Dear editor,

Thank you for your new revision and suggestions to improve this manuscript. We hope we have taken into account every comments of the reviewer in this new version. According to these comments, we have changed the structure of the discussion, now it is divided into 3 parts and the headers of each part have been changed also (*4.1 Effect of clay and biochar on carbon mineralization during composting; 4.2 The presence of worms modifies unexpectedly the effect of clay and biochar on CO_2 emissions during composting; 4.3 Amendment composition and production influences mineralization in soil and total CO_2 emissions*). We have also changed the conclusion, to be in accordance with the discussion. The figures have been improved in term of quality and all the abbreviations have been explained in the legend.

Best regards,

Justine Barthod

The effects of worms, clay and biochar on CO_2 emissions during production and soil application of co-composts

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Abstract

In this study we evaluated CO_2 emissions during composting of green wastes with clay and/or biochar in the presence and absence of worms (species of the *Eisenia* genus), as well as the effect of those amendments on carbon mineralization after application to soil. We added two different doses of clay, biochar or their mixture to pre-composted green wastes and monitored carbon mineralisation during 21 days in absence or presence of worms. The resulting co-composts and vermicomposts were then added to a loamy Cambisol and the CO_2 emissions were monitored during 30 days in a laboratory incubation. Our results indicated that the addition of clay or clay/biochar mixture reduced carbon mineralization during co-composting without worms by up to 44%. In the presence of worms, CO_2 emissions during composting increased for all treatments except for the low clay dose. The effect of the amendments on carbon mineralization after addition to soil was small in the short-term. Overall, composts increased OM mineralization whereas vermicomposts had no effect. The presence of biochar reduced OM mineralization in soil with respect to compost and vermicompost without additives, whereas clay reduced mineralization only in the composts. Our study indicates a significant role of the conditions of composting on mineralization in soil. Therefore, the production of a low CO_2 emission amendment requires optimisation of feedstocks, co-composting agents and worm species.

Keywords: carbon mineralization; worm; composting; biochar; clay; soil.

1. Introduction

Land use changes are responsible for the steady increase of CO_2 in the atmosphere, along with industrial activity and the use of fossil fuels. In this context, massive soil organic matter (OM) loss is observed, leading to the decline of many soil ecosystem services, such as fertility and carbon storage (Smith *et al*, 2015). These global changes of the earth's climate and (agro-)ecosystems have major environmental, agronomic but also social and economic consequences, which could be attenuated by the rebuilding of soil OM stocks (IPCC, 2014). Increasing soil carbon may be possible with the use of composted organic wastes as alternative fertilisers (Barral *et al*, 2009; Ngo *et al*, 2012), which could counterbalance the concentration of greenhouse gases in the atmosphere through soil carbon sequestration (Lashermes *et al*, 2009).

Two well-known aerobic processes based on microbial activity are able to transform organic wastes into valuable soil amendments: composting and vermicomposting. Composting has been traditionally used and leads to stabilized organic amendments with fertilization potential. During vermicomposting the presence of worms induces a continuous aeration resulting in a faster OM transformation (Lazcano *et al*, 2008; Paradelo *et al*, 2009, 2010). However, vermicomposting and composting both emit greenhouse gases such as CO_2, CH_4 and N_2O (Hobson *et al*, 2005; Chan *et al*, 2011; Thangarajan *et al*, 2013). In addition, the final products of these processes lead to greenhouse gas emissions after their application to soil (Cambardella *et al*, 2003; Bustamante *et al*, 2007). These emissions can originate from the mineralization of (vermi)compost OM itself or maybe due to the mineralization of native soil OM following increased microbial development and activity, a mechanism known as priming effect (Bustamante *et al*, 2010).

In order to optimize the recycling of waste carbon, there is a need to enhance OM stabilization during (vermi)composting. Stabilization mechanisms are poorly known for composting processes, while they have been widely studied in soils. Enhancing carbon stabilization in composts could thus benefit from an analogy with the mechanisms known to occur in soils (von Lützow *et al*, 2006): spatial inaccessibility, selective preservation due to chemical recalcitrance, and formation of

organo-mineral associations. Among these processes, the association of OM with minerals is the most efficient for carbon stabilization on long time scales (Kleber *et al*, 2015). Therefore, a variety of minerals has been used to reduce gas emissions (CO_2, CH_4, NH_3 and N_2O) during co-composting (Bolan *et al*, 2012; Chowdhury *et al*, 2015), and co-vermicomposting (Wang *et al*, 2014) of wastes. However, to the best of our knowledge, no studies have been carried out to evaluate the effect of minerals on carbon stability of the resulting organic materials after their addition to soil.

Another material suitable to reduce greenhouse gas emission during co-composting is biochar, which has been reported to decrease these emissions after soil amendment (Bass *et al*., 2016; Ventura *et al*., 2015). Biochar results from the incomplete combustion or pyrolysis of various feedstock materials. The biochar production process transforms OM into aromatic products, which are resistant against microbial decomposition and show increased adsorption properties compared to untransformed OM (Lehman *et al*, 2006). As a result, the use of biochar as co-composting agent leads to a reduction of carbon emissions due to adsorption of organic constituents on the biochar surface (Rogovska *et al,* 2011; Jindo *et al,* 2012; Vu *et al*, 2015).

To further enhance the protection of OM through the formation of organo-mineral or OM-biochar interactions during co-composting, the addition of worms may be a promising avenue. In general, organo-mineral associations and aggregation are enhanced by the presence of worms (Lavelle *et al*, 2006), due to the simultaneous ingestion of OM and minerals (Shipitalo and Protz, 1989). Micro-aggregates formed inside the worm guts improve physical protection of carbon (Bossuyt *et al*, 2005). During co-composting, the addition of worms may thus favour the protection of carbon and prevent its rapid release. On the other hand, earthworms have been shown to increase CO_2 emissions from soils in the short term due to a stimulation of aerobic respiration (Lubbers *et al*, 2013). These contrasting effects may result in a positive or negative global impact of worms on carbon accumulation (Blouin *et al*, 2013). Therefore, we suggest that the assessment of the CO_2 emission potential of co-composts produced in the presence of worms needs to take into account the production process itself and the effects of the amendments on C mineralisation from soil.

In this study we used a model system in order to assess the alterations of the carbon balance of co-composts induced by the presence of worms through the formation of organo-mineral or organo-biochar interactions. We measured CO_2 emissions during two laboratory experiments (1) composting of organic wastes and (2) soil incubation with the amendments. We hypothesized that carbon stabilization during composting would be increased by addition of (a) montmorillonite, a 2:1 clay, able to form organo-mineral associations; (b) biochar, able to protect OM by adsorption and (c) their mixture, which could create synergistic effects. We further hypothesised that the addition of worms additionally influences the magnitude of the CO_2 emissions. The aim of the study was to investigate if worms can be used during co-composting of organic wastes and clay, biochar or their mixture to produce organic amendments with low CO_2 emission potential.

2. Materials and methods

2.1 Compost, additives and worms

A pre-composted green waste was sampled in its maturation phase at BioYvelines service, a platform of green waste composting located 30 km West from Paris (France). The green wastes were a mix of shredded leaves, brushwood and grass cuttings collected from households or firms near the platform. Briefly, the composting process was performed in windrows, which are long narrow piles of green waste. Aerobic conditions and optimal humidity (approximately 45 %) were maintained through mechanical aeration and water sprinkling. The pre-composted material was sampled after 4 months, at the beginning of the maturation phase. Compost pH was 8.5 and the C:N ratio was 13.6 with 205.1 $mg.g^{-1}$ of organic carbon (OC) and 13.3 $mg.g^{-1}$ of nitrogen (N). After sampling, the compost was air-dried and sieved at 3 mm for homogenization.

