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The effects of worms, clay and biochar on CO₂ emissions during production and soil application of co-composts

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Abstract. In this study we evaluated CO₂ emissions during composting of green wastes with clay and/or biochar in the presence and absence of worms (species of the genus *Eisenia*), 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 mineralization over 21 days in the absence or presence of worms. The resulting co-composts and vermicomposts were then added to a loamy Cambisol and the CO₂ emissions were monitored over 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, CO₂ 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 CO₂ emission amendment requires optimization of feedstocks, co-composting agents and worm species.

1 Introduction

Land use changes are responsible for the steady increase in CO₂ 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 in the earth's climate and (agro)ecosystems have major environmental and agronomic, as well as 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 fertilizers (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 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 CO₂, CH₄ and N₂O (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 the 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 timescales (Kleber et al., 2015). Therefore, a variety of minerals have been used to reduce gas emissions (CO₂, CH₄, NH₃ and N₂O) during co-composting (Bolan et al., 2012; Chowdhury et al., 2016) 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 for reducing 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 (Lehmann et al., 2006). As a result, the use of biochar as a co-composting agent leads to a reduction in 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 worm guts improve physical protection of carbon (Bossuyt et al., 2005). During co-composting, the addition of worms may thus favor the protection of carbon and prevent its rapid release. On the other hand, earthworms have been shown to increase CO₂ 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 assess-

ment of the CO₂ 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 mineralization 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 CO₂ 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 hypothesized that the addition of worms additionally influences the magnitude of the CO₂ emissions. The aim of the study was to investigate whether worms can be used during co-composting of organic wastes and clay, biochar or their mixture to produce organic amendments with low CO₂ emission potential.

2 Materials and methods

2.1 Compost, additives and worms

A pre-composted green waste was sampled in its maturation phase at Bio Yvelines Services, a platform of green waste composting located 30 km west of 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 m² g⁻¹. 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 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).

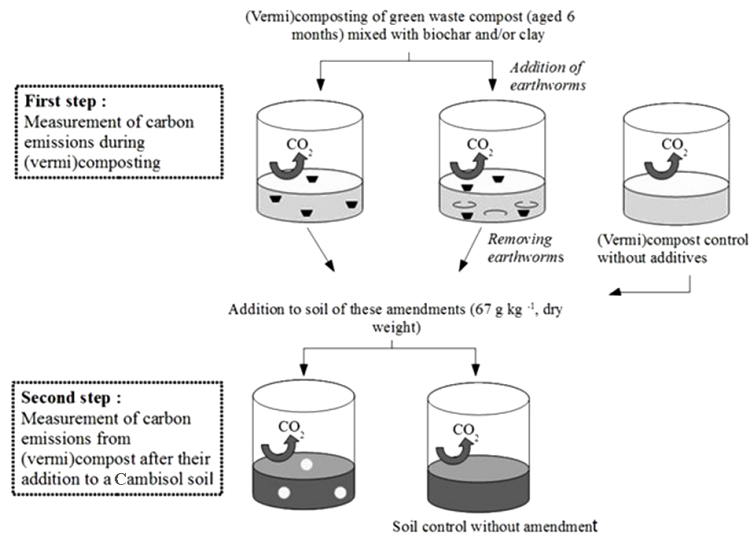


Figure 1. Experimental design to compare CO₂ emissions of different organic materials during composting and after their addition to soil.

Eisenia andrei and *Eisenia fetida* 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; and have short life cycles, high reproductive rates and endurance and resistance to handling (Dominguez and Edwards, 2011).

2.2 Experimental setup

The present study was designed to evaluate and compare the CO₂ emissions of the different organic materials during the production phase and after their addition to soil (Fig. 1)

2.2.1 Composting

Composting was carried out at ambient temperature in the laboratory with 10 treatments and four replicates per treatment as follows (5 treatments for compost and 5 for vermi-compost): (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 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 adding them to the organic material.

The experiments were carried out in 2 L 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 eight worms). A worm can ingest a maximum of its own weight per day (0.5 g).

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

2.2.2 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 southwest of France. This soil was used for crop production for the last 3 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 mg N g⁻¹, carbon content 10.56 mg C 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 2 L glass jars. The mixtures were homogenized. All 10 organic materials obtained during composting were

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. Data with different small letters indicate significant differences between treatments.

| | | pH | TOC | N | DOC | C/N |
|--|-------------------------|-------------------------|---------------------------|--------------------------|----------------------------|--------------------------|
| | | (mg g ⁻¹) | | | | |
| 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 |

applied to soil at a rate of 67 g 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 deionized water. The CO₂ emissions were measured over 30 days as described below.

