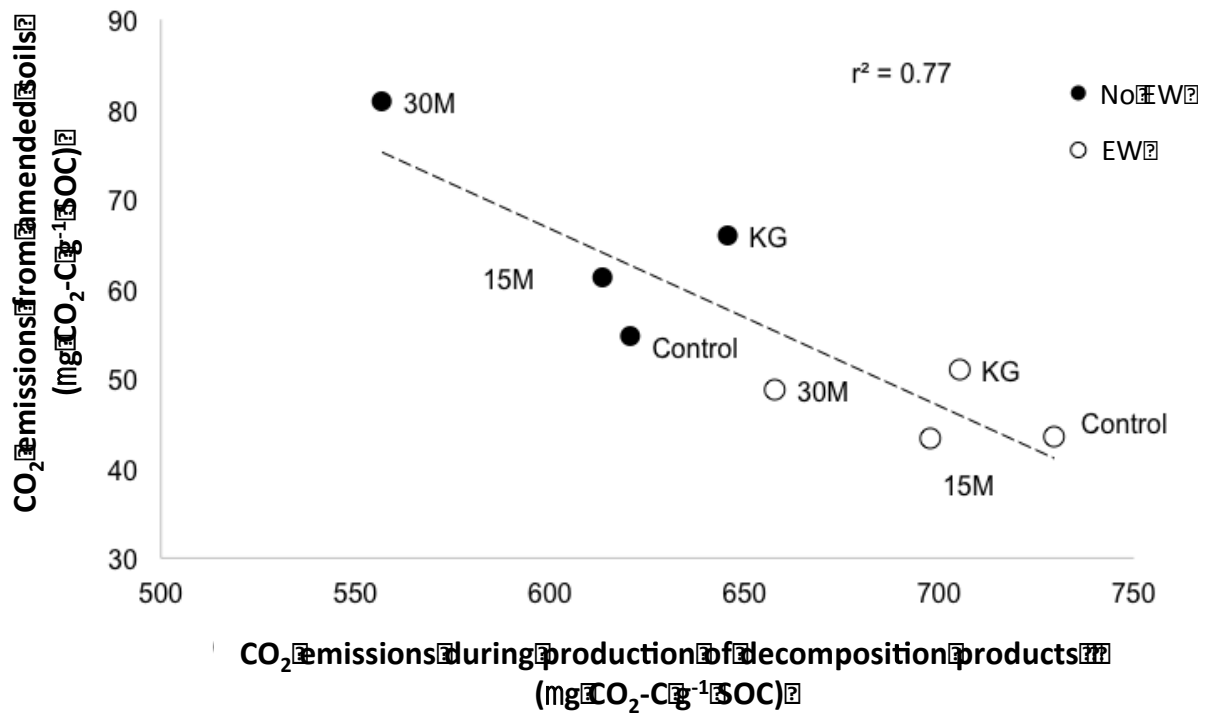


Soil : un-amended soil ; 15M : Soil amended with decomposition products produced in presence of 15% Montmorillonite ; 30M : Soil amended with decomposition products produced in presence of 30% Montmorillonite ; KG : Soil amended with decomposition products produced in presence of a Kaolinite 15%/Goetite 15% mixture

**Fig. 3:** Cumulative carbon mineralization in un-amended soil (soil) and soil amended with the four decomposition products (control, 15M, 30M and KG) produced with (EW) and without (no EW) earthworms predicted for a two year period with the two pool model (Table 5)



**Fig. 4:** SOC distribution in physico-chemical fractions in un-amended soil (Soil) and soil amended with decomposition products produced with minerals (15M, 30M and KG) and without minerals (control) in presence (EW) and absence (No EW) of earthworms. Data represent means of three replicates. Different letters indicate significant differences among treatments for one fraction ( $p < 0.05$ ).



15M : Decomposition products produced in presence of 15% Montmorillonite ; 30M : Decomposition products produced in presence of 30% Montmorillonite ; KG : Decomposition products produced in presence of a Kaolinite 15%/Goetite 15% mixture ; control : Decomposition products produced without minerals

**Fig. 5:** Relationship between CO<sub>2</sub> emissions during production of decomposition products produced with minerals (30M, 15M, KG) and without minerals (control) in presence (EW) and absence (No EW) of earthworms and CO<sub>2</sub> emissions of soil amended with these products