Montmorillonite, a 2:1 clay, was purchased from Sigma-Aldrich. The clay's pH was between 2.5 and 3.5 and its specific surface area (SSA) was 250 m^2/g. Montmorillonite was chosen because organo-mineral interactions depend on clay mineralogy (1:1 clay or 2:1 clay). In general, 2:1 minerals offer a bigger contact area for OM bonding and create stronger bonds with OM than the

1:1 minerals (Kleber *et al*, 2015). Thus numerous organo-mineral associations were expected due to this large SSA.

The biochar was was provided by Advanced Gasification Technology (Italy). It was produced by gasification at 1200°C of a conifer feedstock and had a pH of 9.3 and a C:N ratio of 4030, with 806 mg g^{-1} of OC and 0.2 mg g^{-1} of N (Wiedner *et al*, 2013).

Eisenia andrei and *Eisenia foetida* worms were purchased from La Ferme du Moutta, a worm farm in France. The two species were chosen because they present a high rate of consumption, digestion and assimilation of OM, can adapt to a wide range of environmental factors, have short life cycles, high reproductive rates and endurance and resistance to handling (Dominguez and Edwards, 2011).

2.3 Experimental setup

The present study was designed to evaluate and compare the CO_2 emissions of the different organic materials during the production phase and after their addition to soil (Fig.1)

Composting

Composting was carried out at ambient temperature in the laboratory with 10 treatments and four replicates per treatment: (i) compost alone (control), (ii) compost with 25% (w/w) of montmorillonite (low clay treatment), (iii) compost with 50 % (w/w) of montmorillonite (high clay treatment), (iv) compost with 10% (w/w) of conifer biochar and (v) compost with a mixture of biochar (10% w/w) and montmorillonite (25% w/w). All treatments were established with and without worms (Table 1). Considering that a clay can retain 1 mg C per m^2 (Feng *et al*, 2011), 50% of clay and 25% of clay were chosen in order to theoretically retain 60% and 30% of the total carbon from the compost. In addition, biochar was moistened before addition to compost to avoid worm mortality due to desiccation (Li *et al*, 2011). The addition of 10% of biochar was chosen according to Weyers and Spokas (2011) to avoid negative effects on worms.

Worms were raised in the same compost as used in the experiment. Eight adult worms were chosen

and cleaned to remove adhering soil/compost before estimating their body mass and added to the organic material.

The experiments were carried out in 2L jars. A dry mass of 75 g of pre-composted material was used in each treatment. Water was sprinkled on jars at the beginning of the experiment to reach an optimal moisture level of 80-90% (water content by weight), which was maintained throughout the experimental period. Jars were placed in the dark at ambient temperature (24°C on average). The (vermi)composting was stopped after 21 days, when all the OM should have been ingested (75 g of compost for 8 worms). Indeed a worm can ingest its weight at maximum per day (0.5g).

At the end of the experiment, worms were counted and weighed again. The amount of coccons and juveniles was recorded. The final (vermi)composts were air dried, sieved at 2 mm and an aliquot was ground for further analyses.

Soil incubation

A loamy cambisol soil was collected for the laboratory experiment from the experimental site of a long-term observatory for environmental research (ORE-ACBB) of INRA, near Lusignan in the South-West of France. This soil was used for crop production for the last three years. The soil was collected at 0-10 cm depth, sieved at 4 mm, homogenized and kept at 4°C until the beginning of the experiment. The soil is carbonate-free and has the following characteristics: pH 6.4, N content 1.15 mgN g^{-1}, carbon content 10.56 mgC g^{-1}, sand 11%, clay 17% and silt 72% (Chabbi *et al,* 2009).

For all the treatments, 57 g of dry soil were weighed and placed into 2L glass jars. The mixtures were homogenized. All ten organic materials obtained during composting were applied to soil at a rate of 67g kg^{-1} (dry weight). Amended and unamended soils were incubated in four replicates in the dark at ambient temperature. Soil moisture was adjusted to 18 % (dry weight) and maintained throughout the experiment by compensating weight losses with deionised water. The CO_2 emissions were measured during 30 days as described below.

2.3 Carbon mineralisation

CO_2 emissions were measured in the headspace of the jars according to Anderson (1982). All incubation jars contained a vial with 30 mL of 1M NaOH (composting) or 0.5M (soil incubation) to trap CO_2. The NaOH vials were covered with a tissue to avoid contamination of the NaOH solution by worms. During co-composting step, NaOH traps were replaced at day 1, 2, 3, 4, 8, 11, 14, 16, 18 and 21. During the incubation with soil, vials were replaced at day 1, 2, 4, 7, 14 and 22. Phenolphalein and $BaCl_2$ solution in excess were added to a 10 mL aliquot of NaOH sampled from each vial. The solution was titrated with 1M HCl until neutrality to determine the CO_2-C released. Three empty jars were used as control.

Results are expressed in mg CO_2-C/ g compost (dry weight) or in mg CO_2-C/ g total organic carbon (TOC) according to the formula:

$$\text{Released } CO2 - C = \frac{(B - V) * M * E}{P}$$

where B is the volume of HCl used to titrate the control (mL); V the volume of HCl used to titrate the sample (mL); M the normality of HCL (1M); E (22) the molar mass of CO_2 divided by 2 (because 2 mol of OH^- are consumed by one mol of CO_2) and P the weight of the sample (grams).

2.4 Properties of the final products after composting

OC and N contents were measured using a CHN auto-analyzer (CHN NA 1500, Carlo Erba). A glass electrode (HANNA instruments) was used to measure pH in water extracts of (vermi)-composts (1:5). Dissolved organic carbon (DOC) contents were determined in 0.034 mol L^{-1} K_2SO_4 extracts (1:5 w/v) using a total organic carbon analyzer (TOC 5050A, Shimadzu).

2.5 Calculations and statistical analysis

The amount of CO_2-C mineralized was expressed as mgC per g of TOC. TOC includes for composting compost carbon and biochar carbon. For soil incubation, it includes soil carbon,

compost carbon and biochar carbon. Finally, a global carbon balance was done and calculated on the basis of the CO_2 emissions from the composting phase and the soil incubation after amendment. These results are expressed as mgC per g of TOC, including soil carbon, compost carbon and biochar carbon.

Additionally, for composting, the amount of CO_2-C mineralized was expressed as mgC per g of compost in order to focus on the carbon from the pre-composted material (the amount of biochar and clay was not included). Biochar is not supposed to be mineralized during this step because it is produced at high temperatures and therefore its carbon is supposed to have a high chemical recalcitrance against biological decomposition (McBeath and Smernik, 2009). Biochar produced at high temperatures showed a very low carbon emissions during a 200 days incubation in soil (Naisse *et al*, 2015), so that we can hypothesize that its mineralization can be neglected compared to OM mineralization during 21 days.

A first-order model was used to describe the rate of carbon mineralization during composting (step 1):

$$C = C_0 (1- e^{(-kt)}), \qquad \textit{equation 1}$$

where C is the cumulative amount of CO_2-C mineralized after time t (mgC g^{-1} compost), C_0 is the initial amount of organic carbon (mgC g^{-1} compost), t is the incubation time (days), and k is the rate constant of CO_2-C mineralization (day^{-1}).

All reported data are the arithmetic means of four replicates. A Kruskal-Wallis test was performed to assess the significance of differences of CO_2 emissions from the different treatments. A Student t test was run to investigate the influence of the different substrates on the worm development. Significance was declared at the 0.05 level. Statistical analyses were carried out using the R 3.12 statistical package for Windows (http://www.r-project.org).

3.Results

3.1 Properties of the co-(vermi)composts

Total N and OC contents, DOC and pH of initial material and the different composts are shown in Table 2. The pH of the treatments ranged from 7.9 to 8.7, with no significant effect of neither additives nor worms, in contrast to results obtained by other authors (Frederickson *et al*, 2007; Lazcano *et al*, 2008). The contrasting results may be explained by a lower production of CO_2 and organic acids by micro-organisms in our experiment due to the almost mature pre-composted material used compared to the fresh green wastes used in previous experiments. The lowest pH was observed for the high clay treatments due to the addition of acidic clay material (pH 2.5 to 3.5). Co-composting with biochars did not lead to any change in pH (Table 2). This may be due to the alkaline pH of the initial material and the low amount of biochar added.

