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. The stability of the final products as well as their effect on carbon mineralization in soil were evaluated. The aim of the study was to test the following hypotheses: (1) interactions between clay and biochar and organic wastes lead to reduced CO_2 emissions during the composting process, (2) these interactions are enhanced in the presence of worms, and (3) more carbon is sequestered in soil after the use of the resulting compost/vermicompost as amendments. We added two different doses of clay, biochar and their mixture to pre-composted green wastes and monitored carbon mineralisation during 21 days in presence or absence of worms (species of the *Eisenia* genus). The organic materials 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%. However, in the presence of worms, CO_2 emissions increased for all treatments except for the low clay dose. The production conditions had more influence on carbon mineralization in soil for composts than for vermicomposts except for the low clay treatment, which showed a more reduced CO_2 emissions for vermicompost compared to regular compost.

In summary, the addition of worms during co-composting with clay and biochar speeds up CO_2 emissions in most cases. Therefore, the production of a low CO_2 emission amendment requires optimisation of OM source, co-composting agents and worm species. The effect of the resulting material on soil fertility has to be evaluated.

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 (Ngo *et al*, 2011, 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*, 2012). 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 (v. 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; Wang *et al*, 2014; Chowdhury *et al*, 2015), e.g. clay minerals during composting of poultry manure (Bolan *et al*, 2012) or zeolite during (vermi)composting of wastes (Wang *et al*, 2014). However, to the best of our knowledge, no studies have been carried out to evaluate the effect of minerals on carbon stability in the resulting organic materials after their addition to soil.

In addition, many recent studies explored the potential benefits of biochar as soil amendment due to its physical and chemical properties (Chan *et al*, 2007; Kookana *et al*, 2011). 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 associations during co-composting, the addition of worms may be a promising avenue. In general, organo-mineral associations are enhanced by the presence of worms, 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). However, these interactions have only been evidenced for soil earthworms and have never been evaluated as a strategy to reduce CO_2 emissions during co-composting. One study investigated the effect of biochar on worm activity during vermicomposting of a sludge biochar mixture (Malinska *et al*, 2016). However, to the best of our knowledge, no studies have investigated the effect on carbon emissions of biochar as a co-composting agent during vermicomposting.

In this study, we assessed carbon emission potential of organic soil amendments produced by composting and vermicomposting. To do so, we measured CO_2 emissions during the two following 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 evaluated if the addition of worms influences the magnitude of the CO_2 emissions. We hypothesised that the addition of worms reduces CO_2 emissions during co-composting and after the addition of co-composts to soil due to the formation of stable OM-clay or OM-biochar interactions by worm activity. The aim of the study was to investigate (1) the magnitude of CO_2 emissions during co-composting with different additives and after addition of co-composts to soil and (2) if the presence of worms during composting with additives leads to enhanced carbon stabilisation.

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 m² (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:

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

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 applied 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. 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 C-containing biochar increased OC concentrations and decreased N concentrations by dilution.

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 treatments (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 3 cocoons and one juvenile.

3.3 Carbon mineralisation during composting

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. 3). 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 3). The cumulative CO_2 emissions during composting did not reach a plateau for any treatment (Fig. 3 and 4), but the duration of the experiment was limited by worm activity since worms had processed all organic material after 21 days.

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.

The addition of worms increased carbon mineralisation in most treatments with cumulative CO_2 emissions ranging from 7.9 to 12.0 mg CO_2-C g^{-1} compost in treatments with worms (Fig. 3 and 4). The presence of worms (Fig. 2) 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

(Fig. 3 and 4), 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. Organic amendments produced with high clay addition induced similar carbon emissions from soil. Compost produced in the presence of biochar showed the lowest mineralization in 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 addition. 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.

Figure 6 shows the correlation between the amount of carbon mineralized from the amended soil and the DOC of the respective organic material. The relationship was stronger for the soil amended with composts compared to the soil amended with vermicomposts (Fig.6, respectively R^2=0.67 and R^2=0.07).