2.3 Carbon mineralization

CO₂ emissions were measured in the headspace of the jars according to Anderson (1982). All incubation jars contained a vial with 30 mL of 1 M NaOH (composting) or 0.5 M (soil incubation) to trap CO₂. 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 1–4, 8, 11, 14, 16, 18 and 21. During the incubation with soil, vials were replaced at day 1, 2, 4, 7, 14 and 22. Phenolphthalein and BaCl₂ solution in excess were added to a 10 mL aliquot of NaOH sampled from each vial. The solution was titrated with 1 M HCl until neutrality to determine the CO₂-C released. Three empty jars were used as control.

Results are expressed in mg CO₂-C g⁻¹ compost (dry weight) or in mg CO₂-C g⁻¹ total organic carbon (TOC) according to the following formula:

$$\text{Released CO}_2\text{-C} = \frac{(B - V) \cdot M \cdot E}{P},$$

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 (1 M), E (22) the molar mass of CO₂ divided by 2 (because 2 mol of OH⁻ is consumed by 1 mol of CO₂) and P the weight of the sample (g).

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 K₂SO₄ extracts (1 : 5 w/v) using a total organic carbon analyzer (TOC 5050A, Shimadzu).

2.5 Calculations and statistical analysis

The amount of CO₂-C mineralized was expressed as mg C g⁻¹ 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 CO₂ emissions from the composting phase and the soil incubation after amendment. These results are expressed as mg C g⁻¹ of TOC, including soil carbon, compost carbon and biochar carbon.

Additionally, for composting, the amount of CO₂-C mineralized was expressed as mg C g⁻¹ 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 very low carbon emissions during a 200-day incubation in soil (Naisse et al., 2015); thus, we can hypothesize that its mineralization can be neglected compared to OM mineralization over 21 days.

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

$$C = C_0 \left(1 - e^{(-kt)}\right), \quad (1)$$

where C is the cumulative amount of CO₂-C mineralized after time t (mg C g⁻¹ compost), C_0 is the initial amount of organic carbon (mg C g⁻¹ compost), t is the incubation time (days), and k is the rate constant of CO₂-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 CO₂ 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 either additives or 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 CO₂ and organic acids by microorganisms 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 2.5–3.5). 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 to 241.9 mg g⁻¹ and total N from 8.5 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

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

| | k (10 ⁻³ day ⁻¹) | SE (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 |

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 a biochar–clay mixture.

The 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. However, it decreased 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.

