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Justine Taverne

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THESE

Pour l'obtention du grade de
DOCTEUR DE L'UNIVERSITE PIERRE ET MARIE CURIE

Ecole doctorale « Science de l'Environnement d'Ile-de-France »

Laboratoire iEES Paris

**Innovative waste treatment by composting with minerals
and worms: effects on carbon storage, soil properties and
plant growth**

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Soutenue le 17 octobre 2017

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*A ma sœur,
Pour m'avoir pourchassée et terrorisée avec des vers de terre ;
A mes parents, pour n'avoir rien fait contre...*

« Si un jour tu tombes, je serai là... »

Le sol

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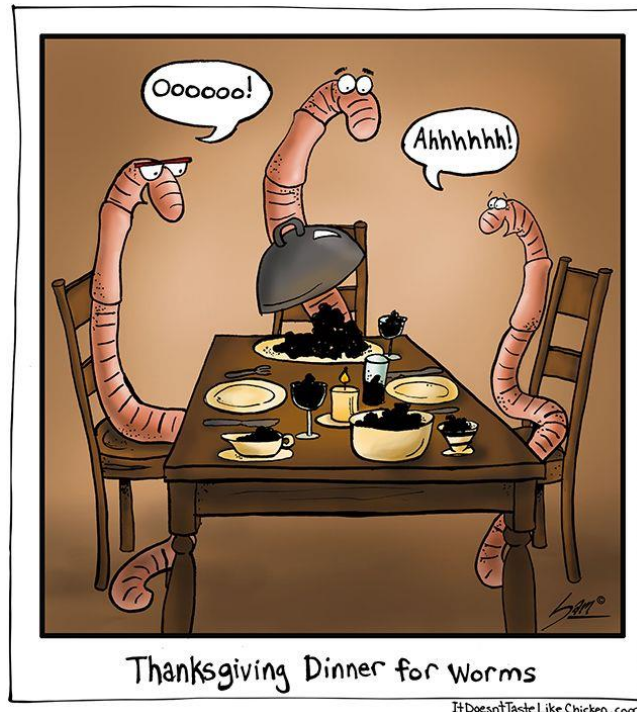
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milles et une façons de tuer un vers » ... !). Vos petits sourires devant un trognon de pomme et une feuille de salade vont me manquer...



RESUME

Procédé de compostage innovant en présence de vers et de minéraux : effets sur le stockage de carbone, les propriétés du sol après amendement et la croissance des végétaux.

Basés sur la bio-oxidation de la matière organique, le compostage et le vermicompostage émettent des quantités non négligeables de CO₂. Ainsi, ce travail s'intéresse (1) à la stabilisation potentielle du carbone de la matière organique compostable due à l'ajout de minéraux, avec et sans vers, et (2) l'influence des minéraux sur la maturité et la qualité du compost final. La première expérience consista en une incubation de matière organique, permettant d'obtenir différents composts et vermicomposts, avec et sans minéraux, de suivre les émissions de carbone et d'analyser les produits obtenus. Une deuxième incubation a été menée pour étudier l'effet de ces produits en tant qu'amendement sur un sol. Enfin, le suivi de la croissance de *A.Thaliana* en présence de co-composts et co-vermicomposts en tant que fertilisant fut réalisé.

La présence de minéraux lors du compostage induit une baisse des émissions de carbone, probablement due à la formation d'associations organo-minérales ou bien par un changement des communautés microbiennes. De plus, les co-composts, en tant qu'amendement semblent augmenter les stocks de carbone du sol, comparé à un compost classique. Enfin, bien que l'aspect fertilisant des co-composts obtenus soit optimum, ils n'ont pas amélioré la croissance d'*A.Thaliana*. La présence de vers lors du co-compostage a augmenté les émissions de carbone mais l'effet positif des minéraux sur la minéralisation était toujours observable. De plus, les vers ont augmenté la biodisponibilité des nutriments et le co-vermicompost a permis d'améliorer les propriétés du sol ainsi que la croissance des végétaux comparé à un (vermi)compost classique et un co-compost.

Keywords : compost; vers; minéraux; carbone; croissance des plantes; sol; stock de carbone; amendement; fertilisant; analyses physico-chimiques; analyses microscopiques.

