



Can worms be used to produce amendments with reduced CO₂ emissions during co-composting with clay and biochar and after their addition to soil?

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Abstract :

In this study we evaluated CO₂ emissions during co-composting and co-vermicomposting of green wastes with clay and/or biochar. The stability of the final products as well as their effect on C mineralization in soil have been evaluated. The aim of the study was to test the following hypothesis: (1) interactions between clay and biochar and organic wastes would lead to reduced CO₂ emissions during the composting process, (2) these interactions would be enhanced in the presence of worms, and (3) more carbon would be 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 C mineralisation during 21 days in presence or absence of worms (*Eisenia* species). The organic materials were then added to a loamy Cambisol and the CO₂ 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, CO₂ emissions increased for all treatments except for the low clay dose. The production conditions had more influence on C mineralization in soil for composts than for vermicomposts except for the low clay treatment, which showed a more reduced CO₂ emissions compared to a regular compost.

In summary, the addition of worms during co-composting with clay and biochar may be a promising technology for reducing CO₂ emissions and increasing soil carbon storage. We suggest that the production of a low CO₂ 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

Industrial activity, deforestation and the utilization of fossil fuels are responsible for a steady increase of CO₂ in the atmosphere. In this context, massive soil organic matter (OM) loss is observed, leading to decline of many soil ecosystem services, such as fertility and carbon storage
40 (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 C may be possible with the use of composted organic wastes, which have been proposed as alternative fertilisers (Ngo *et al.*, 2011, 2012), and which could counterbalance the concentration of greenhouse gases in the atmosphere
45 through soil C 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 organic
50 matter transformation. 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 the mineralization of native soil
55 organic matter following increased microbial development and activity, a mechanism known as priming effect (Bustamante *et al.*, 2010).

In order to optimize the recycling of waste C, 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 C stabilization in composts could thus benefit
60 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 C stabilization on long time scales (Kleber *et al*, 2015). Therefore, a variety of minerals has been used to reduce gas emissions (CO₂, CH₄, NH₃ and N₂O) during co-composting (Bolan *et al*, 2012, Wang *et al*, 2014, Chowdhury *et al*, 2015), e.g. clay minerals during composting of
65 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 the resulting organic material after their addition to soil.

In addition, many recent studies explored the potential benefits of biochar as soil amendment due to
70 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 organic matter (Lehman *et al*, 2006). As a result, the use of biochar as co-composting agent leads to a reduction of C emissions
75 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
80 ingestion of organic matter and minerals (Shipitalo and Protz, 1989). Micro-aggregates formed inside the worm guts improve physical protection of C (Bossuyt *et al*, 2005). However, these interactions have only been evidenced for soil earthworms and have never been evaluated as a strategy to reduce CO₂ emissions during co-composting. Moreover, to the best of our knowledge, no studies have investigated the effect of biochar as a co-composting agent during vermicomposting.

85 The objective of this study was to evaluate if the addition of clay, biochar and their mixture to pre-composted wastes can reduce CO₂ emissions during (vermi)composting and after use of the final products as soil amendments. We hypothesized that C stabilization may be increased by addition of



(a) 2:1 clay such as montmorillonite, able to form organo-mineral associations; (b) biochar, able to protect organic matter by adsorption and (c) their mixture, which could create synergistic effects. In addition, we tested the effect of two different amounts of clay on the reduction of CO₂ emissions during co-composting with and without worms and after addition to soil.

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. 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 and 13.3 mg.g⁻¹ of nitrogen. After sampling, the compost was air-dried and sieved at 3 mm for homogenization.

