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

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## Abstract

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

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

## Introduction

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

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

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

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

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

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

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

The aim of the study was to evaluate:

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

## Material and Methods

### *Decomposition products*

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

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

#### *Soil sampling and preparation*

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

#### *Incubation experiment*

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

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

### *Physico-chemical fractionation*

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

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

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

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

### *Carbon mineralisation*

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

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

#### *Microbial biomass*

Microbial biomass was determined on days 2, 15, 30 and 79 using the chloroform-fumigation extraction method (Vance *et al.*, 1987). Briefly, 5 g of fresh soil were fumigated with CHCl<sub>3</sub> in a desiccator during 16h, allowing for lysis of microbial cells. Fumigated and non-fumigated 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 analyzed for DOC (see above). Microbial biomass carbon (MBC) was calculated using the following equation:

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

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

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

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

$$\text{CUE} = (\text{MBC}_{\text{treatment}} - \text{MBC}_{\text{soil}}) / (\text{MBC}_{\text{soil}} + \text{C-CO}_2 \text{ treatment mineralised}),$$

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

#### *Statistical analysis*



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

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

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),  $C_1$  (mg C-CO<sub>2</sub> g<sup>-1</sup> soil) is the labile/fast mineralisable carbon pool, associated with the rate 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, associated with the rate constant  $k_2$  (day<sup>-1</sup>).

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

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

## Results

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

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

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

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

#### *Microbial biomass*

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

#### *Carbon mineralisation*

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

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

In order to describe the dynamics of carbon mineralization, carbon mineralization data were fitted with a two pool model. According to this model, the labile carbon pool ( $\text{C}_1$ ) was higher in amended soils than in un-amended soils. Moreover, it was lower in EW treatments as compared to no EW treatments. The degradation rates associated with this pool ( $k_1$ ) showed the opposite behavior (no EW treatments < EW treatments). For instance, the application of 30M (no EW) to soil led to the highest labile carbon pool (32.7  $\text{mg g}^{-1}$  soil) while its degradation rate was the lowest (0.124  $\text{day}^{-1}$ ). The second model pool ( $\text{C}_2$ ) represents the slowly mineralisable carbon fraction. This fraction increased when in amended soil, except

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

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

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

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

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

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

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

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

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

## Discussion

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

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

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

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

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

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

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

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

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

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

While the importance of endogeic and anaecic earthworm species for SOM stabilization through their cast forming activity has been known for long (Shapiro et al., 1989), epigeic earthworms used in our study were thought to contribute little to SOM stabilisation as they are assumed to produce mineral-free casts (Huang et al., 2020). The results from our study suggest that epigeic earthworms during OM decomposition in presence of minerals may favor the formation of material, capable of slowing down SOC mineralization and concomitantly increase CUE after soil addition. The beneficial activity of earthworms for stabilizing microbial necromass has recently been highlighted (Angst et al., 2019). For epigenic earthworm species, this process may be important in no till agricultural, grassland and forest soils at the litter-soil interphase and should be considered when assessing carbon stabilization potential of these (agro-)ecosystem types. As this ecological earthworm group is frequently used for organic waste transformation into soil amendment (vermicomposts), the results of our study could thus also be valuable in the context of ecotechnological applications as for example the development of low emission organic fertilizers (Calabi Floody et al., 2018; 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> +		WSOC*	
				$\square g g^{-1}$		$\mu g g^{-1}$					
		No EW	EW	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>e</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>e</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.