ABSTRACT

Innovative waste treatment by composting with minerals and worms: effects on carbon storage, soil properties and plant growth

Due to bio-oxidative mechanisms, composting and vermicomposting produce a large amount of CO₂ emissions. Therefore, in this study we aimed to assess (1) the potential stabilization of carbon of fresh organic matter due to mineral presence, with and without worms, and (2) the influence of minerals on maturity and quality of the finished products. The first experiment was based on a laboratory incubation of fresh organic matter, which allowed to obtain composts and vermicomposts, with and without minerals, to monitor carbon emissions and to analyse the final products. A second incubation was carried out to investigate the effect of these products as organic amendment on an arenic cambisol. Finally, the last experiment was carried out to assess the use of co-composts and co-vermicomposts as potting media, by following the growth of *A.Thaliana*.

The presence of minerals during composting induced a decrease of carbon emission, likely due to the formation of organo-mineral associations or shift of microbial communities. Moreover, the use of co-compost as soil conditioner may allow to increase soil carbon storage, compared to a regular compost. Although the physico-chemical characteristics of the end-products were optimum for its use as potting media, no beneficial aspects on *A.Thaliana* growth has been observed. In presence of worms during co-composting, the carbon emissions were increased due to worm activities but the positive effect of minerals was still observed. In addition, worms improved the nutrient availability in the end products. Therefore, co-vermicompost tended to improve soil properties and to enhance plant growth compared to a regular (vermi)compost.

Keywords : compost; worms; minerals; carbon; plant growth; soil; carbon storage; amendment; organic fertilizer; physico-chemical analyses; microscopic analyses.

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GENERAL INTRODUCTION

The increase in world human population coupled to a higher consumption of goods and services, has driven rapid increase of the quantities of produced wastes such as organic household, municipal or industrial and agricultural wastes (Hoornweg *et al*, 2013). This situation generates a serious environmental issue, calling to find clean and sustainable strategies to treat these wastes, from their production to their recycling or elimination.

In parallel to this issue, land use changes are responsible for the steady increase of 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 of the earth's climate and of (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 stable exogenous organic matter (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).

One of the main challenges is to solve these two issues together. In this way, two well-known aerobic processes based on microbial activity are able to transform organic wastes into valuable soil amendments: composting and vermicomposting. Both these processes require low investment and are efficient to recycle organic matter. Composting has been traditionally used and leads to stabilized organic amendments with fertilization potential. During vermicomposting the presence of worms induces a continuous aeration resulting in a faster OM transformation (Lazcano *et al*, 2008; Paradelo *et al*, 2009, 2010). Though beneficial, 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 organic matter itself or maybe due to the mineralization of native soil organic matter following increased microbial development and activity, a mechanism known as priming effect (Bustamante *et al*, 2010).

Composting is an aerobic process, performed at large-scale in windrows or piles (Hobson *et al*,

2005), or at local scale with a home composter (Andersen *et al*, 2011). Composting is used to treat manures, green wastes or municipal solid wastes and their fractions (Goyal *et al*, 2005). Vermicomposting, involving the presence of worms, is also used to treat organic wastes, such as organic household wastes or manure (Chan *et al*, 2011; Garg *et al*, 2006; Ngo *et al*, 2011). Some organic wastes, such as sewage sludge are co-composted with various additives that are either organic (Gabhane *et al*, 2012), mineral (Wong *et al*, 1995) or biological through the inoculation of micro-organisms (Wakase *et al*, 2008). Additives are used to enhance the composting process by reducing leaching (Steiner *et al*, 2010) and odour release (McCroory *et al*, 2001), improving compost aeration, or accelerating organic matter degradation (Sánchez-García *et al*, 2015) and improving nutrient content and availability (Gabhane *et al*, 2012) of the final product. Nevertheless, until now, few studies worked on carbon stabilization with additives to limit CO₂ production during composting (Bolan *et al*, 2012; Chowdhury *et al*, 2016) and none during vermicomposting. The reasons explaining this lack in the literature is that biogenic CO₂ is not accounted as GHG emissions (IPCC, 2014). However, approximately 50% of the initial waste carbon is lost through mineralization during composting (Tiquia *et al*, 2002), impacting in similar way as non-biogenic emissions the environment.