Total OC in all treatments ranged from 118.6 mg g^{-1} to 241.9 mg g^{-1} and total N from 8.5 mg g^{-1} to 13.5 mg g^{-1}. Addition of clay produced lower OC and N concentrations due to dilution, whereas the addition of the carbon-containing biochar increased OC concentrations and decreased N concentrations by dilution. The C:N ratio was significantly higher in treatments with biochar, due to addition of carbon enriched material. Worms had no effect on the OC and N concentrations. These results are in line with those obtained by Ngo *et al* (2013), who suggested that the elemental composition and the chemical structures present in different composts and vermicomposts could be similar.

DOC contents in the treatments ranged from 15.0 to 29.1 mg g^{-1} TOC. The presence of additives significantly decreased the DOC during composting. The lowest DOC concentrations were recorded for composts produced with biochar/clay mixture.

Presence of worms during the composting phase had no effect on pH. Compared to initial material, OC was decreased significantly after 21 days of vermicomposting while N concentrations and DOC content remained unchanged. In treatments with clay, biochar and their mixture, similarly to pH, the presence of worms had no effect on OC or N of the final products. It decreased however DOC concentrations by 12% in the high clay treatment and by 16% in the low clay treatment. Worms also had an effect on compost morphology: compost showed a compact aspect, whereas OM had been

processed into a homogeneous and aerated material in the presence of worms, illustrating the positive effects of worms on the physical structure of the final product.

3.2 Worm growth and reproduction

The number of worms and their total weight were measured before and after 28 days of composting. The number of worms did not vary (p-value > 0.07) and neither did their total weight (p-value = 0.34). Cocoons and juveniles were separated manually from the substrates and counted at the end of composting. The number of cocoons and juveniles in treatments ranged from none to 4. High and low clay treatments did not differ significantly from the control (p-value= 0.39). No cocoon and no juvenile were counted in the biochar treatment. Finally, in treatments with clay/biochar mixture, the number of cocoons and juveniles was significantly higher (p-value=0.003) compared to the treatment with biochar alone with an average of three cocoons and one juvenile.

3.3 Carbon mineralisation during composting

The cumulative CO_2 emissions during composting did not reach a plateau for any treatment (Fig. 2 and 3). Cumulative carbon emissions at the end of the experiment ranged from 6.4 to 11.9 mg CO_2-C g^{-1} compost in treatments without worms (Fig. 2). In the compost treatment without additives (control), the amounts of carbon mineralized after 21 days was about 12 mg CO_2-C g^{-1} compost. Composting with clay led to a significant decrease of the carbon emissions compared to the controls: in the low clay treatment, emission decreased by 15% and in the high clay treatment emissions decreased by 43%. Biochar addition reduced CO_2 emissions during composting by 24% with biochar alone and by 46 % with biochar/clay mixture (Fig. 2 and 4).

Rate constants of carbon mineralization during composting, obtained with the first-order kinetic model (eq. 1), are listed in Table 2. Highest rate constants were observed for composts produced with clay and clay/biochar mixture. Biochar alone decreased carbon mineralization in compost treatments.

In treatments with worms, cumulative CO_2 emissions ranged from 7.9 to 12.0 mg CO_2-C g^{-1} compost (Fig. 3 and 4). The presence of worms (Fig. 4) had contrasting effects on carbon mineralisation (mg g^{-1} TOC) in the different treatments: 1) no change in treatments free of additives (control); 2) decrease in the low clay treatments and 3) increase in the treatments with high clay and biochar/clay mixture. Worms further reduced CO_2 emissions in the low clay treatment up to 34% compared to the control, and increased CO_2 emissions in the high clay treatment. In general, the presence of worms increased rate constants, except for the control and low clay treatments, which showed the lowest rate constants.

3.4 Carbon mineralisation during incubation with soil

Carbon emissions from the soil amended with the organic materials are shown in Figure 5. Cumulative emissions at day 30 ranged from 8.95 to 18.20 mg g^{-1} TOC. Generally, the application of organic materials to soil led to a larger amount of carbon mineralized compared to the soil without amendments. The carbon emissions were influenced by the compost production procedure (additives and worms). The highest emissions were recorded for soil amended with composts free of additives. Compost produced in the presence of biochar showed the lowest CO_2 release. Compost and Vermicompost produced with high clay dose induced similar carbon emissions from soil. Compared to soil amended with regular composts, vermicomposts decreased the carbon emissions from amended soil only when produced without additives or with low clay dose. When biochar was mixed with clay, the final product induced lower carbon emissions from soil when produced in the presence of worms compared to those produced without worms.

4.Discussion

4.1 Effect of clay and biochar on carbon mineralization during composting

Addition of clay reduced CO_2 emissions during composting (Fig. 4). This confirms our initial hypothesis, stating that carbon mineralisation would be reduced due to the formation of organo-

mineral interactions formed in the presence of 2:1 clay. Carbon storage in soil generally increases linearly with increasing clay concentration (Hassink, 1997), and similar results have been obtained by other authors during co-composting with clay additives (Bolan *et al*, 2012).

Biochar addition led to a reduction of CO_2 emissions up to 44% compared to the control (Fig. 4), in agreement with the capacity of biochar to adsorb and protect labile organic compounds from degradation (Augustenborg *et al*, 2012; Ngo *et al*, 2013; Naisse *et al*, 2015) and its capacity to enhance aggregation (Plaza *et al*, 2016; Ngo *et al*, 2016). In turn, some studies showed no significant reduction of CO_2 emissions when biochar was used for co-composting (Sánchez-García *et al*, 2015). These contrasting results may be explained by variable physico-chemical properties of biochar: the biochar used in this study was produced by gasification while Sánchez-García *et al*, (2015) used a biochar produced by pyrolysis.

When clay and biochar were added together during composting, lower CO_2 emissions were recorded as compared to their use alone (Table 3), therefore the effect of each material is additive.

4.2 The presence of worms modifies unexpectedly the effect of clay and biochar on CO_2 emissions during composting

The addition of worms to green wastes did not modify mineralization during composting: treatments C and V have similar k values (Table 2) and CO_2 emissions (Table 3), and thus it is expected that they would not have an effect when clay or biochar are added neither. However, this was not the case. The presence of worms reduced CO_2 emissions when the low dose of clay was added: this shows that worm activity most probably increases the formation of organo-mineral associations (Bossuyt *et al*, 2005), leading to higher reduction of CO_2 emissions compared to regular composting (Fig. 3). These results are in line with our initial hypothesis indicating that the protective capacity of clay minerals may be enhanced by worm activity.

However, the same was not observed when the high dose of clay was employed, and in this case the presence of worms produced an increase of CO_2 emissions (Fig. 4). Since similar worm biomass

existed in both treatments, it is clear that high clay contents have had a negative effect on worm activity that interfered with the formation of stable organo-mineral associations. This hypothesis is supported by the results of Klok *et al* (2007), who showed that *Lumbricus rubellus* worms can have their life cycle influenced by a high content of clay in soil, leading to anaerobic conditions and soil compaction. Given that species of the *Eisenia* genus (*Fetida and Andrei*) belong to the epigenic worm species living at the soil surface in leaf litter, they might not be well adapted to process high amounts of minerals. Thus, the enhancement of organic matter protection by worms may occur up to a threshold of the clay:OM ratio, above which species of the *Eisenia* genus are no longer able to reduce CO_2 emissions. The optimal clay:OM ratio to allow for maximal reduction of CO_2 emissions remains to be assessed as well as the possibility to use other worm species more adapted to ingestion of minerals.