4.Discussion

4.1 Effect of worms and additives on compost properties

The presence of additives and worms had no effect on the pH (Table 2), all the treatments tending to a slightly alkaline pH. By contrast, some authors observed a decrease in pH during vermicomposting of household wastes (Frederickson *et al*, 2007) or cattle manure (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 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.

4.2 Effect of additives on carbon mineralization during composting

Addition of clay and biochar reduced carbon emissions during composting (Fig. 3). This is in line with 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 and due to OM adsorption in the presence of biochar. Similar results were obtained by other authors for co-composting with clay additives (Bolan *et al*, 2012). Carbon storage generally increases linearly with increasing clay concentration (Hassink, 1997). This is in line with our results, showing higher CO_2 decrease, when clay content and thus potentially available surface area increased. Biochar addition led to a reduction of CO_2 emissions up to 44% compared to the control (Fig. 3), in accordance 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). However, other 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 biochar was used in mixture with clay during the composting procedure, lower CO_2 emissions were recorded as compared to the additives used alone (Table 3). This suggests synergistic effects due to combined processes induced by both materials in agreement with our initial hypothesis.

4.3 Effect of worms on carbon mineralization during composting with clay

Data recorded (Fig. 3 and 4) for control treatments indicated in contrast to what is generally observed (e.g. Chan *et al*, 2011), that the presence of worms did not lead to higher CO_2 emissions during composting. This is probably due to the OM used, which was almost mature compost and, may thus be characterised by lower degradability than the organic wastes originating from households usually used for composting.

In the presence of worms, carbon mineralization was most reduced in the low clay treatment. Thus, 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, CO_2 emissions in treatments with worms were more reduced for the low clay compared to the high clay treatment and to regular compost (Fig. 4). As we observed similar worm biomass in both treatments, we hypothesize that high clay contents may have negative effects on worm activity and therefore the formation of 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. Our results suggest that a 50% proportion of montmorillonite also impacts the activity of *Eisenia andrei* and *foetida* species. Therefore, enhancement of OM 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. Species of the *Eisenia* genus (*Fetida and Andrei*) belong to the epigenic worm species living at the soil surface in leaf litter, one of the three ecological lifetypes described by Bouché (1977). Therefore, they are well adapted to process pure OM and may be less suited for co-composting with minerals. 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.

4.4 Effect of worms on carbon mineralization during composting with biochar and biochar/clay mixture

In the presence of worms, the addition of biochar and biochar/clay mixture induced higher CO_2 emission (p-value > 0.1) compared to regular composting (Fig. 3 and 4). This is in contrast to our initial hypothesis stating that the OM-biochar interactions may be enhanced by worm activity similar to OM-clay interactions. 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 may be supported by the increased 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 Malinska *et al*. (2016) study)..

In line with our results, the presence of biochar has already been described as a potential risk for earthworm development (Liesch *et al*, 2010). In soil, the negative effects of biochar on worm activity 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 these 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.

4.5 Effect of co-(vermi)compost production conditions on carbon mineralization in soil and total carbon balance

CO_2 emitted from soil after the addition of amendments may originate from two sources: the mineralization of added carbon and the mineralization of native soil OM. Differences compared to the control (soil without amendments) may be explained by positive or negative priming effect, induced by microbial reaction to OM addition.

In the case of amendments produced with biochar alone, a negative priming effect could be observed, because the mineralization rate observed for this treatment was lower than for the control soil incubation. 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 accordance with our initial hypothesis, the presence of worms during compost production reduced CO_2 emissions as well as priming effects after their addition to soil (second experiment only). Negative priming was not observed for co-composts produced in the presence of worms.

In order to evaluate the positive or negative effect of each additive on carbon mineralization, the

CO_2 emissions during composting and during incubation of amended soil were summed up and expressed as mgC g^{-1} TOC. The carbon emissions during both experiments were influenced differently by the compost production procedure. The lowest total carbon emissions were recorded for compost produced in presence of biochar. If our initial hypothesis that the presence of worms produced amendments with more stable carbon was verified (experiment 2), when the total carbon balance is considered (CO_2 emissions during both experiments summed up), the presence of worms during composting increased total CO_2 emissions in all treatments due to higher emissions during the production step.