Stabilization mechanisms are poorly known for composting processes, while they have been widely studied in soils (Sollins *et al*, 1996). Understanding carbon stabilization in composts in order to enhance it could thus benefit from an analogy with the mechanisms known to occur in soils (von Lützow *et al*, 2006): spatial inaccessibility, selective preservation due to chemical recalcitrance, and formation of organo-mineral associations. Among these processes, the association of OM with minerals is the most efficient for carbon stabilization on long time scales (Kleber *et al*, 2015).

Therefore, the aim of this thesis is to investigate the influence of minerals on the decomposition of fresh organic matter during composting and vermicomposting and their potential effects on carbon stabilization at short and long terms. As an ecosystem engineer, worms could play a significant role in carbon stabilization. Several minerals, with different characteristics, have been tested, alone or combined: montmorillonite, a 2:1 clay; kaolinite, a 1:1 clay and goethite, an iron oxide. Other additives obtained from industrial wastes were also tested, red mud and fly ash. This work will specifically focus on the organo-mineral associations potentially formed during composting according to the type of minerals and worm's absence/presence (*Eisenia Andrei* and *Eisenia foetida*). Studying organo-mineral associations during composting process

with minerals has the advantage of constituting a simplified “model”: all initial parameters (characteristics of organic matter, properties of minerals, number of worms...) are controlled. Therefore, impacts of worms on organo-mineral associations may be easier to observe than in a complex soil environment. Finally, the physical and chemical carbon protection potentially induced by minerals and worms during composting will be assessed after their use as organic amendments in soil.

Another aim of this thesis is to investigate the use of these new composts and vermicomposts as potting media and thus, their effect on plant development. Composting and vermicomposting recycle wastes into a suitable and marketable organic fertilizer. In this way, adding minerals to a compost pile in order to limit carbon emissions must not degrade the quality of the finished product. In the present study, investigating how minerals and worms influenced the quality and maturity of the end-product is achieved by combining chemical methods (nutrient contents, heavy metals...) with plant growth experiments.

The present work is divided into 6 parts. The first chapter is a state-of-the-art on composting, vermicomposting and how the use of additives can improve these processes. In order to understand how worms and minerals can affect the decomposition/preservation of organic matter during composting, the mechanisms of carbon stabilization in soils are also presented and discussed in this chapter. The second chapter focuses on the carbon stabilization during the composting process and GHG emissions released in the presence of minerals, with or without worms. The third chapter describes the quality and maturity of the co-composts, in a perspective of use as soil amendment or as potting media. The fourth chapter is dedicated to soil application of co-composts and focus on the soil properties modified by the application of these organic amendments. In the fifth chapter, plant development obtained with compost and vermicompost produced with montmorillonite is characterized. Finally, the last chapter assess, in a perspective of developing a low cost and feasible process, the influence of two industrial wastes on composting and vermicomposting (red mud and fly ash).

Chapter 1

-State of the art-

Improving the environmental footprint and properties of compost and vermicompost through mineral, organic and biological additives.

This chapter is based on the article “Improving the environmental footprint and properties of composts and vermicomposts through mineral, organic and biological additives. A review”, submitted to Agronomy for Sustainable Development

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1. Principle and differences of two waste management strategies: composting and vermicomposting

1.1. Definition of the composting process

According to Zucconi and Bertoldi (1987), composting can be defined as a bio oxidative process leading to organic matter (OM) mineralization and transformation. The end-product is considered as stabilised and free of phytotoxic agents and pathogens (Wichuk, and McCartney, 2007). Composting typically consists of 3 phases: initial activation, thermophilic and maturation phase (**Fig.1**). The initial activation generally lasts 1-3 days during which simple organic compounds such as sugars are mineralised by microbes, producing CO₂, NH₃, organic acids and heat (Bernal et al, 2009). During this phase, the temperature in the composting pile increases. Thereafter, during the thermophilic phase, the temperature reaches a maximum. The optimum temperature range for composting is 40-65°C (de Bertoldi *et al*, 1983), allowing to kill pathogens above 55°C. During this phase, thermophilic microorganisms degrade fats, cellulose and lignin (Bernal et al, 2009). Finally, during the mesophilic phase or maturation, temperature slowly decreases due to a reduction of the microbial activity associated to a decrease of biodegradable compounds. Composting involves a succession of microorganisms (Mehta *et al*, 2014), according to the temperature of the composting pile. For instance, fungi are not present at temperature above 60°C, when bacteria predominate (Klamer and Bååth, 1998). Thus, the relative abundance of specific microorganisms and temperature are good indicators of the compost evolution.