Regarding biochar, the presence of worms during composting increased OM mineralization, irrespectively the dose of biochar. Thus, our initial hypothesis stating that OM-biochar interactions might be enhanced by worm activity similarly to OM-clay interactions is not supported by results. Yet, this is in line with other studies showing that the presence of biochar accelerates the composting process (Sanchez-Garcia *et al*, 2015; Czekala *et al*, 2016). Three processes might explain that worms drastically modify the complex interactions between clay, biochar and OM: 1) the microbial colonization of biochar might be enhanced in the worm gut decreasing their long-term resistance to bio-degradation; 2) biochars might enhance worm activity, as suggested by Augustenborg *et al* (2012) to explain the increase of CO_2 emissions when biochar was added to soil in the presence of worms; 3) during composting with biochar/clay mixture, the worms might increase in their gut the contact between clay and biochar, leading to the partial saturation of clay surfaces with carbon compounds originating from biochar and thus to a reduction of the available surface area. This hypothesis is supported by the fact the we observed higher worm reproduction rates when biochar was used in combination with clay.

The incidence of these three hypotheses probably depends on the biochar quality, which influences

the effects of biochar on worm activity. Indeed, biochar addition had contradictory effects on worm reproduction. Biochar alone reduced the number of juveniles and cocoons of *Eisenia* to zero, contrary to what Malińska *et al*. (2016) observed during the vermicomposting of a sewage sludge-biochar mixture. These contrasting influences of biochar on worm activity may be explained by the different biochar chemical characteristics due to specific production processes (gasification in our study and pyrolysis in the study by Malińska *et al*, 2016).

In line with our results, the presence of biochar has already been described as a potential risk for earthworm development (Liesch *et al*, 2010). Negative effects of biochar on worm activity in soil have been suggested to originate from a) a lack of nutrients following their adsorption on biochar, b) the presence of toxic compounds such as polycyclic aromatic hydrocarbons (PAH) mainly, or c) a lack of water (Li *et al*, 2011). In our experiment, the lack of nutrients was balanced by the presence of compost and the lack of water was avoided by a preliminary humidification of biochar before their addition. The presence of PAH or other potentially toxic substances might thus explain the effects that we observed. Although PAH and dioxine contents of the biochar used in this study were reported to be under the official limits (Wiedner *et al*, 2013), further analyses and longer experiments should be carried out in order to investigate the reasons for the adverse effects. Testing the influence of biochar of various origins (initial material and process) on vermicomposting with clay compared with similar composting treatments would be necessary to elucidate the mechanisms responsible for their influence on carbon mineralization.

4.3 Amendment composition and production influences mineralization in soil and total CO_2 emissions

The complicated trends observed in CO_2 emissions during composting were not reproduced once the amendments were added to soil. Here, the most important factor to explain the differences were the presence/absence of worms during compost production. Thus, differences in OM mineralization among amendments were more important in the case of the composts than in the case of

vermicomposts (Figure 4). Overall, vermicomposts did not change OM mineralization after addition to soil (with the only exception of the vermicompost with 10% biochar), whereas composts increased it. This is likely a consequence of the higher stabilization of the amendments during vermicomposting due to the effect of worms; it is an effect commonly observed in the literature (Paradelo *et al*, 2012; Ngo *et al*, 2013). Our initial hypothesis that amendments produced in the presence of worms contain more stable carbon was therefore verified.

Regarding the additives in the composts, the presence of biochar reduced OM mineralization in soil with respect to the compost and vermicompost without additives, whereas clay reduced mineralization only in the composts. The lowest total carbon emissions were recorded for compost produced in presence of biochar (Fig. 4). This result is in line with many other studies reporting reduced mineralization of native soil OM after biochar amendment (Zimmerman *et al*, 2011). Our data evidenced that this phenomenon may also occur after addition of composts to soil, when biochar is used as co-composting agent.

In order to evaluate the overall positive or negative effect of each additive on carbon mineralization, CO_2 emissions during composting and incubation of amended soil were summed up and expressed as mg C g^{-1} TOC (Table 3). The lowest total carbon emissions were recorded for compost produced in presence of biochar. Overall, the results may be explained by the content and composition of labile compounds in the substrates, which is generally linked to the mineralisation rate (Chaoui *et al*, 2003; Paradelo *et al*, 2010). The labile DOC content was in the case of vermicomposts lower than that of composts (Table 1) and not related to CO_2 emissions. Figure 5 shows the correlation between the amount of carbon mineralized from the amended soil and the DOC of the respective organic material. The relationship was evident only for the soil amended with composts (Fig. 5). The lack of correlation between DOC and CO_2 emitted after addition to soil of vermicomposts suggests contrasting properties of DOC in composts and vermicomposts (Lazcano *et al*, 2008, Kalbitz *et al*, 2003). Clay and biochar addition may thus have an effect on CO_2 emissions through adsorption of labile compounds of composts, while these processes are no longer controlling CO_2

emissions when these co-composts were produced in the presence of worms.

5. Conclusion

This study tested the influence of clay and biochar and their mixture on CO_2 emission potential of organic soil amendments produced during composting of green wastes. We established the complete carbon balance taking into account production of amendments and their impact after addition to soil. Clay was found to decrease CO_2 release during composting, while inducing positive priming after soil amendment. Biochar also decreased CO_2 emissions during composting, while inducing negative priming when used alone as a co-composting agent. We conclude that the use of additives may have the potential to greatly reduce CO_2 emissions during co-composting. We also tested the effect of worm species of the *Eisenia* genus on CO_2 emissions during composting. Worms generally increased carbon mineralization, except in treatments with low clay dose. Our results thus evidenced a threshold of clay concentrations for *Eisenia* worms, above which CO_2 emitted is no longer reduced. Addition of biochar did not lead to reduced CO_2 emissions in the presence of worms. When added to soil, vermicomposts did not increase OM mineralization most probably because of the high stabilization of the amendments during their production. Our study suggests a significant role of the production conditions on total carbon balance of amendments. We therefore suggest that the carbon balance before (production process) and after addition to soil has to be taken into account when evaluating their CO_2 emission potential. A low emission potential of organic amendments may be achieved by optimisation of their production conditions. Further work needs to be done to assess the long-term effect of composts and vermicomposts produced with additives on mineralization and on soil fertility and plant growth.

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7. References

Anderson J.P.E (1982) Soil Respiration. *Methods of Soil Analysis, part 2. Chemical and Microbiological Properties*, second ed. p. 331-871.

Augustenborg C.A., Hepp S., Kammann C., Hagan D., Schmidt O. and Müller C. (2012) Biochar and earthworm effect on soil nitrous oxide and carbon dioxide emissions. *Journal of Environmental Quality* 41, 1203-1209.

Barral M.T., Paradelo R., Moldes A.B., Domínguez M. and Díaz-Fierros F. (2009) Utilization of MSW compost for organic matter conservation in agricultural soils of NW Spain. *Resources, Conservation and Recycling* 53, 529-534.

Bass A.M., Bird M.I., Kay G. and Muirhead B. (2016) Soil properties, greenhouse gas emissions and crop yield under compost, biochar and co-composted biochar in two tropical agronomic systems. *The Science of the Total Environment* 550, 459-470.

Blouin M., Hodson M.E., Delgado E.A., Baker G., Brussaard L., Butt K.R., Dai J., Deendooven L., Peres G., Tondoh J.E., Cluzeau D. and Brun J.-J. (2013) A review of earthworm impact on soil function and ecosystem services. *European Journal of soil science* 64, 161-182.

Bolan N.S., Kunhikrishnan A., Choppala G.K, Thangarajan R. and Chung J.W (2012) Stabilization of carbon in composts and biochars in relation to carbon sequestration and soil fertility. *Science of the Total Environment* 424, 264-270.

Bossuyt H., Six J. and Hendrix P.F. (2005) Protection of soil carbon by microaggregates within earthworm casts. *Soil Biology & Biochemistry* 37, 251-258.

Bouché, M.B. (1977) Stratégies lombriciennes. In : Lohm, U., Perssonn, T., Soil Organisms as Components of Ecosystems. *Ecological Bulletin* 25, 122-132.