The clay used was a 2:1 clay, purchased from Sigma-Aldrich. The clay's pH was between 2.5-3.5 and its specific surface area (SSA) was 250 m²/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 used was the product of gasification at 1200°C of a conifer feedstock and it was provided by Advanced Gasification Technology (Italy). Biochar had a pH of 9.3 and a C:N ratio of 4030, with 806 mg g⁻¹ of organic carbon and 0.2 mg g⁻¹ of nitrogen (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
115 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 CO₂ emissions of the different organic
120 materials during the production phase and after their addition to soil (Fig.1)

First step

Co-(vermi)composting was carried out at ambient temperature in the laboratory with 10 treatments
and four replicates per treatment: (i) compost alone, (ii) compost with 25% (w/w) of
montmorillonite (low clay treatment), (iii) compost with 50 % (w/w) of montmorillonite (high clay
125 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
130 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 grown 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.

135 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 organic matter should have been



140 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 weighted again. The amount of cocoons and juvenils was recorded. The final (vermi)composts were airdried, sieved at 2 mm and an aliquot was ground for further analyses.

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Second step

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
150 collected at depth 0-10 cm, 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, nitrogen 1.15 mgN g⁻¹, carbon 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 through thorough mixing. All ten organic materials obtained during the first step
155 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 CO₂ emissions were measured during 30 days as described below.

160 2.3 Carbon mineralisation

CO₂ 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 (first step) or 0.5M (second step) to trap CO₂. The NaOH vials were covered with a tissue to avoid contamination of the NaOH solution by worms. During the first (vermi)composting step, NaOH traps were replaced at day 1, 2, 3, 4, 8, 11,
165 14, 16, 18 and 21. During the incubation with soil, vials were replaced at day 1, 2, 4, 7, 14 and 22.



Phenolphalein and BaCl₂ 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 CO₂-C released. Three empty jars were used as control.

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

$$\text{Released CO}_2 - \text{C} = \frac{(B - V) * N * E}{P}$$

where B is the volume of HCl used to titrate the control (mL); V the volume of HCl used to titrate the sample (mL); N the normality of HCL (1M); E (22) the molar mass of CO₂ divided by 2 (because 2 mol of OH⁻ are consumed by one mol of CO₂) and P the weight of the sample (grams).

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2.4 Properties of the final products after co-(vermi)composting

Organic carbon and nitrogen 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).

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2.5 Calculations and Statistical analysis

The amount of CO₂-C mineralized was expressed as mgC per g of TOC, including for step 1, (vermi)compost C and biochar C and for step 2 soil C, (vermi)compost C and biochar C. 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 mgC per g of TOC, including soil C, (vermi)compost C and biochar C.

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Additionally, for the composting (step 1), the amount of CO₂-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

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because it is produced at high temperatures and therefore its C is supposed to have a high chemical recalcitrance against biological decomposition (McBeath and Smernik, 2009).

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

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

195 where C is the cumulative amount of CO₂-C mineralized after time t (mgC g⁻¹ compost), C₀ is the initial amount of organic carbon (mgC 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
200 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

205 3.1 Properties of the co-(vermi)composts

Total N, OC, DOC and pH of initial pre-composted material and of the different co-(vermi)composts are shown in Table 2. The pH of composts and vermicomposts ranged from 7.9 to 8.7. The lowest pH was observed for the high clay treatments and the highest pH was recorded for control treatments and (vermi)composts with biochar. Compared to the initial pH of pre-composted
210 organic material (8.5 ± 0.1), the high clay treatment led to a significant decrease of pH. Presence of worms during the composting phase had no effect on pH.

Total OC in composts and vermicomposts 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. Compared to initial pre-composted material, OC was decreased significantly after 21 days of (vermi)composting in both control treatments while N concentrations
215 remained unchanged. 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. Similarly to pH, the presence of worms had no effect on OC or N of co-vermicomposted material.

DOC in composts and vermicomposts ranged from 15.04 to 29.08 mg g⁻¹ TOC. DOC was similar to
220 the pre-composted material after 21 days of (vermi)composting for the two controls whereas the presence of additives significantly decreased the DOC in all other treatments. The lowest DOC concentrations were recorded for compost and vermicompost produced with biochar/clay mixture. The presence of worms had only an effect on DOC for compost produced with clay, decreasing its concentration by 12% (high clay treatment) and 16% (low clay treatment).