Biogenic CO₂ emissions during composting derive from organic matter aerobic decomposition and CH₄ oxidation by aerobic methanotrophic bacteria (Sánchez *et al*, 2015). CO₂ emissions are the most important emissions during composting (Haug, 1993). CH₄ emissions are mostly recorded during the initial and thermophilic phases (Beck-Friis, 2000), due to the formation of anaerobic spots. N₂O emissions can occur during the whole process due to nitrification. NH₃ emissions may also occur during composting, generally during the first 2 weeks, then they decrease when the easily degradable materials are exhausted and the degradation rate decreases (Morand *et al.*, 2005; Szanto *et al.*, 2007).

The composting process and the quality and maturity of the compost are controlled by several parameters influencing the microbial activity, such as the initial pH and C:N ratio of the feedstock, their particle size and their distribution, as well as the aeration and moisture control

of the compost pile (Bernal *et al*, 2009, Onwosi *et al*, 2017).

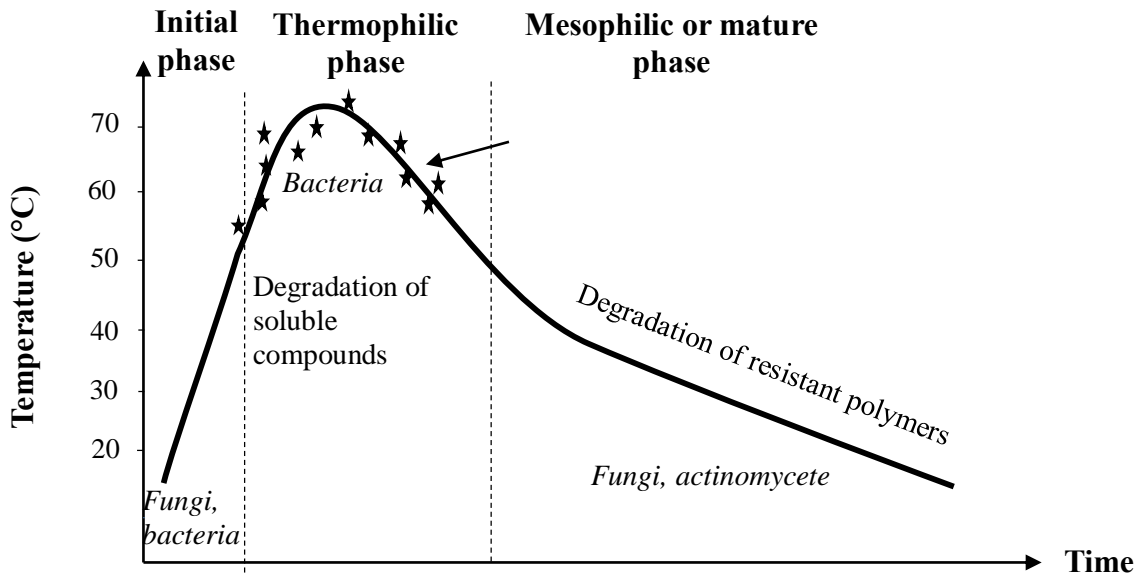


Figure 1 Temperature changes during composting, and corresponding microbial communities and degraded compounds. Stars indicate the phases when the removal of pathogen microorganisms takes place.

1.2. Definition of the vermicomposting process

During vermicomposting, the degradation of organic matter is enhanced by the presence of worms. This process becomes popular due to its low cost and efficient transformation of organic wastes (Dominguez *et al*, 2010). Furthermore, the end-product is often reported to be characterised by a higher stability against microbial degradation (Lazcano *et al*, 2008) and a higher nutrient content, in forms that are more available for plants as compared to a regular compost (Dominguez and Gomez-Brandon, 2013; Ghosh *et al*, 1999).