Bustamante M.A., Pérez-Murcia M.D., Pareded C., Moral R., Pérez-Espinosa A. and Moreno-Caselles J. (2007) Short-term carbon and nitrogen mineralization in soil amended with winery and distillery organic wastes. *Bioresource Technology* 98, 3269-3277.

Bustamante M.A., Said-Pullicino D., Paredes C., Cecilia J.A. and Moral R. (2010) Influences of

winery-distillery waste compost stability and soil type on soil carbon dynamics in amended soils. *Waste Management* 30, 1966-1975.

Cambardella C.A., Richard T.L and Russel A. (2003) Compost mineralization in soil as a function of composting process conditions. *European Journal of Soil Biology* 39, 117-127.

Chabbi A., Kögel-Knabner I. and Rumpel C. (2009) Stabilised carbon in subsoil horizons is located in spatially distinct parts of the soil profile. *Soil Biology and Biochemistry* 41, 256-261.

Chan K.Y., Zwieten L. V., Meszaros I., Downie A. and Joseph S. (2007) Agronomic values of greenwaste biochar as a soil amendment. *Australian Journal of Soil Research*, 45, 629-634.

Chan Y.C., Sinha R.K. and Wang W.J. (2011) Emission of greenhouse gases from home aerobic composting, anaerobic digestion and vermicomposting of household wastes in Brisbane (Australia). *Waste Management and Research* 29(5), 540-548.

Chaoui H.I., Zibilske L.M. and Ohno T. (2003) Effects of earthworms casts and compost on soil microbial activity and plant nutrient availability. *Soil Biology and Biochemistry* 35, 295-302

Chowdhury S., Bolan N.S., Seshadri B., Kunhikrishnan A., Wijesekara H., Xu Y., Yang J., Kim G-H., Sparks D. and Rumpel C. (2015) Co-composting solid biowastes with alkaline materials to enhance carbon stabilization and revegetation potential. Environmental Science and Pollution Research, 1-12.

Czekala W., Malinska K., Caceres R., Janczak D., Dach J. and Lewicki A. (2016) Co-composting of poultry manure mixtures amended with biochar - The effect of biochar on temperature and C-CO2 emission. *Bioressource Technology* 200, 921-927.

Dias B.O., Silva C.A., Higashikawa F.S., Roig A. and Sánchez-Monedero M.A. (2010) Use of biochar as bulking agent for the composting of poultry manure: effect on organic matter degradation and humification. *Bioresource Technology* 101, 1239-1246.

Dominguez J. and Edwards C.A. (2011) Biology and ecology of earthworm species used for vermicomposting. Taylor and Francis Group.

Frederickson J., Howell G. and Hobson A.M. (2007) Effect of pre-composting and

vermicomposting on compost characteristics. *European Journal of Soil Biology* 43, S320-S326.

Hassink J. (1997) The capacity of soils to preserve organic C and N by their association with clay and silt particles. *Plant and Soil* 191: 77-87.

Hobson A.M., Frederickson J. and Dise N.B. (2005) CH4 and N2O from mechanically turned windrow and vermicomposting systems following in-vessel pre-treatment. *Waste Management* 25, 345-352.

IPCC (2014) Climate change 2014. Synthesis Report.

Jindo K., Suto K., Matsumoto K., García C., Sonoki T. and Sánchez-Monedero M.A. (2012) Chemical and biochemical characterisation of biochar-blended composts prepared from poultry manure. *Bioresource Technology* 110, 396-404.

Kalbitz K., Schmerwitz J., Schwesig D. and Matzner E. (2003) Biodegradation of soil-derived dissolved organic matter as related to its properties. *Geoderma* 113, 273-291.

Kleber M., Eusterhues K., Keiluweit M., Mikutta C., Mikutta R. and Nico P.S. (2015) Mineral-Organic Associations: formation, properties and relevance in soil environments. *Advances in Agronomy* 130, 1-140.

Klok C., Faber J., Heijmans G. and Bodt J. (2007) Influence of clay content and acidity of soil on development of the earthworm *Lumbricus rubellus* and its population level consequences. *Biol. Fertil. Soils* 43: 549-556.

Kookana R.S., Sarmah A.K., Zwieten L.V., Krull E. and Singh B. (2011) Biochar application to soil: agronomic and environmental benefits and unintended consequences. *Advances in Agronomy*, Volume 112, chapitre 3.

Lashermes G., Nicolardot B., Parnaudeau V., Thuriès L., Chaussod R., Guillotin M.L., Linères M., Mary B., Metzger L., Morvan T., Tricaud A., Villette C. and Houot S. (2009) Indicator of potential residual carbon in soils after exogenous organic matter application. *European Journal of Soil Science* 60, 297-310.

Lavelle, P., Decaens, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., *et al.* 2006. Soil invertebrates

and ecosystem services. *European Journal of Soil Biology* 42, 3–15.

Lazcano C., Gomez-Brandon M. and Dominguez J. (2008) Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere* 72, 1013-1019.

Lehmann J., Gaunt J. and Rondon M. (2006) Biochar sequestration in terrestrial ecosystems- a review. *Mitigation and adaptation strategies for global change* 11, 403-427.

Li D., Hockaday W.C., Masiello C.A. and Alvarez P.J.J. (2011) Earthworm avoidance of biochar can be mitigated by wetting. *Soil Biology & Biochemistry* 43, 1732-1737.

Liesch A. M., Weyers S.L., Gaskin J.W. and Das K.C (2010) Impact of two different biochars on earthworm growth and survival. *Annals of Environmental Science*, Vol 4, 1-9.

Lubbers I.M., van Groeningen K.J., Fonte S.J., Six J., Brussaard L. and van Groeningen J.W. 2013 Greenhouse-gas emissions from sois increased by earthworms. Nature Climate Change, 3, 187-194.

Malińska K., Zabochnicka-Światek M., Cáceres R. and Marfà O. (2016) The effect of precomposted sewage sludge mixture amended with biochar on the growth and reproduction of *Eisenia fetida* during laboratory vermicomposting. *Ecological Engineering* 90, 35-41.

McBeath A.V. and Smernik R.J. (2009) Variation in the degree of aromatic condensation of chars. *Organic Geochemistry* 40, 1161-1168.

Naisse C., Girardin C., Davasse B., Chabbi A. and Rumpel C. (2015) Effect of biochar addition on C mineralisation and soil organic matter priming in two subsoil horizons. *J Soils Sediments* 15, 825-832.

Ngo P-T, Rumpel C., Doan T.T. and Jouquet P. (2012) The effect of earthworms on carbon storage and soil matter composition in tropical soil amended with compost and vermicompost. *Soil Biology and Biochemistry* 50, 21-220.

Ngo P-T, Rumpel C., Ngo Q-A., Alexis M., Vargas V.G., De la Luz Mora Gil M., Dang D-K and Jouquet P. (2013) Biological and chemical reactivity and phosphorous forms of buffalo manure

compost, vermicompost and their mixture with biochar. *Bioresource Technology* 148, 401-407.

Ngo P-T., Rumpel C., Dignac M-F., Billou D.,Tran Duc T. and Jouquet P. (2011) Transformation of buffalo manure by composting or vermicomposting to rehabilitate degraded tropical soils. *Ecological Engineering* 37, 269-276.

Ngo P-T., Rumpel C., Janeau J-L., Dang D-K., Doan T.T. and Jouquet P. (2016) Mixing of biochar with organic amendments reduces carbon removal after field exposure under tropical conditions. *Ecological Engineering* 91, 378-380.

Owojori O.J., Reinecke A.J. and Rozanov A.B. (2010) Influence of clay content on bioavailability of copper in the earthworm *Eisenia fetida*. *Ecotoxicology and Environmental Safety* 73, 407-414.

Paradelo, R., Moldes, A.B. and Barral, M.T. (2009) Amelioration of the physical properties of slate processing fines using grape marc compost and vermicompost. *Soil Science Society of America Journal* 73, 1251-1260.

Paradelo, R., Moldes, A.B., Prieto, B., Sandu, R.-G., and Barral, M.T. (2010) Can stability and maturity be evaluated in finished composts from different sources? *Compost Science and Utilization* 18, 22-31.