The production conditions had more influence on carbon mineralization after addition to soil for composts than vermicomposts (Table 3). Clay and biochar reduced the concentration of labile compounds in composts and vermicomposts leading to decreased DOC concentrations of the final amendments (Table 1). But the CO_2 emissions after addition to soil were only reduced by clay and biochar addition when the compost was produced without worms (Fig 5). The rate of mineralization of organic amendments is generally linked to the labile carbon compounds (Chaoui *et al*, 2003) as was observed for the compost addition (Fig. 6). The lack of correlation between DOC and CO_2 emitted after addition to soil of vermicomposts suggests contrasted properties of DOC in composts and vermicomposts (Lazcano *et al*, 2008, Kalbitz *et al*, 2003).

5. Conclusion

This study tested the influence of clay and biochar and their mixture during composting of green wastes. We established the complete carbon balance taking into account production of amendments as well as the effect after their addition to soil. Moreover, we tested the effect of the use of worm species of the *Eisenia* genus during composting on CO_2 emissions. Clay and biochar were found to decrease CO_2 release during composting, while inducing positive priming after soil amendment except for composts produced with biochar alone. Biochar/clay mixture showed synergistic effects.

Worms generally speed up carbon mineralization during composting except in treatments with low clay dose. In the presence of worms, 25% of clay led to greater OM protection than 50%. The opposite was observed in the absence of worms. Our results thus evidenced a threshold of clay concentrations for *Eisenia* worms, above which CO_2 emitted during composting is no longer reduced. Biochar had a negative effect on carbon emissions for all treatments with worms, except, when used in mixture. We conclude that the use of additives may have the potential to greatly reduce CO_2 emissions during co-composting. The effect of the amendments on carbon mineralization after addition to soil was small in the short-term. We suggest that production conditions during composting have to be optimized in terms of total CO_2 reduction by choosing the minerals, their optimal ratio with OM and testing different worm species. The effects of these amendments on soil fertility and plant growth remain to be investigated. Further work need to be done to assess the long-term effect of these amendments.

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Table 1: Mean values of pH, DOC, content of total nitrogen and organic carbon 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, $p < 0.005$).

	pH	**C (mg g^{-1})**	**N (mg g^{-1})**	**DOC (mg g^{-1} TOC)**	**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}

Table 2: Effect of the addition of clay and/ or biochar on the rate constant k (day^{-1}) during composting and vermicomposting.

	k (10^{-3} day^{-1})	Std. Error (10^{-5})
Compost treatments		
C	3.069 ^{a}	4.429
C + 25 % M	2.588 ^{cd}	4.539
C + 50 % M	1.699 ^{g}	2.776
C + 10 % B	2.313 ^{ef}	2.204
C + 10 % B+ 25 % M	1.762 ^{g}	5.265
Vermicompost treatments		
V	3.036 ^{ab}	4.089
V + 25 % M	1.973 ^{fg}	3.783
V + 50 % M	2.431 ^{de}	3.616
V + 10 % B	2.855 ^{ab}	4.869
V + 10 % B+ 25 % M	2.798 ^{bc}	4.251

Table 3: Carbon balance. Data are presented as means and standard error (n=4).