During vermicomposting, a bio-oxidative process as well, worms intensively interact with microorganisms (**Fig.2.**), thus accelerating organic matter degradation (Dominguez *et al*, 2010). Worms change the physical and chemical properties of wastes by ingesting fresh organic matter, thus increasing its accessibility to microorganisms and microbial degradability and also the rate of decomposition. Vermicomposting consists of 2 phases: first, worms, by physically altering organic matter, enhance the microbial activity and accelerate organic matter decomposition (Lores *et al*, 2006; Aira *et al*, 2007); second, when readily assimilable compounds have decreased, worms feed on micro-organisms, decreasing the organic matter mineralization and stabilizing the end-product.

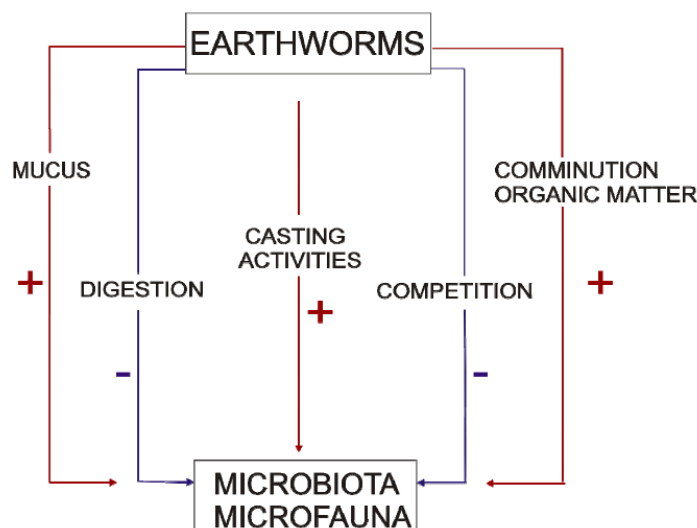


Figure 2 Positive (+) and negative (-) effects of earthworms on microbiota and microfauna (from Dominguez *et al*, 2010).

During vermicomposting, temperature ranges from 25 to 37°C, depending on the worm species and generally, worm activities and development are negatively affected by higher or lower temperatures.

Four species, two tropical and two temperate, are extensively used in vermicomposting facilities: *Eudrilus eugenia* (Reinecke *et al*, 1992; Kurien and Ramasamy, 2006), *Perionyx excavates* (Suthar and Singh, 2008), *Eisenia andrei* and *Eisenia fetida* (Kaushik and Garg, 2003; Garg *et al*, 2006). These species have been classified as epigeic worms and correspond to worms which mainly feed on fresh organic matter as found in forest litter, manure and compost (Bouché 1977). The main characteristics of these species are summed up in the **Table 1**.

Most vermicomposting facilities and most studies are using the worms *Eisenia Andrei* and *Eisenia fetida* due to their high rate of consumption, digestion and assimilation of OM, adaptation to a wide range of environmental factors, short life cycles, high reproductive rates and endurance and resistance to handling (Domínguez and Edwards, 2011).

Combining vermicomposting to composting favours the removal of pathogen microorganisms and improves the OM decomposition rate (Frederickson *et al*, 1996, 2007; Wang *et al*, 2014). Wastes are composted during a few weeks, until the end of the thermophilic phase. Then, after some days at high temperature, pre-mature compost is cooled by spreading it on vermicomposting beds (thin layers).

Vermicomposting produces GHG, as well as composting. Worm activity accelerates and enhances organic matter decomposition leading to higher CO₂ emissions than those of regular composting (Nigussie *et al*, 2017; Chan *et al*, 2011). The N₂O emissions are either increased

(Hobson *et al* 2005) or decreased (Wang *et al* 2014; Nigussie *et al* 2017) with vermicomposting compared to composting, probably depending on the feedstock materials.

As already noted for composting, the vermicomposting parameters must be controlled because they influence the rate of decomposition, the quality of the end-product and the GHG emissions. In addition, for vermicomposting, worm mortality must be avoided, by controlling in particular the temperature and the moisture content.