4 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 juve-

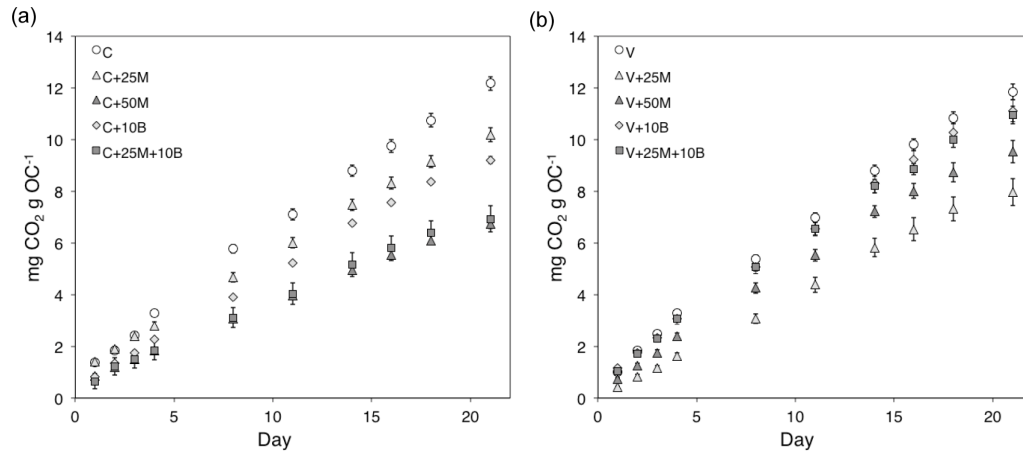


Figure 2. (a) Cumulative CO₂ emissions during composting without worms of pre-composted material alone (C), with 25 % clay (C + 25 M), with 50 % clay (C + 50 M), with 10 % biochar (C + 10 B) or with 25 % clay and 10 % biochar (C + 25 M + 10 B). (b) Cumulative CO₂ emissions during composting with worms of pre-composted material alone (V), with 25 % clay (V + 25 M), with 50 % clay (V + 50 M), with 10 % biochar (V + 10 B) or with 25 % clay and 10 % biochar (V + 25 M + 10 B). Bars represent twice the standard deviation of the mean ($n = 4$).

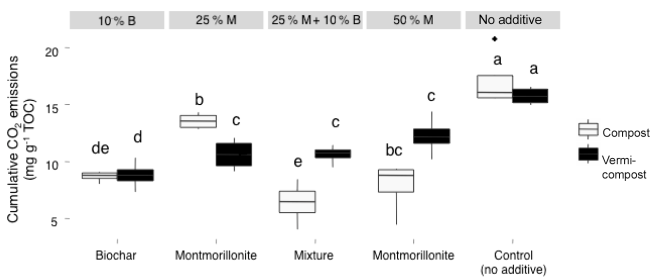


Figure 3. Total CO₂ emissions after 21 days of composting. Different letters (a, b, c, d, e and f) indicate statistically significant differences.

niles in treatments ranged from none to four. High- and low-clay treatments did not differ significantly from the control (p value = 0.39). No cocoons and no juveniles were counted in the biochar treatment. Finally, in treatments with a 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.

4.1 Carbon mineralization during composting

The cumulative CO₂ emissions during composting did not reach a plateau for any treatment (Figs. 2, 3). Cumulative carbon emissions at the end of the experiment ranged from 6.4 to 11.9 mg CO₂-C g⁻¹ compost in treatments without worms (Fig. 2). In the compost treatment without additives (control), the amount of carbon mineralized after 21 days was about 12 mg CO₂-C g⁻¹ compost. Composting with clay led to a significant decrease in the carbon emissions compared to the controls: in the low-clay treatment, emission decreased

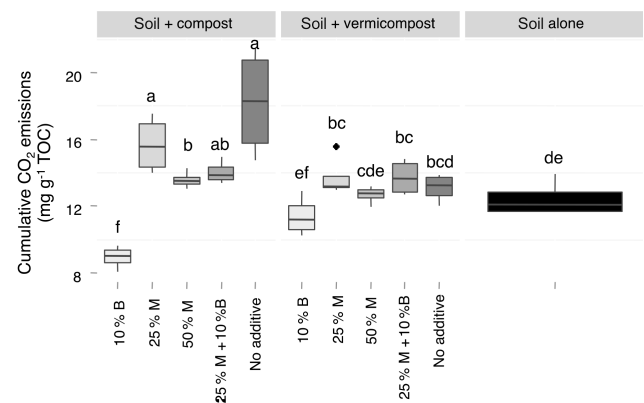


Figure 4. Cumulative CO₂ emissions after 30 days of incubation of the soil alone or amended with composts and vermicomposts with 25 % of clay (25 % M), 50 % of clay (50 % M), 10 % of biochar (10 % B) or 25 % of clay and 10 % of biochar (25 % M + 10 % B). Different letters (a, b, c, d, e and f) indicate statistically significant differences.

by 15 % and in the high-clay treatment emissions decreased by 43 %. Biochar addition reduced CO₂ emissions during composting by 24 % with biochar alone and by 46 % with a biochar–clay mixture (Figs. 2, 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 a clay–biochar mixture. Biochar alone decreased carbon mineralization in compost treatments.

In treatments with worms, cumulative CO₂ emissions ranged from 7.9 to 12.0 mg CO₂-C g⁻¹ compost (Figs. 3, 4). The presence of worms (Fig. 4) had contrasting effects on

carbon mineralization ($\text{mg g}^{-1} \text{TOC}$) 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 a biochar–clay mixture. Worms further reduced CO₂ emissions in the low-clay treatment by up to 34 % compared to the control and increased CO₂ 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.

4.2 Carbon mineralization during incubation with soil

Carbon emissions from the soil amended with the organic materials are shown in Fig. 5. Cumulative emissions at day 30 ranged from 8.95 to 18.20 $\text{mg g}^{-1} \text{TOC}$. 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 CO₂ 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.