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3.2 Worm growth and reproduction

The number of worms and their total weight were measured before and after the experiment. The number of worms did not vary after vermicomposting (p-value > 0.07) and neither did their total weight (p-value = 0.34). Cocoons and juveniles were separated manually from the substrates and
230 counted at the end of the experiments. 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
235 one juvenile.

3.3 Carbon mineralisation during co-(vermi)composting

During the composting phase (Fig. 2), the presence of worms did not change the C mineralisation (mg g⁻¹ TOC) in treatments free of additives. In the low clay treatments, the presence of worms decreased the amount of C mineralized. In contrast, increased C mineralization was noted for the
240 high clay and the biochar/clay mixture (56 % and 66% increase). The cumulative CO₂ emissions (mg g⁻¹ compost) during composting and vermicomposting did not reach a plateau for any treatment



(Fig. 3 and 4), but the experimental period was limited by worms activity since worms had processed all organic material after 21 days.

245 Rate constants obtained with the first-order kinetic model (eq. 1) are listed in Table 2. Similarly high rate constants suggest a rapid carbon mineralization from compost or vermicompost without additives (control). In general, treatments with worms showed higher rate constants than those without, except for the low clay treatment and control treatments. With worms, the lowest rate constant was observed for the low clay treatment. Without worms lower degradation rates as
250 compared to the control were recorded for treatments with high clay and clay/biochar mixture. Biochar alone decreased C mineralization more in treatments without worms.

In treatments without worms, cumulative carbon emissions at the end of the experiment ranged from 6.4 to 11.9 mg CO₂-C g⁻¹ compost, whereas in treatments with worms values ranged from 7.9 to 12.0 mg CO₂-C g⁻¹ compost (Fig. 3 and 4). In both control treatments without substrate additions,
255 the amounts of carbon mineralized after 21 days were similar, about 12 mg CO₂-C g⁻¹ (vermi)compost. Co-(vermi)composting with clay led to a significant decrease of the carbon emissions compared to the controls. The cumulative carbon emissions were decreased by 15% in the low clay treatment without worms and by 34% in the same treatment with worms (Fig. 3 and 4). In the high clay treatment, CO₂ emissions were reduced by 43% without worms and by 24% when
260 worms were present.

3.3 Carbon mineralisation during incubation with soil

Carbon emissions from the soil amended with the organic materials (step 2) 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
265 without amendments. The carbon emissions were influenced by the (vermi)compost production procedure (additives and worms). The highest emissions were recorded for soil amended with (vermi)composts free of additives. Compared to soil amended with composts, vermicomposts



decreased the carbon emissions from amended soil only when produced without additives or with
low clay addition. High clay addition during co-composting with and without worms produced
270 organic amendments which induced similar C emissions from soil. Compost produced in the
presence of biochar showed the lowest effect on mineralization in soil. When biochar was mixed
with clay, the co-vermicompost induced lower C emissions from soil compared to the co-compost.
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
275 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

280 After 21 days of vermicomposting, OM had been processed into a homogeneous and aerated
material whereas composts had a compact aspect, illustrating the positive effects of worms on the
physical structure of the final product. Co-composting with biochars did not lead to any change in
pH of the final product (Table 2). This may be due to the alkaline pH of the pre-composted material
and the low amount of biochar added. In contrast, the addition of acidic clay (pH 2.5 to 3.5) to
285 slightly alkaline pre-composted material tended to reduce the pH of the final (vermi)compost (Table
2). The presence of worms in our experiments had no effect on the pH, 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
290 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 (vermi)composts produced in the presence of
biochar, due to addition of carbon enriched material. No difference was observed in vermicompost



treatments compared to compost treatments, concerning the OC and the total N. These results are in
295 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 worms and additives on carbon mineralization during (vermi)composting (step1)

Data recorded (Fig. 3 and 4) for control treatments indicated in contrast to what is generally
300 observed (e.g. Chan *et al*, 2011), that the presence of worms did not lead to higher CO₂ 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 (vermi)composting.