	Composting phase (mgC g^{-1} TOC)	*Soil incubation phase* (mgC g^{-1} TOC)	*Total carbon mineralized* (mgC 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}

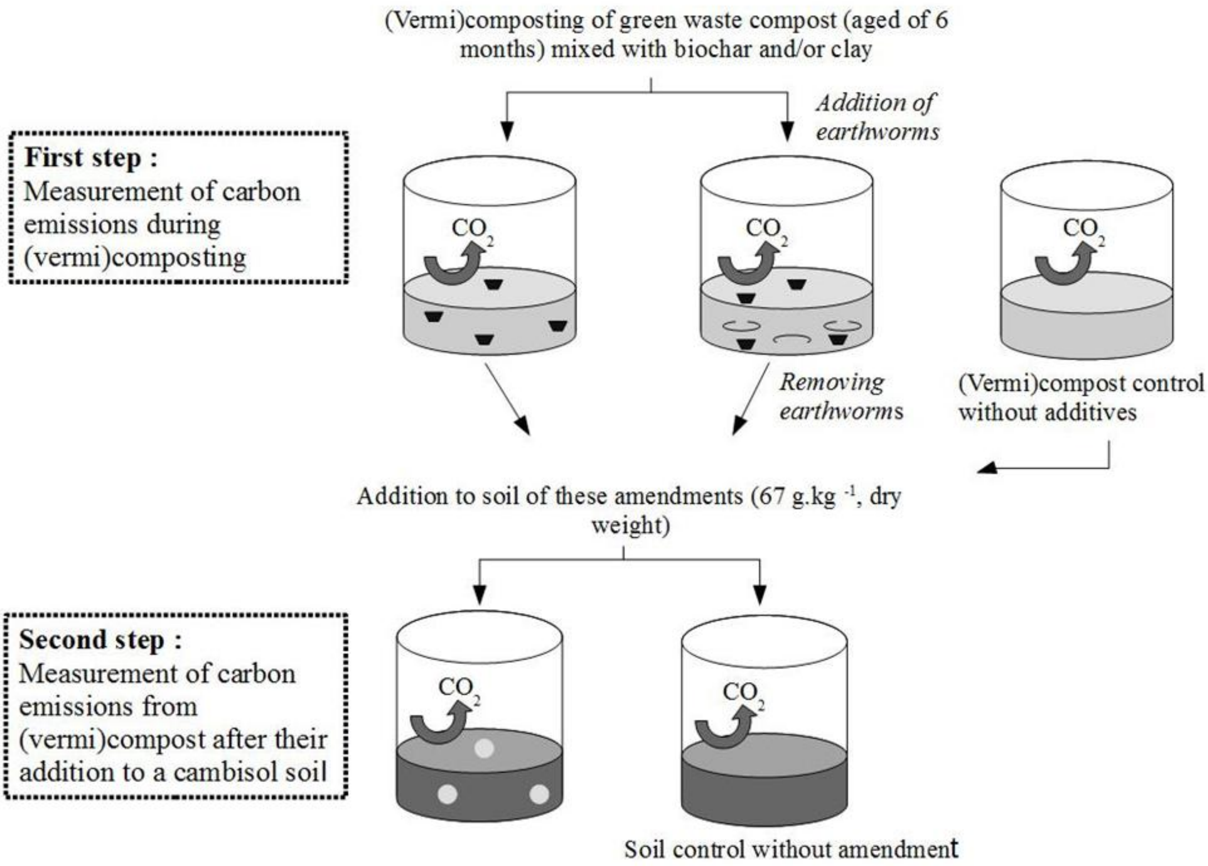

Figure 1. Experimental design to compare CO_2 emissions of different organic materials during composting and after their addition to soil.