Characteristics	Values
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

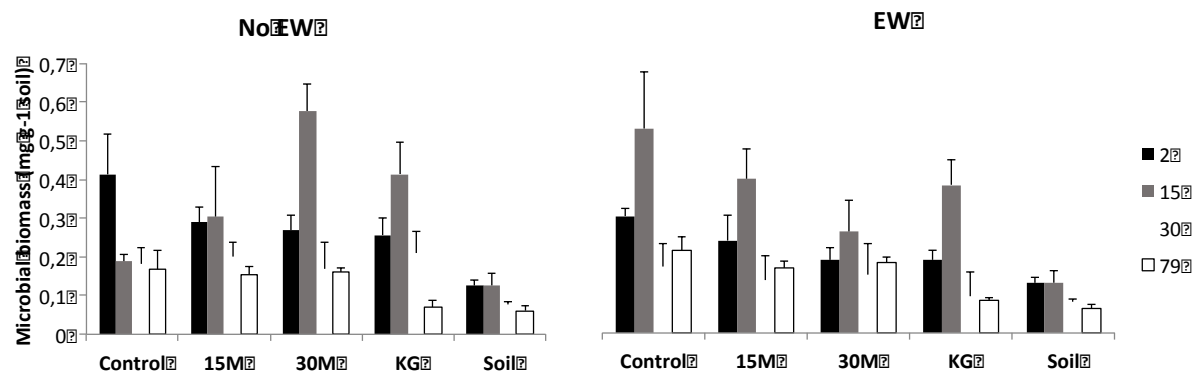
Amendment type	C	N	C/N	DOC	NO <sub>3</sub>	NH <sub>4</sub>	Cmic/Corg	CUE
	<i>mg g<sup>-1</sup></i>	<i>mg g<sup>-1</sup></i>		<i>μg g<sup>-1</sup></i>	<i>μg g<sup>-1</sup></i>	<i>μg g<sup>-1</sup></i>		
soil	10.3	1.0	10.1	15.4	12.2	5.3	0.6	
<b>Soil with decomposition products (no EW)</b>								
Control	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
15M	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
30M	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
KG	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>								
Control	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
15M	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
30M	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
KG	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)

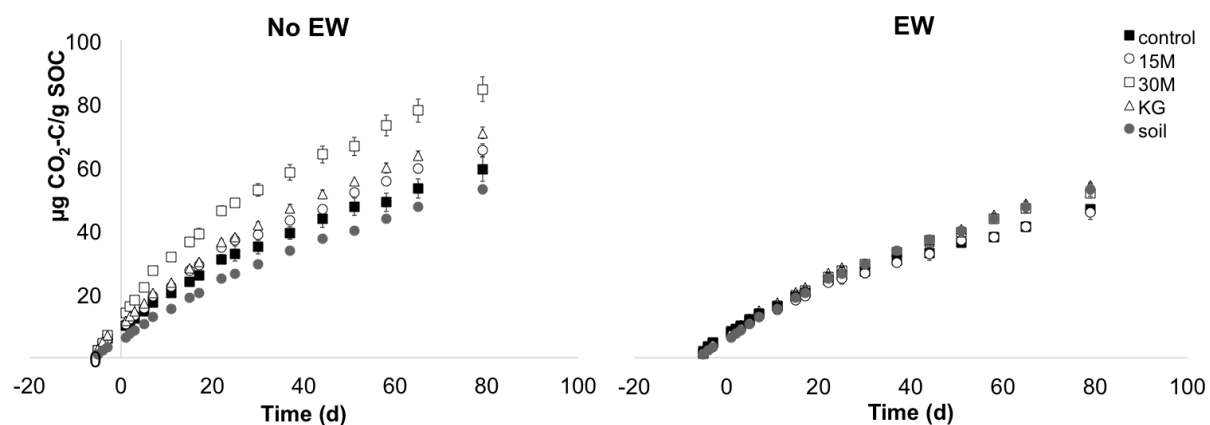
<b>Treatment</b>	<b>C1</b>	<b>K1</b>	<b>T1/2</b>	<b>C2</b>	<b>K2</b>	<b>T1/2</b>	<b>r<sup>2</sup></b>
<b>Control (EW)</b>	12.0	0.149	4.6	192.5	0.002	347	0.99
<b>Control (No EW)</b>	13.3	0.152	4.6	116.2	0.005	139	0.99
<b>15M (EW)</b>	10.6	0.157	4.4	87.7	0.006	115	0.99
<b>15M (No EW)</b>	17.4	0.139	5.0	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