Addition of clay and biochar reduced carbon emissions during composting (Fig. 3). Similar results
305 were obtained by other authors for co-composting in absence of worms with clay additives (Bolan
et al, 2012) or biochar (Dias *et al*, 2010). These data may indicate carbon stabilization by physico-
chemical protection of OM on clay and/or biochar surfaces. Carbon storage generally increases
linearly with increasing clay concentration (Hassink, 1997). This is in line with our results, showing
proportional CO₂ decrease, when clay content and thus surface area was doubled.

310 By contrast, in the presence of worms, C mineralization was more reduced for the low clay
compared to the high clay treatment (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
315 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*
species. In contrast, in the low clay treatment, worm activity most probably increases the formation
of organo-mineral associations, thus leading to higher reduction of CO₂ emissions compared to
regular composting without worms (Fig. 3). These results indicate that the protective capacity of



320 clay minerals may be enhanced by worm activity, up to a threshold of the clay:OM ratio, above
which *Eisenia* species are no longer able to reduce CO₂ emissions. *Eisenia* species belong to the
epigenic worm species living at the soil surface in leaf litter, one of the three ecological lifestyles
described by Bouché (1977). Therefore, they are well adapted to process pure organic matter and
may be less suited for co-composting with minerals. The optimal clay: OM ratio to allow for
325 maximal reduction of CO₂ emissions remains to be assessed as well as the possibility to use other
worm species more adapted to ingestion of minerals.

When biochars were added, alone or in mixture with clay, contrasting results were observed among
co-composting processes with and without worms. In general biochar addition led to a reduction of
330 CO₂ emissions up to 44% compared to the regular (vermi)compost (Fig. 2 and 3). In absence of
worms, a 24% decrease was observed in treatments with biochar and a 46 % decrease with
biochar/clay mixture (Fig. 2 and 3). These results are in contrast with other studies showing no
significant reduction of CO₂ emissions when biochar was used for co-composting (Sánchez-García
et al., 2015). However, biochar effects may depend on its physic-chemical properties, which are
335 depending on the production conditions. Therefore, the biochar produced by gasification used in
this study may have different effects compared to biochar produced by pyrolysis used in the other
study. Reduced CO₂ emissions in presence of biochar are in line with observation by other authors
concerning biochar effects on microbial activity and OM mineralisation. Decrease of OM
mineralization induced by biochar was explained by its capacity to adsorb labile organic
340 compounds, which may otherwise be degraded (Augustenborg *et al.*, 2012; Ngo *et al.*, 2013; Naisse
et al., 2015).

In the presence of worms, the addition of biochar and biochar/clay mixture induced higher CO₂
emission (p-value > 0.1) compared to regular co-composting (Fig. 2 and 3). Three hypothesis might
explain that worms drastically modify the complex interactions between clay, biochar and pre-
345 composted OM: 1) 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; 2) the microbial colonization of biochar might be enhanced in the worm gut decreasing their long-term resistance to bio-degradation; 3) biochars might enhance worm activity, as suggested by Augustenborg *et al* (2012) to explain the increase of
350 CO₂ emissions when biochar was added to soil in the presence of worms.

The incidence of these three hypotheses probably depends on the biochar quality, which influences the affinity of worms for biochar. The properties of the biochar and its effects on the worms might be dependent upon their production process, for example upon the initial feedstock or the conditions of pyrolysis. In our case, biochar had no effect on worm biomass, but it had contradictory effects on
355 worm reproduction. The addition of biochar alone reduced the number of juveniles and cocoons of *Eisenia* to zero, indicating a high stress for worms. For the development of soil earthworms, the presence of biochar has already been described as a potential risk (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
360 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 negative effects that we observed. Further analyses and longer experiments should be carried out in order to investigate the reasons for
365 these stressful effects and for the increasing reproduction rates observed when biochar was used in combination with clay. Testing the influence of biochar with contrasted 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 C mineralization.