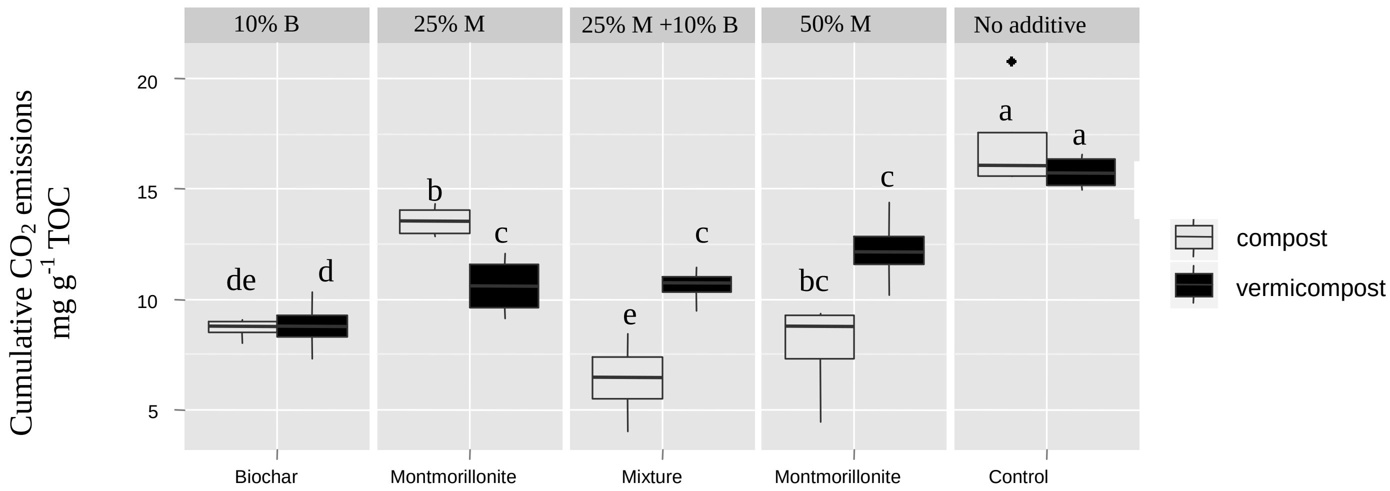

Figure 2. Cumulative CO_2 emissions at day 21 from composts and vermicomposts. Different letters (a, b, c, d, e and f) indicate statistically significant differences.