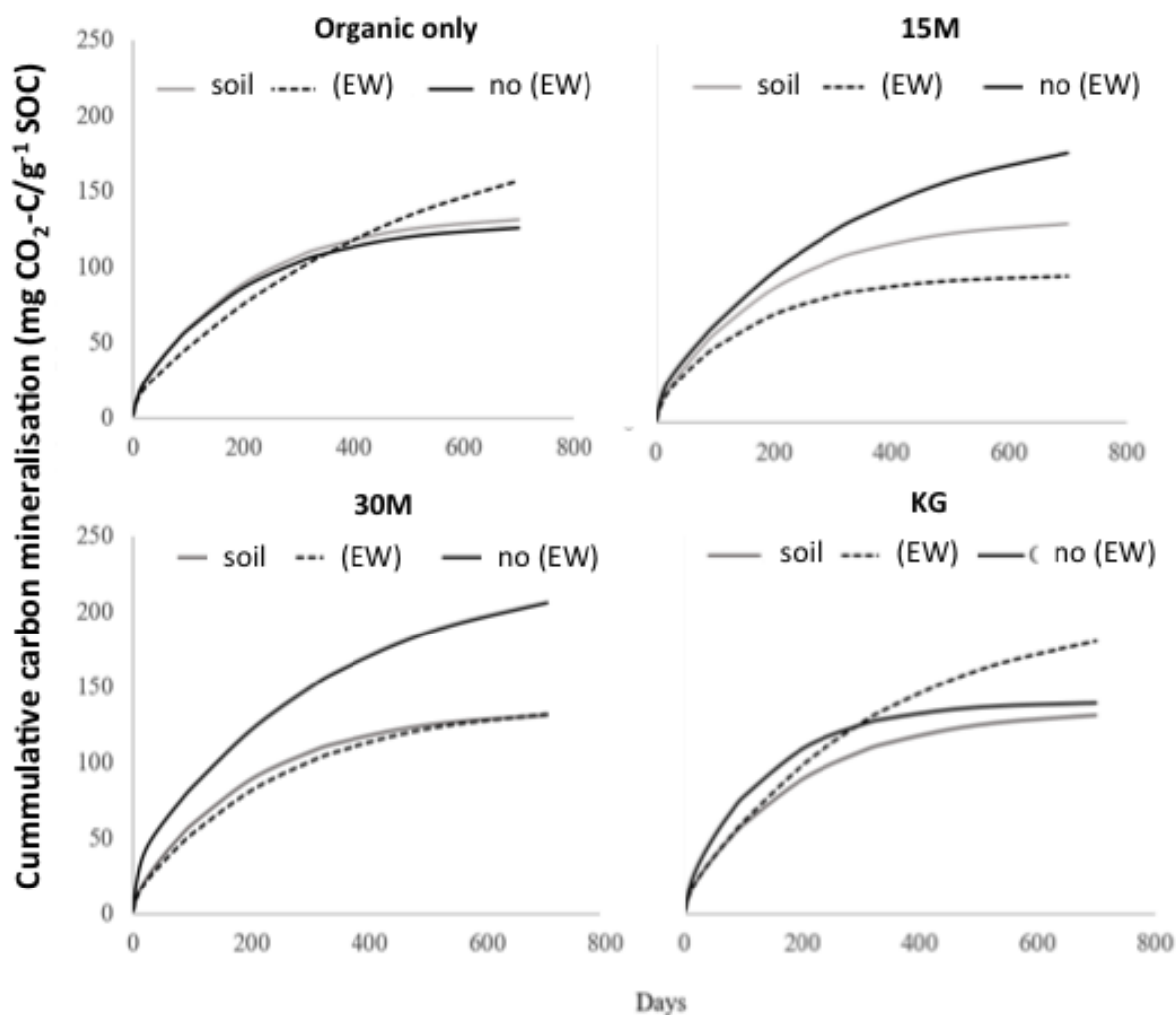
**Fig. 1:** Microbial biomass in soil amended with different types of decomposition products produced with and without earthworms at different times after the beginning of incubation.