370 **4.3 Effect co-(vermi)compost production conditions on carbon mineralization in soil (step 2) and total carbon balance**



The production conditions had more influence on C mineralization in 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
375 (Table 1). But the CO₂ 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. The lack of correlation between DOC and CO₂ emitted after addition to soil of vermicomposts suggests contrasted properties of DOC in composts and
380 vermicomposts (Lazcano *et al*, 2008, Kalbitz *et al*, 2003).

CO₂ 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.

385 In 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. 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 for composts, when biochar is used as co-composting agent. This was not observed for co-
390 composts produced in the presence of worms. However, the addition of worms may attenuate these effects of the final product.

In order to evaluate the positive or negative effect of each additive and process (with or without worms) on C mineralization, the emitted CO₂ of both steps (composting phase and incubation of soil with amendments) was summed up and expressed as mgC g⁻¹ TOC. The carbon emissions
395 during step 1 and step 2 were influenced differently by the (vermi)compost production procedure (additives and worms). The lowest total carbon emissions were recorded for compost and vermicompost produced in presence of biochar. Low clay treatment more efficiently reduced carbon



emissions in the presence of worms. In all other treatments, except the control, CO₂ emissions were higher for vermicompost due to higher emissions during the production step.

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5. Conclusion

This study tested the possibility to use the worm species *Eisenia* to reduce CO₂ emissions during co-composting of green wastes with clay, biochar or their mixture. We established the complete carbon balance taking into account production of amendments as well as the effect after their
405 addition to soil. Most additives were found to decrease CO₂ release during co-(vermi)composting. 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 CO₂ emissions are no longer reduced. Biochar had a positive effect on carbon storage for all treatments. Biochar/clay mixture resulted in synergistic effects for treatment
410 without worms. We conclude that the use of additives may have the potential to greatly reduce CO₂ emissions during co-composting. Worms further reduced CO₂ emissions only in treatment with low clay dose. The effect of the amendments on C mineralization after addition to soil was small in the short-term. We suggest that production conditions during co-(vermi)composting have to be optimized in terms of total CO₂ reduction by choosing the minerals, their optimal ratio with OM
415 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.

	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.

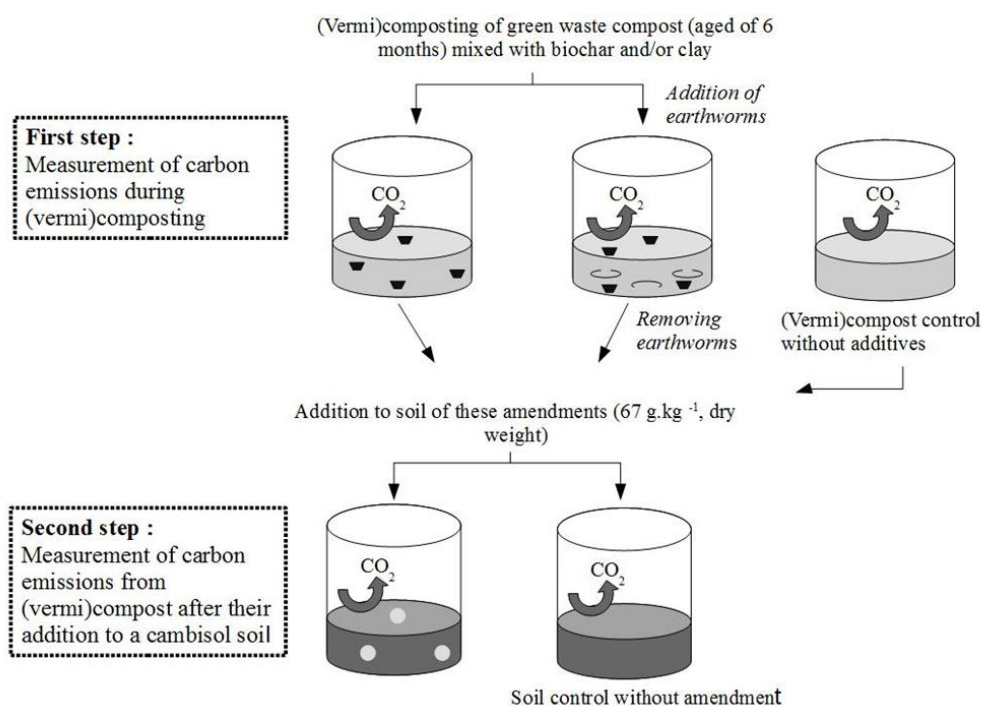
	k (10 ⁻³ day ⁻¹)	Std. Error (10 ⁻⁵)
Compost treatments		
C	3.069 ^a	4.429
C + 25 % M	2.588 ^{cd}	4.539
C + 50 % M	1.699 ^g	2.776
C + 10 % B	2.313 ^{ef}	2.204
C + 10 % B+ 25 % M	1.762 ^g	5.265
Vermicompost treatments		
V	3.036 ^{ab}	4.089
V + 25 % M	1.973 ^{fg}	3.783
V + 50 % M	2.431 ^{de}	3.616
V + 10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