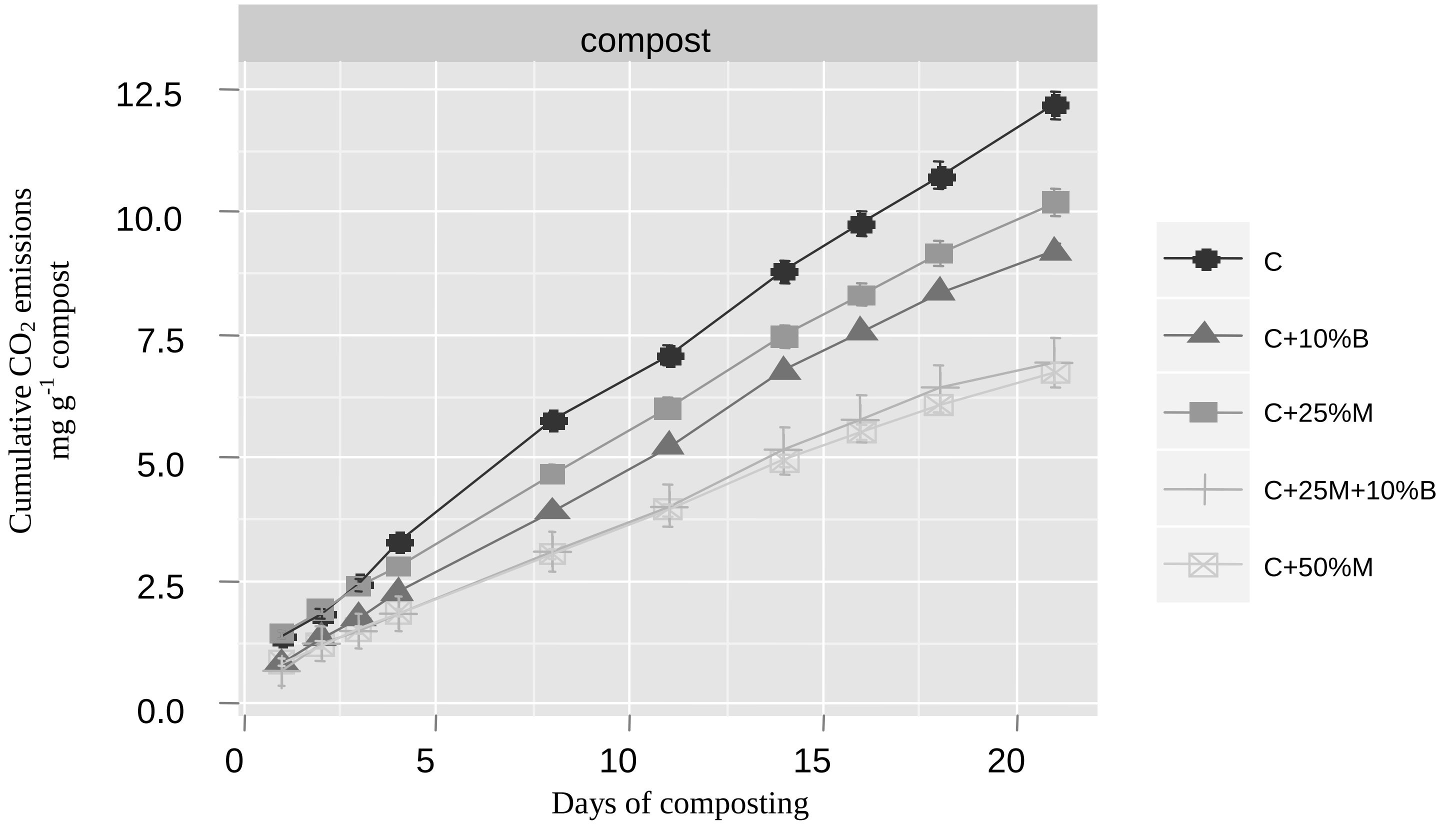

Figure 3: Cumulative CO_2 emissions during composting without worms of pre-composted material alone (C), with 25% of clay (C+25% M), with 50% of clay (C+ 50% M), with 10% of biochar (C+ 10%B) and, with 25% of clay and 10% of biochar (C+25%M + 10% B).