Paradelo R., Moldes A.B., González D. and Barral M.T. (2012) Plant tests for determining the suitability of grape marc composts as components of plant growth media. *Waste Management & Research* 30, 1059-1065.

Plaza C., Giannetta B., Fernández J.M, López-de-Sá E.G., Polo A., Gasco G., Méndez A. and Zaccone C. (2016) Response of different soil organic matter pools to biochar and organic fertilizers. *Agriculture, Ecosystems & Environment* 225, 150-159.

Rogovska N., Laird D., Cruse R., Fleming P., Parkin T. and Meek D. (2011) Impact of biochar on manure carbon stabilization and greenhouse gas emissions. *Soil Biology and Biochemistry* 75, 871-879.

Sánchez-García M., Alburquerque J.A., Sánchez-Monedero M.A., Roig A. and Cayuela M.L. (2015) Biochar accelerates organic matter degradation and enhances N mineralisation during

composting of poultry manure without a relevant impact on gas emissions. *Bioressource Technology* 192, 272-279.

Shipitalo M.J. and Protz R. (1989) Chemistry and micromorphology of aggregation in earthworm casts. *Geoderma* 45, 357-374.

Smith P., House J.I., Bustamante M., Sobocka J., Harper R., Pan GG., West P.C., Clark J.M., Adhya T., Rumpel C., Paustian K., Kuikman P., Cotrufo M.F., Elliott J.A., McDowell R., Griffiths R.I., Asakawa S., Bondeau A., Jain A.K., Meersmans J. and Pugh T.A.M. (2015) Global change pressures on soils from land use and management. Global Change Biology 22, 1008-1028.

Speratti, A. B. and Whalen, J. K. 2008. Carbon dioxide and nitrous oxide fluxes from soil as influenced by anecic and endogeic earthworms. *Applied Soil Ecology* 38, 27–33.

Thangarajan R., Bolan N.S., Tian G., Naidu R. and Kunhikrishnan A. (2013) Role of organic amendment application on greenhouse gas emission from soil. *Science of the Total Environment* 465, 72-96.

Ventura M., Alberti G., Viger M., Jenkins J., Girardin C., Baronti S., Zaldei A., Taylor G., Rumpel C., Miglietta F., Tonon G. (2015) Biochar mineralization and priming effect on SOM decomposition in two European short rotation coppices. *Global Change Biology Bioenergy* 7, 1150-1160, DOI: 10.1111/gcbb.12219.

von Lützow M., Kögel-Knaber I., Ekschmitt K., Matzner E., Guggenberger G., Marschner B. and Flessa H. (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. *European Journal of Soil Science* 57, 426-445.

Vu Q.D., de Neergaard A., Tran T. D., Hoang H. T., Vu V.T. and Jensen L.S. (2015) Greenhouse gas emissions from passive composting of manure and digestate with crop residues and biochar on small-scale livestock farms in Vietnam. *Environmental Technology* 36, 2924-2935.

Wang J., Hu Z., Xu X., Jiang X., Zheng B., Liu X., Pan X. and Kardol P. (2014) Emissions of ammonia and greenhouse gases during combined pre-composting and vermicomposting of duck

manure. *Waste Management* 34, 1546-1552.

595 Weyers L.S. and Spokas A.K. (2011) Impact of biochar on earthworms populations: a review. *Applied and Environmental Soil Science*, Volume 2011, Article ID 541592.

Wiedner K., Rumpel C., Steiner C., Pozzi A., Maas R. and Glaser B. (2013) Chemical evaluation of chars produced by thermochemical conversion (gasification, pyrolysis and hydrothermal carbonization) of agro-industrial biomass on a commercial scale. *Biomass and Bioenergy* 59,

600 264-278.

Zimmerman A.R., Gao B. and Ahn M-Y. (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology and Biochemistry* 43, 1169-1179.

605 Table 1. Mean values of pH, total nitrogen, total organic carbon (TOC) and dissolved organic carbon (DOC) after 21 days of co-(vermi)composting. Data are presented as means and standard error (n=4). Different small letters indicate significant differences between treatments (Kruskal-Wallis test, $p < 0.005$). C: compost; V: vermicompost; B: biochar; M: clay.

	pH	**TOC (mg g^{-1})**	**N (mg g^{-1})**	**DOC (mg g^{-1})**	**C/N**
Pre-composted material	8.5 ± 0.1 ^{c}	205.1 ± 3.0 ^{b}	13.3 ± 0.2 ^{a}	29.08 ± 0.86 ^{a}	15.4 ± 0.1 ^{bc}
	Organic materials after 21 days of co-composting				
Compost treatments					
C	8.7 ± 0.1 ^{ab}	188.2 ± 9.1 ^{c}	13.5 ± 0.8 ^{a}	28.85 ± 0.38 ^{a}	13.5 ± 0.6 ^{d}
C + 25 % M	8.2 ± 0.1 ^{d}	153.1 ± 9.5 ^{d}	10.6 ± 0.5 ^{c}	21.77 ± 1.57 ^{b}	14.4 ± 0.8 ^{d}
C + 50 % M	7.9 ± 0.1 e	118.6 ± 2.9 ^{e}	8.5 ± 0.1 ^{e}	19.32 ± 0.94 ^{c}	14.0 ± 0.3 ^{d}
C + 10 % B	8.7 ± 0.1 ^{a}	241.9 ± 15.1 ^{a}	12.4 ± 0.5 ^{b}	21.26 ± 0.78 ^{b}	19.5 ± 0.8 ^{ab}
C + 10 % B+ 25 % M	8.2 ± 0.1 ^{d}	197.8 ± 5.9 ^{b}	10.0 ± 0.2 ^{cd}	15.04 ± 0.68 ^{e}	19.7 ± 0.3 ^{a}
Vermicompost treatments					
V	8.6 ± 0.1 ^{b}	185.0 ± 8.3 ^{c}	13.0 ± 0.6 ^{ab}	26.83 ± 0.49 ^{a}	14.3 ± 0.4 ^{d}
V + 25 % M	8.2 ± 0.1 ^{d}	150.2 ± 5.2 ^{d}	10.4 ± 0.5 ^{cd}	18.41 ± 0.66 ^{cd}	14.5 ± 0.3 ^{d}
V + 50 % M	7.9 ± 0.1 ^{e}	121.4 ± 6.0 ^{e}	8.6 ± 0.1 ^{e}	17.16 ± 0.7 ^{d}	14.1 ± 0.7 ^{d}
V + 10 % B	8.7 ± 0.1 ^{ab}	247.6 ± 12.3 ^{a}	12.5 ± 0.5 ^{b}	19.68 ± 0.49 ^{bc}	19.9 ± 0.9 ^{a}
V + 10 % B+ 25 % M	8.3 ± 0.1 ^{d}	206.0 ± 11.4 ^{b}	9.9 ± 0.3 ^{d}	15.18 ± 0.43 ^{e}	20.8 ± 1.4 ^{a}