560



Table 3: Carbon balance. Data are presented as means and standard error (n=4). Different small letters indicate significant differences between treatments (Kruskal-Wallis test, $p < 0.005$)

	<i>Composting phase</i> (mgC g ⁻¹ TOC)	<i>Soil incubation phase</i> (mgC g ⁻¹ TOC)	<i>Total carbon mineralized</i> (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}



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

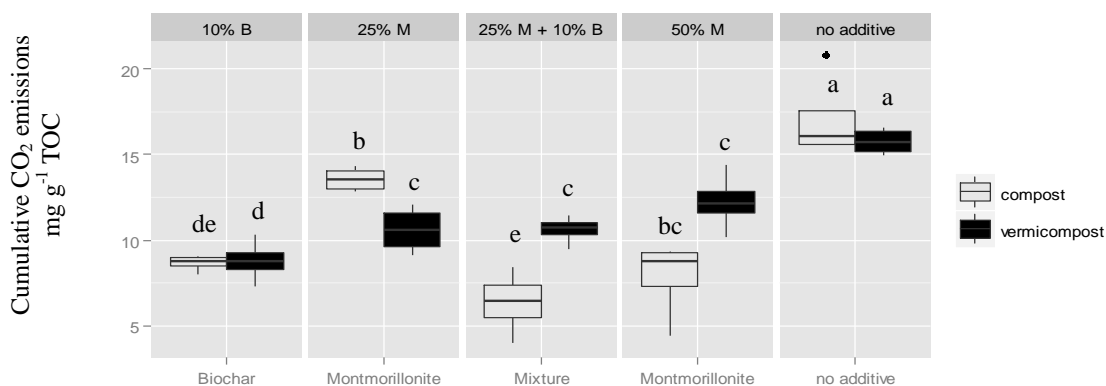
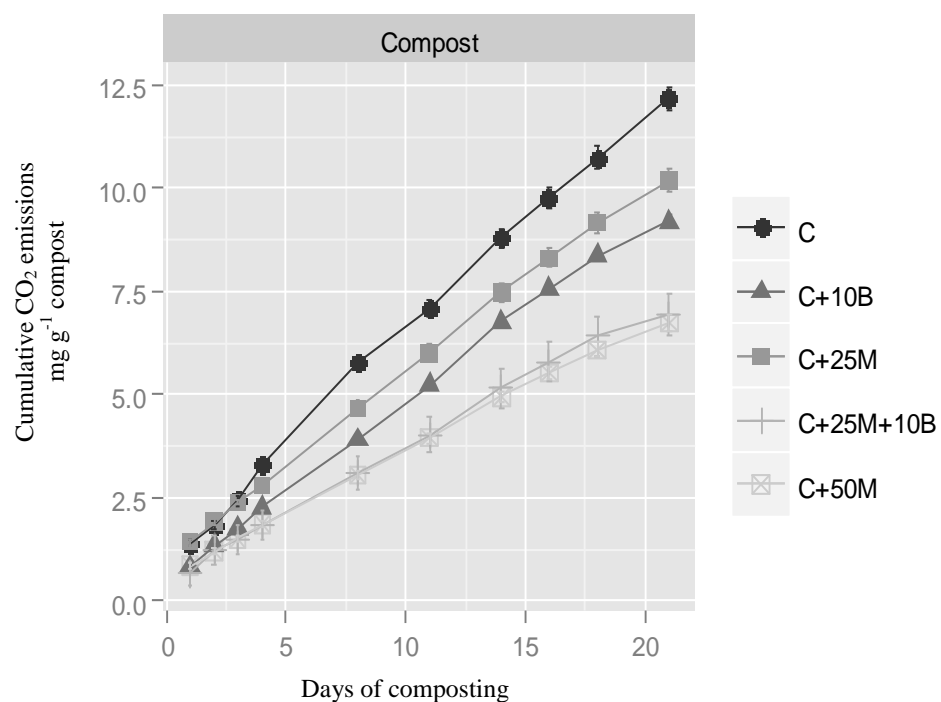
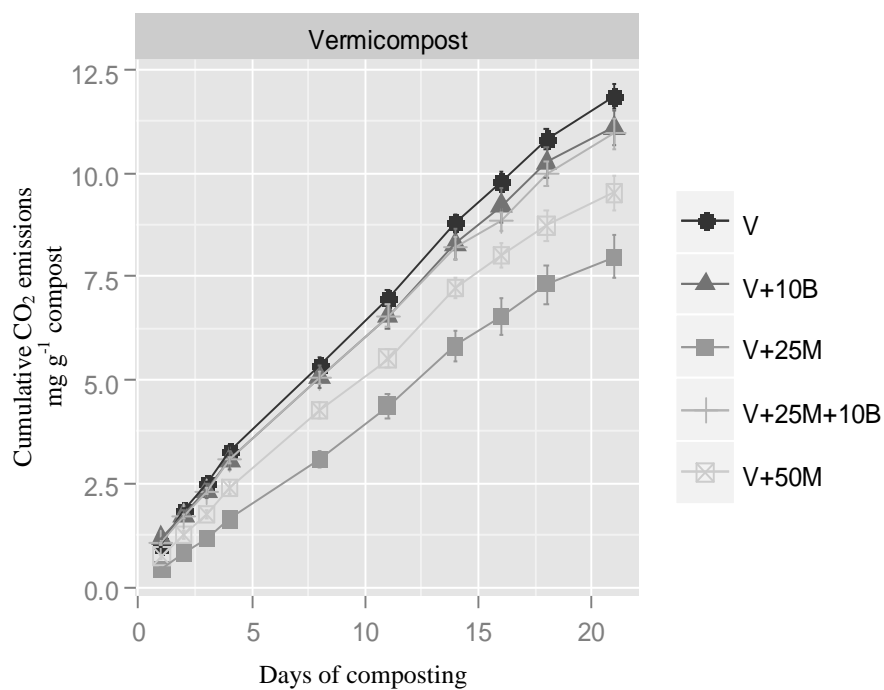


Figure 2. Cumulative CO₂ emissions at day 21 from composts and vermicomposts. Letters a,b,c, d, e and f means the statistical difference.