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

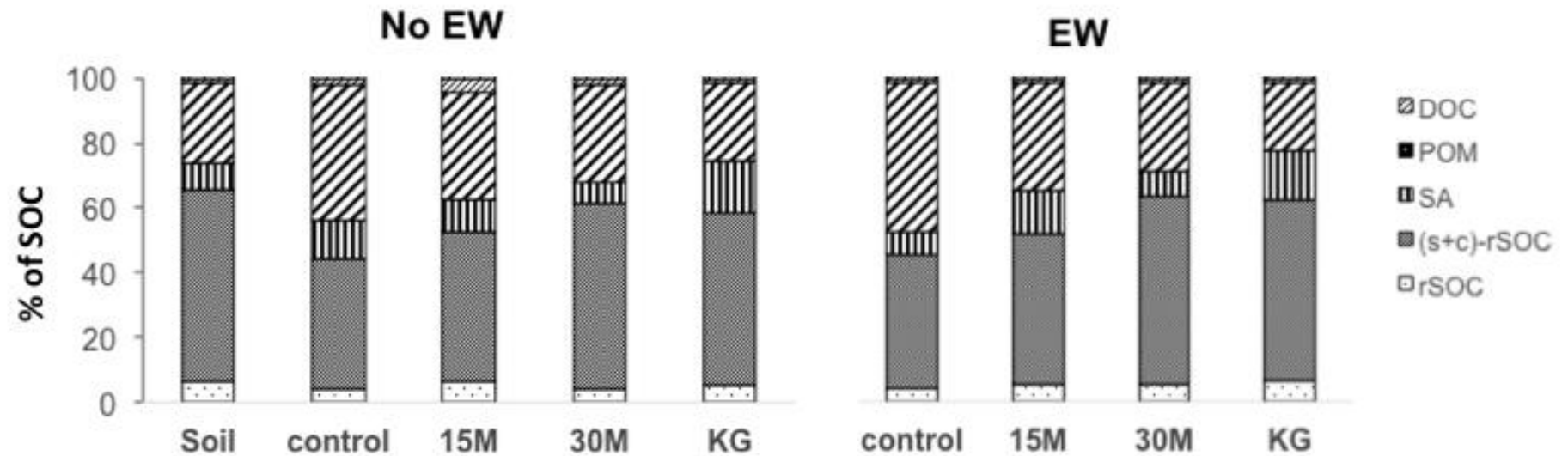
**Fig. 2:** Cumulative carbon mineralization in un-amended soil (soil) and soils amended with decomposition products produced in the presence (EW) and absence (no EW) of earthworms



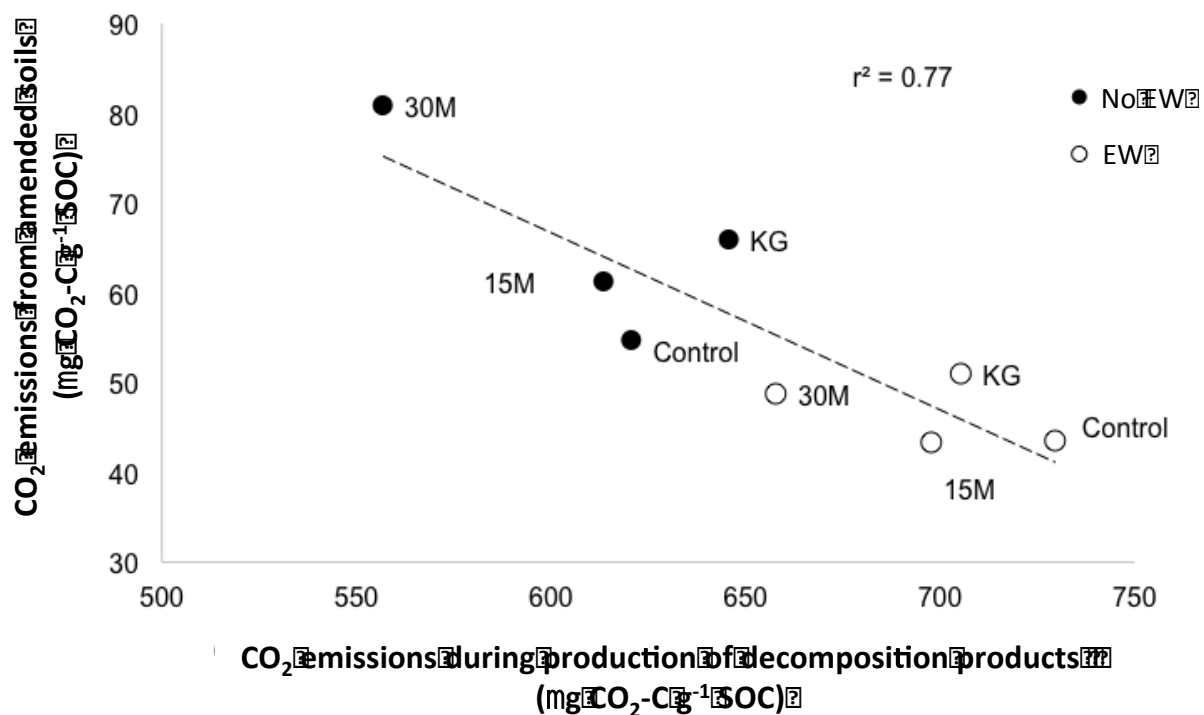


Soil : un-amended soil ; 15M : Soil amended with decomposition products produced in presence of 15% Montmorillonite ; 30M : Soil amended with decomposition products produced in presence of 30% Montmorillonite ; KG : Soil amended with decomposition products produced in presence of a Kaolinite 15%/Goetite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