610

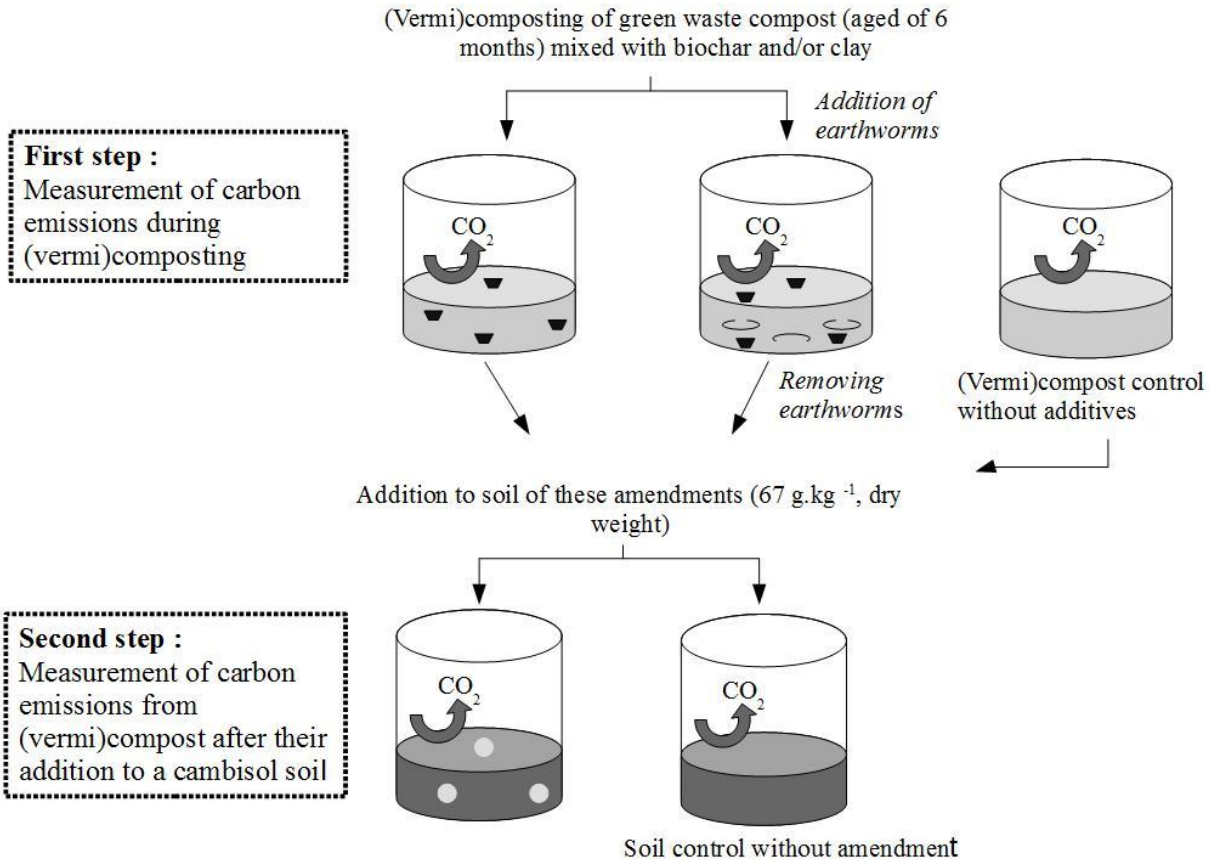
Table 2. Effect of the addition of clay and/or biochar on the rate constant k (day^{-1}) during composting and vermicomposting. Different small letters indicate significant differences between treatments (Kruskal-Wallis test, $p< 0.005$). C: compost; V: vermicompost; B: biochar; M: clay.

	k (10^{-3} day^{-1})	Std. Error (10^{-5})
Compost treatments		
C	3.069 ^{a}	4.4
C + 25 % M	2.588 ^{cd}	4.5
C + 50 % M	1.699 ^{g}	2.8
C + 10 % B	2.313 ^{ef}	2.2
C + 10 % B+ 25 % M	1.762 ^{g}	5.3
Vermicompost treatments		
V	3.036 ^{ab}	4.1
V + 25 % M	1.973 ^{fg}	3.8
V + 50 % M	2.431 ^{de}	3.6
V + 10 % B	2.855 ^{ab}	4.9
V + 10 % B+ 25 % M	2.798 ^{bc}	4.3

615

Table 3. CO_2 emissions during the experiments. C: compost; V: vermicompost; B: biochar; M: clay.

	Composting phase (mg CO_2 g^{-1} TOC)	*Soil incubation phase* (mg CO_2 g^{-1} TOC)	*Total carbon mineralized* (mg CO_2 g^{-1} TOC)
Compost treatments			
C	17.11 ^{a}	18.20 ^{a}	35.31 ^{a}
C + 25 % M	13.55 ^{b}	15.68 ^{ab}	29.23 ^{a}
C + 50 % M	7.83 ^{bc}	14.03 ^{bc}	21.87 ^{de}
C + 10 % B	8.67 ^{de}	8.95 ^{f}	17.62 ^{f}
C + 10 % B+ 25 % M	6.36 ^{e}	13.58 ^{c}	19.94 ^{ef}
Vermicompost treatments			
V	15.75 ^{a}	13.11 ^{cd}	28.87 ^{ab}
V + 25 % M	10.59 ^{c}	13.72 ^{c}	24.31 ^{cd}
V + 50 % M	12.23 ^{bc}	13.73 ^{c}	25.96 ^{bc}
V + 10 % B	8.81 ^{d}	11.42 ^{ef}	20.22 ^{ef}
V + 10 % B+ 25 % M	10.59 ^{c}	12.67 ^{de}	23.27 ^{cd}



620 Figure 1. Experimental design to compare CO_2 emissions of different organic materials during composting and after their addition to soil.

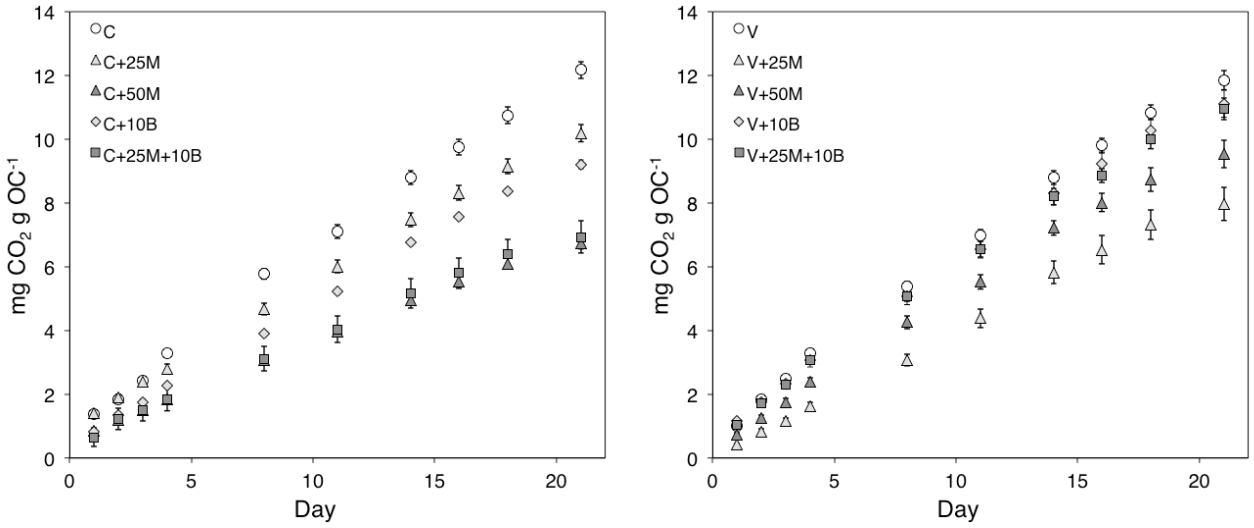


Figure 2. A) Cumulative CO_2 emissions during composting without worms of pre-composted material alone (C), with 25% of clay (C+25M), with 50% of clay (C+50M), with 10% of biochar (C+10B) or with 25% of clay and 10% of biochar (C+25M+10B). B) Cumulative CO_2 emissions during composting with worms of pre-composted material alone (V), with 25% of clay (V+25M), with 50% of clay (V+50M), with 10% of biochar (V+10B) or with 25% of clay and 10% of biochar (V+25M+10B). Bars represent twice the standard deviation of the mean (n=4).