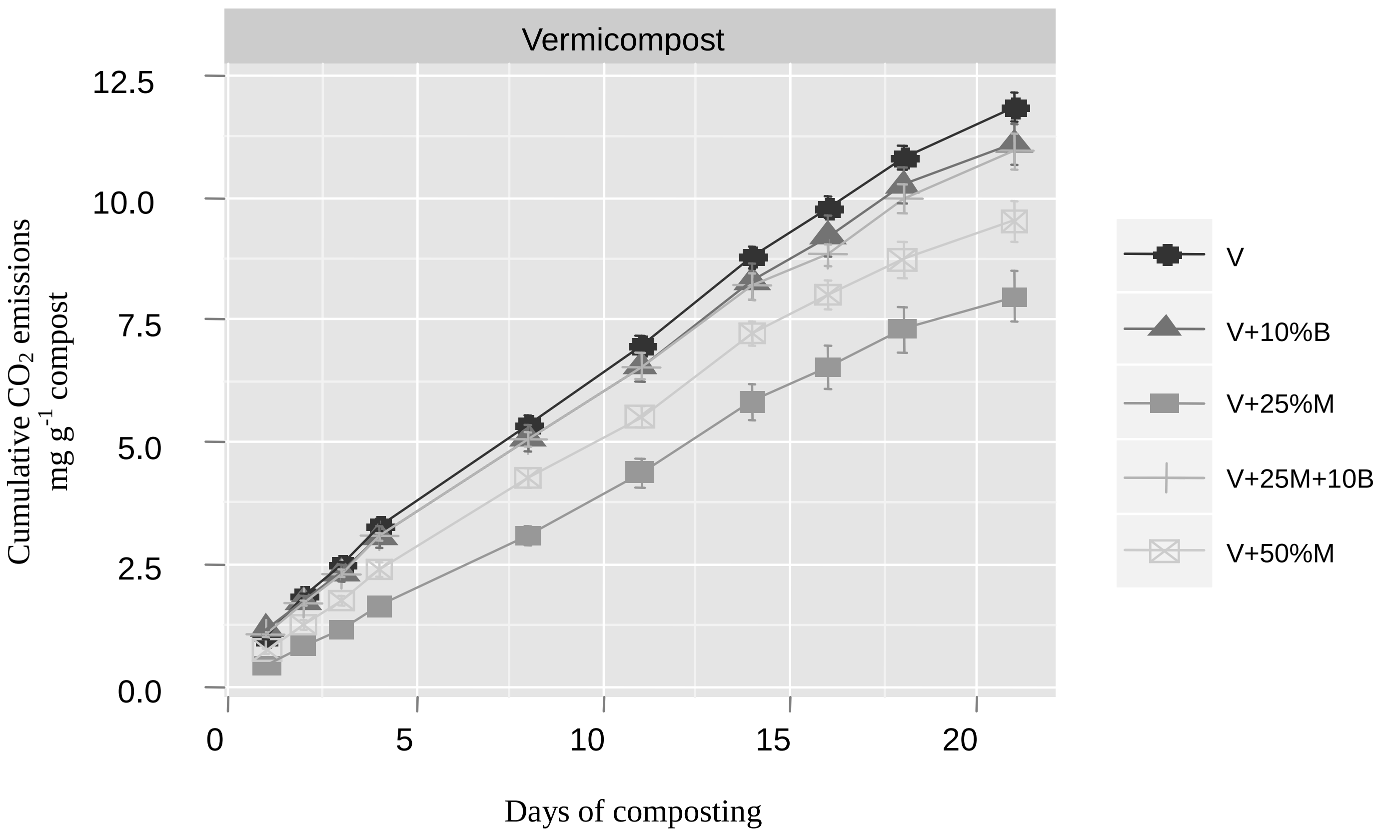

Figure 4: Cumulative CO_2 emissions during composting with worms of pre-composted material alone (V), with 25% of clay (V+25% M), with 50% of clay (V+ 50% M), with 10% of biochar (V+ 10%B) and, with 25% of clay and 10% of biochar (V+25%M + 10% B).