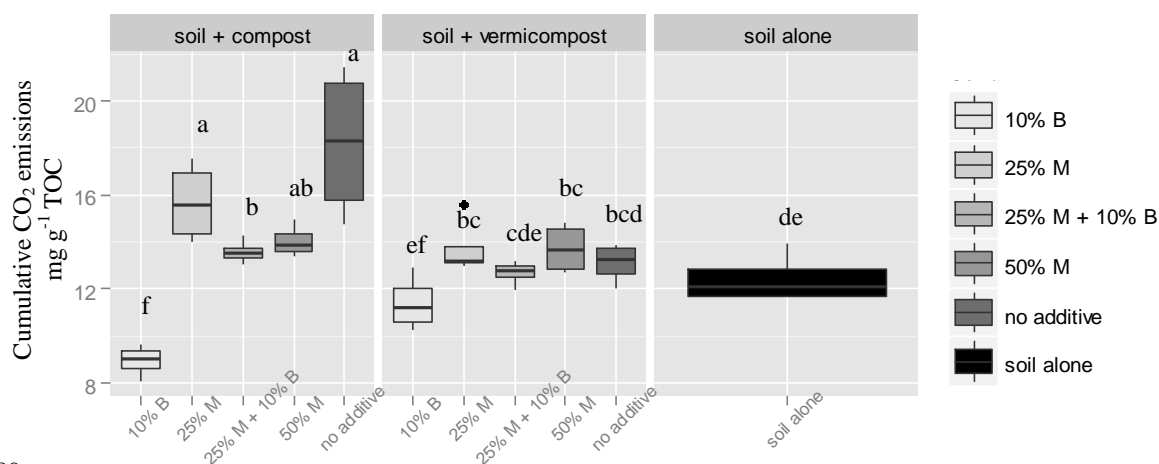


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Figure 3: Cumulative CO₂ emissions during composting without worms of pre-composted material alone (C), with 25% of clay (C+25% M), with 50% of clay (C+ 50% M), with 10% of biochar (C+ 10%B) and, with 25% of clay and 10% of biochar (C+25%M + 10% B).



575 **Figure 4: Cumulative CO₂ 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).**



580

Figure 5. Cumulative CO₂ emissions at day 30 from composts and vermicomposts in soil.

Letters a,b,c, d, e and f means the statistical difference.

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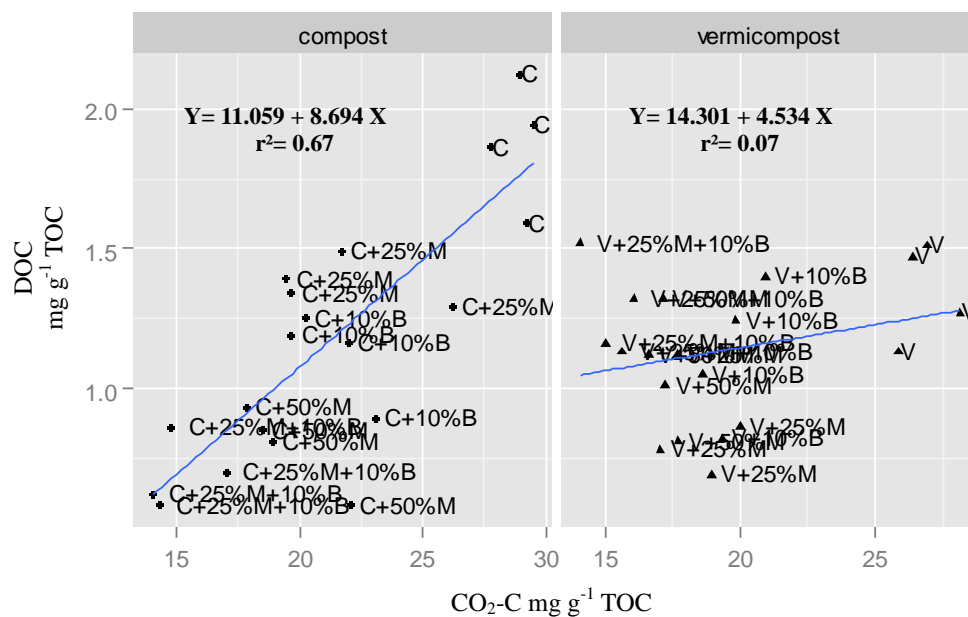


Figure 6. Comparison between cumulative CO₂ emissions at day 30 from composts and vermicomposts in soil and DOC from these amendments.

590