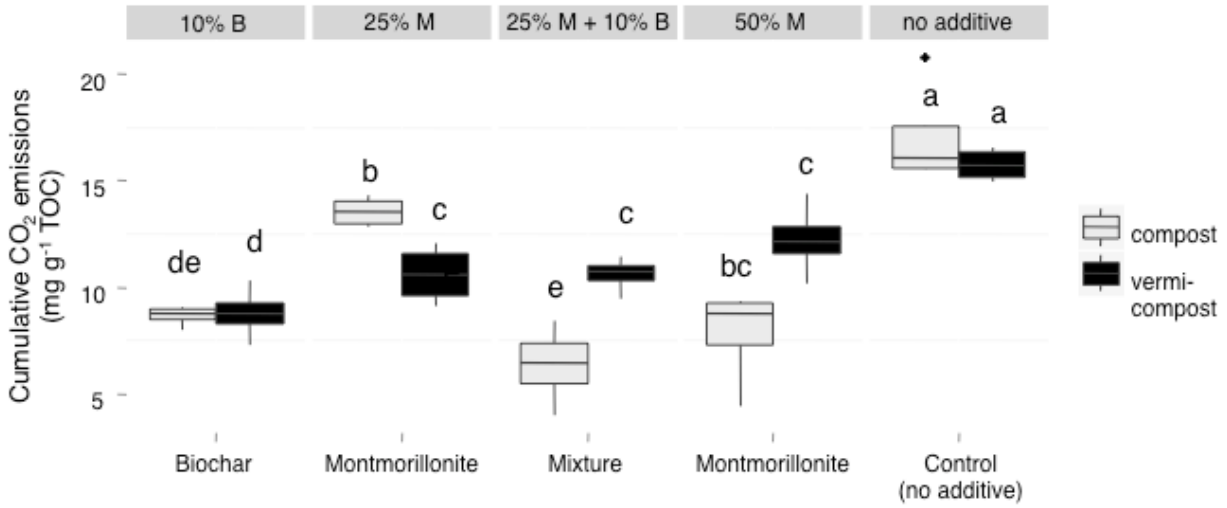


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Figure 3. Total CO_2 emissions after 21 days of composting. Different letters (a, b, c, d, e and f) indicate statistically significant differences.

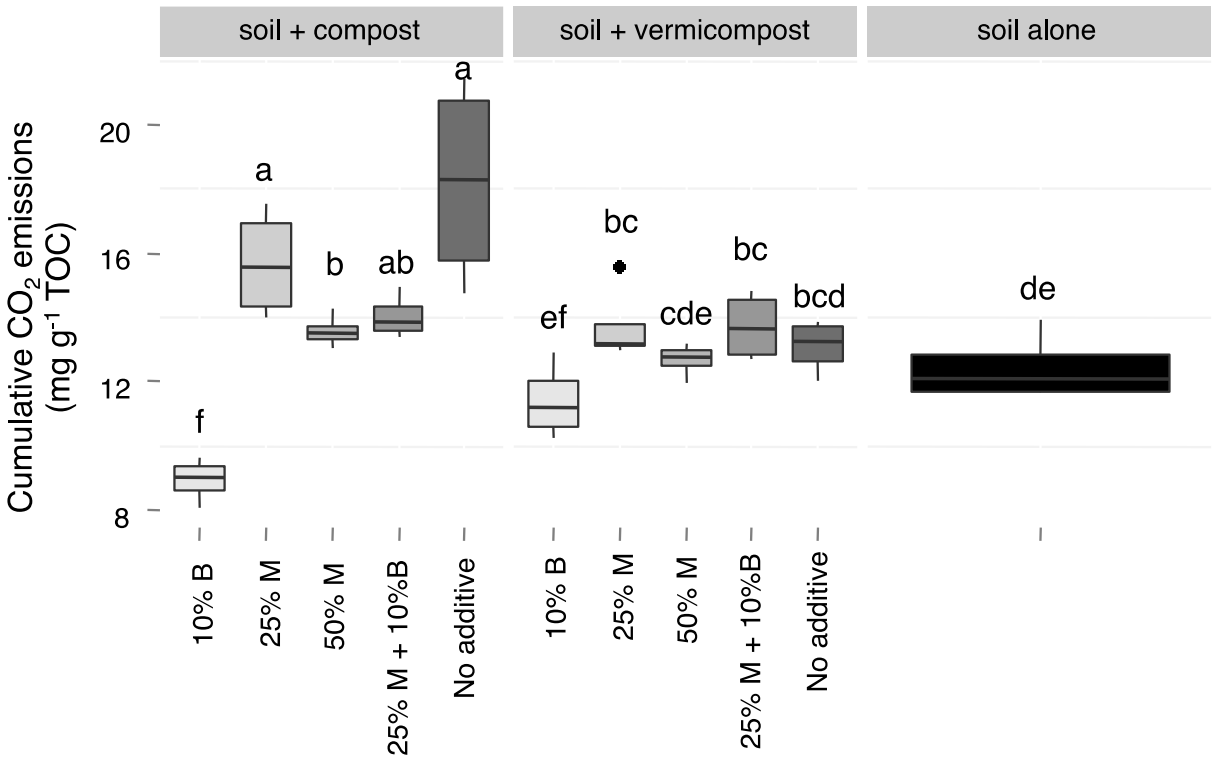

Figure 4. Cumulative CO_2 emissions after 30 days of incubation of the soil alone or amended with composts and vermicomposts with 25% of clay (25M), 50% of clay (50M), 10% of biochar (10B) or 25% of clay and 10% of biochar (25M+10B). Different letters (a, b, c, d, e and f) indicate statistically significant differences.

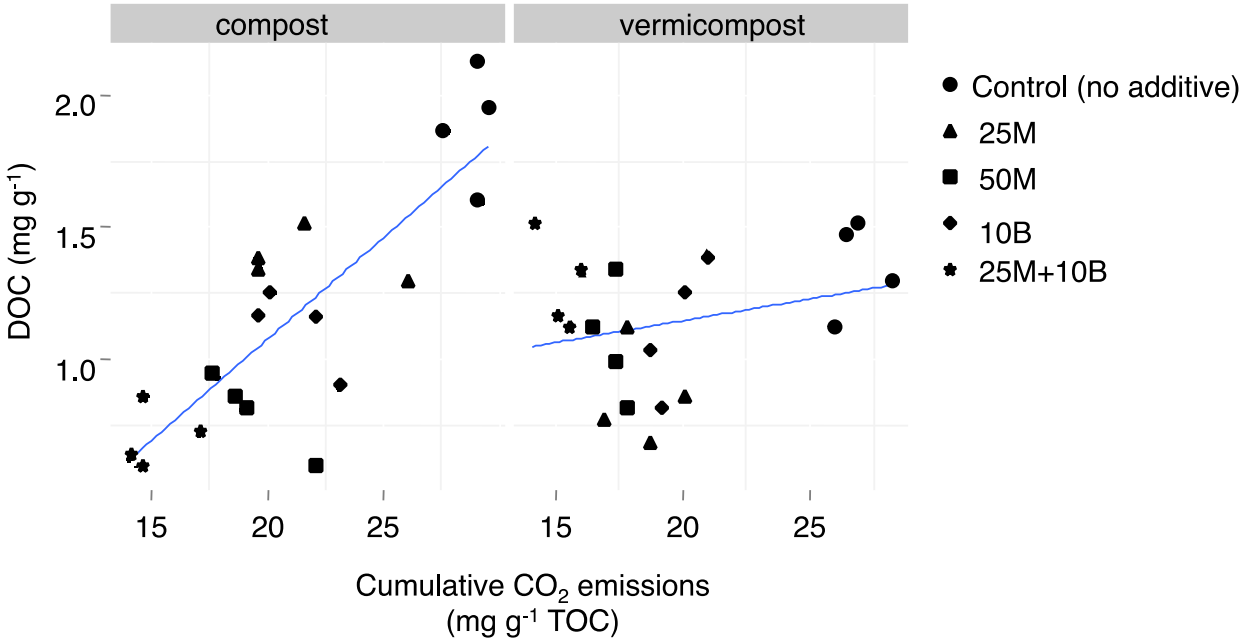

Figure 5. Comparison between total CO_2 emissions (composting + incubation with soil) and dissolved organic carbon (DOC) in the amendments. Key as in previous figures.

650