5 Discussion

5.1 Effect of clay and biochar on carbon mineralization during composting

Addition of clay reduced CO₂ emissions during composting (Fig. 4). This confirms our initial hypothesis, stating that carbon mineralization 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 in CO₂ 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 in CO₂ emissions when biochar was used for co-composting (Sánchez-García et al., 2015). These contrasting results may be explained by variable physicochemical properties of biochar: the biochar used in this study was produced by gasification,

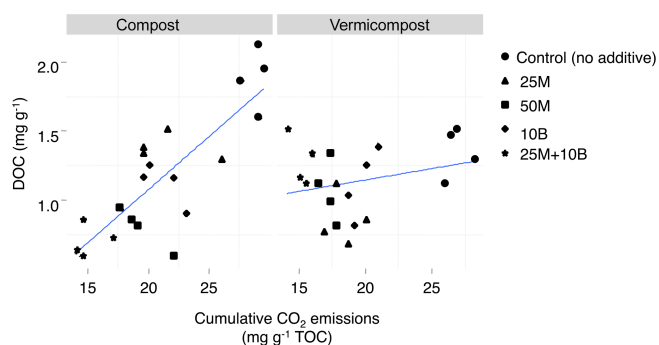


Figure 5. Comparison between total CO₂ emissions (composting + incubation with soil) and dissolved organic carbon (DOC) in the amendments. Key as in previous figures.

Table 3. CO₂ emissions during the experiments. C: compost; V: vermicompost; B: biochar; M: clay. Data with different small letters indicate significant differences between treatments.

| | Composting phase | Soil incubation phase | 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 ^c | 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} |

while Sánchez-García et al. (2015) used a biochar produced by pyrolysis.

When clay and biochar were added together during composting, lower CO₂ emissions were recorded as compared to their use alone (Table 3); therefore, the effect of each material is additive.

5.2 The presence of worms unexpectedly modifies the effect of clay and biochar on CO₂ 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 CO₂ emissions (Table 3), and thus it is expected that they would not have an effect when clay or biochar are added either. However, this was not the case. The presence of worms reduced CO₂ 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 in CO₂ 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 in CO₂ 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 genus *Eisenia* (*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 *Eisenia* are no longer able to reduce CO₂ emissions. The optimal clay : OM ratio to allow for maximal reduction in CO₂ 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, irrespective of 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. However, this is in line with other studies showing that the presence of biochar accelerates the composting process (Sánchez-García et al., 2015; Czekala et al., 2016). Three processes might explain why the 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 biodegradation; (2) biochars might enhance worm activity, as suggested by Augustenborg et al. (2012) to explain the increase in CO₂ emissions when biochar was added to soil in the presence of worms; and (3) during composting with a biochar–clay mixture, the worms might increase the contact between clay and biochar in their gut, leading to the partial saturation of clay surfaces with carbon compounds originating from biochar and thus to a reduction in the available surface area. This hypothesis is supported by the fact that 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 (PAHs), 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 PAHs or other potentially toxic substances might thus explain the effects that we observed. Although PAHs and dioxin 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.

5.3 Amendment composition and production influences mineralization in soil and total CO₂ emissions

The complicated trends observed in CO₂ 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 (Fig. 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; such 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 the 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 showed that this phenomenon may also occur after addition of composts to soil, when biochar is used as a co-composting agent.

In order to evaluate the overall positive or negative effect of each additive on carbon mineralization, CO₂ 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 the 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 mineralization 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 CO₂ 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 CO₂ 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 CO₂ emissions through adsorption of labile compounds of composts, while these processes are no longer controlling CO₂ emissions when these co-composts were produced in the presence of worms.

6 Conclusion

This study tested the influence of clay and biochar and their mixture on CO₂ 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 CO₂ release during composting, while inducing positive priming after soil amendment. Biochar also decreased CO₂ 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 CO₂ emissions during co-composting. We also tested the effect of worm species of the genus *Eisenia* on CO₂ 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 CO₂ emitted is no longer reduced. Addition of biochar did not lead to reduced CO₂ 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 evalu-

ating their CO₂ emission potential. A low emission potential of organic amendments may be achieved by optimization 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.

7 Data availability

Data are available upon request to the corresponding author.

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