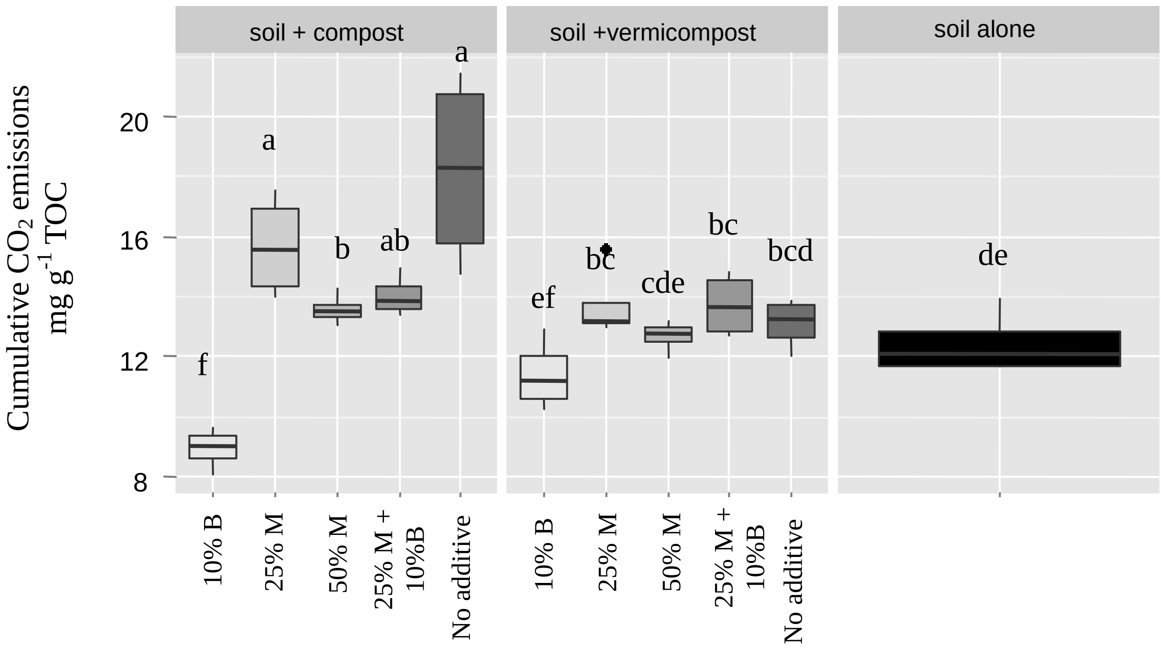

Figure 5. Cumulative CO_2 emissions at day 30 from composts and vermicomposts co-composting with 25% of clay (25% M), with 50% of clay (50% M), with 10% of biochar (10%B) and, with 25% of clay and 10% of biochar (25%M + 10% B) in soil. Different letters (a, b, c, d, e and f) indicate statistically significant differences.

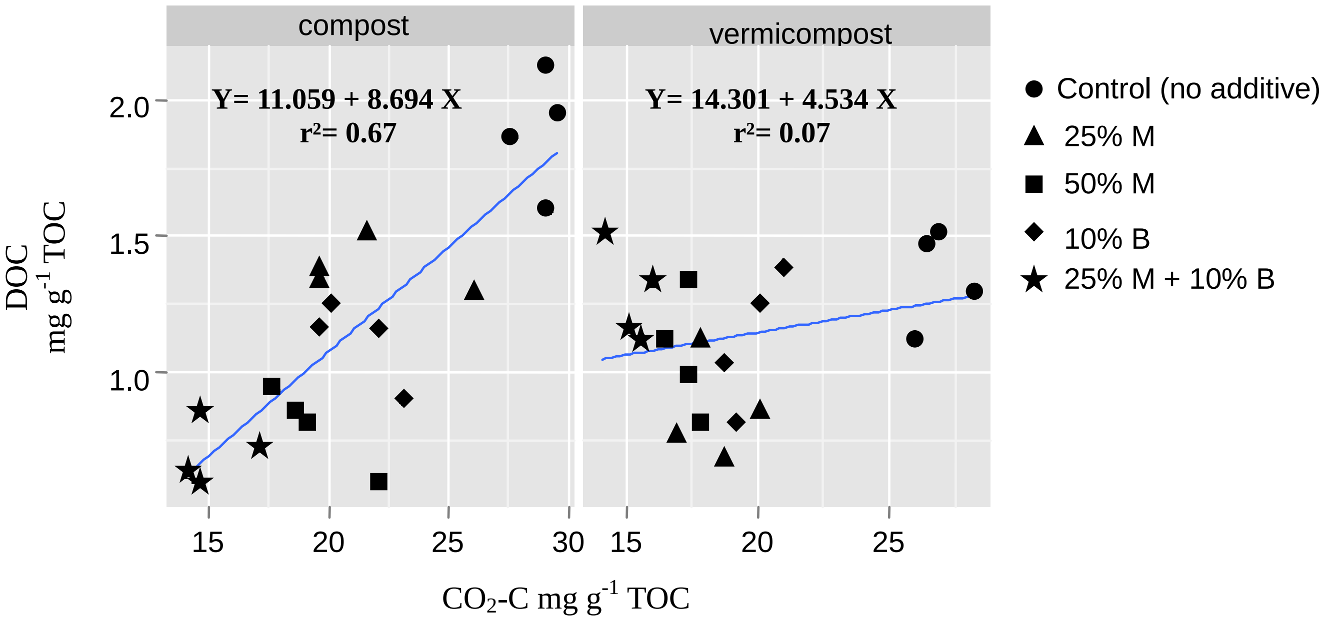

Figure 6. Comparison between cumulative CO_2 emissions at day 30 from composts and vermicomposts in soil and DOC from these amendments co-composting with 25% of clay (25% M), with 50% of clay (50% M), with 10% of biochar (10%B) and, with 25% of clay and 10% of biochar

625 (25%M + 10% B).