The parameters controlling both composting and vermicomposting may be influenced by the addition of mineral, organic or biological substrates (Suthar, 2009). For vermicomposting, the choice of additives is also driven by their possible negative impacts on worm development (Malinska *et al*, 2016).

Table 1 Main characteristics of the species used for vermicomposting (Dominguez and Edwards, 2011)

	Tropical species		Temperate species	
	<i>Eudrilus eugeniae</i>	<i>Perionyx excavatus</i>	<i>Eisenia andrei</i>	<i>Eisenia fetida</i>
Color	Reddish brown	Reddish brown	Brown and buff bands	Red
Size of adult earthworms	5-7 mm x 80-190 mm	4-5 mm x 45-70 mm	4-8 mm x 50-100 mm	4-8 mm x 50-100 mm
Mean weight of adults	2.7 - 3.5 g	0.5 -0.6 g	0.55 g	0.55 g
Time to maturity (days)	40- 49	28-42	28-30	21-28
Number of cocoons day -1	0.42 - 0.51	1.4	0.35-0.5	0.35-0.5
Number of worms cocoon - 1	2-2.7	1-1.1	2.5-3.8	2.5-3.9
Life cycle (days)	50 - 70	40 - 50	45-51	45-51
Limit and optimal temperature	25°C (16°C-30°C)	25°C-37°C	25°C (0°C-30°C)	25°C (0°C-30°C)
Limit and optimal moisture	80% (70% - 85%)	-	80%-85% (70%-90%)	80%-85% (70%-90%)

1.3. Advantages and disadvantages of these processes

Composting and vermicomposting are both efficient strategies to recycle organic matter. According to the literature, the use of compost or vermicompost as a potting media improves soil health, plant growth and is therefore an alternative to synthetic fertilizers (Jouquet *et al*, 2010; Arancon *et al*, 2008; Paradelo *et al*, 2012). However, the presence of worms during the process generally leads to a product richer in bioavailable nutrients. Ghosh *et al* (1999) observed that the transformation of phosphorus into available forms was considerably higher in vermicompost than in a regular compost. These results underline the fact that worms are able to increase the availability of nutrients by decreasing their fixation into insoluble organic forms. Inversely, during vermicomposting, the heavy metals are more efficiently removed from the organic material than during composting. For instance, He *et al* (2016) observed that worms

could regulated Zn and Cu within their tissues, increasing the exchangeable fraction and decreasing soluble forms of non-essential elements such as Cr and Pb. Similar results were obtained by Wang *et al* (2013) who concluded that vermicomposting was more efficient than regular composting to decrease the metal bioavailability. Thus, worms seemed to bioaccumulate heavy metals within their tissue, reducing the metal concentrations in the compost. Although composting is a friendly technology to recycle organic matter, this process releases significant amounts of greenhouse gases such as N₂O, CH₄ and CO₂. Generally, a composting pile with a low aeration rate release more CH₄ than a well-aerated composting pile (Jiang *et al*, 2013). Moreover, presence of worms during composting affects the GHG emissions. For instance, Chan *et al* (2010) and Luth *et al* (2011) indicated that the total GHG emissions were the lowest during vermicomposting compared to aerobic composting. Similar results have been observed by Komakech *et al* (2015) who found that vermicomposting process caused 78% lesser GHG emissions than composting. These results are mainly due to the fact that worm activities allow a constant aeration of the organic matter and therefore decrease N₂O and CH₄ emissions. However, several authors showed that N₂O emissions increased with worms (Hobson *et al* 2005) while others observed the opposite trends (Wang *et al* 2014; Nigussie *et al* 2017), probably due to the different composted materials. Finally, CO₂ emissions are generally higher during vermicomposting than composting due to high worm and microbial activities (Nigussie *et al*, 2017; Chan *et al*, 2011). Composting and vermicomposting allow to transform organic wastes into organic fertilizers, directly applicable to soil or as potting media. Nevertheless, one of the major problems with the application to soil of compost and vermicompost is their relatively fast rate of degradation. Coupled with the priming effect involved by their addition to soil (Thangarajan *et al*, 2013), and the biological degradation during composting, this technology may become more a source of carbon than a sink (**Fig.3**).

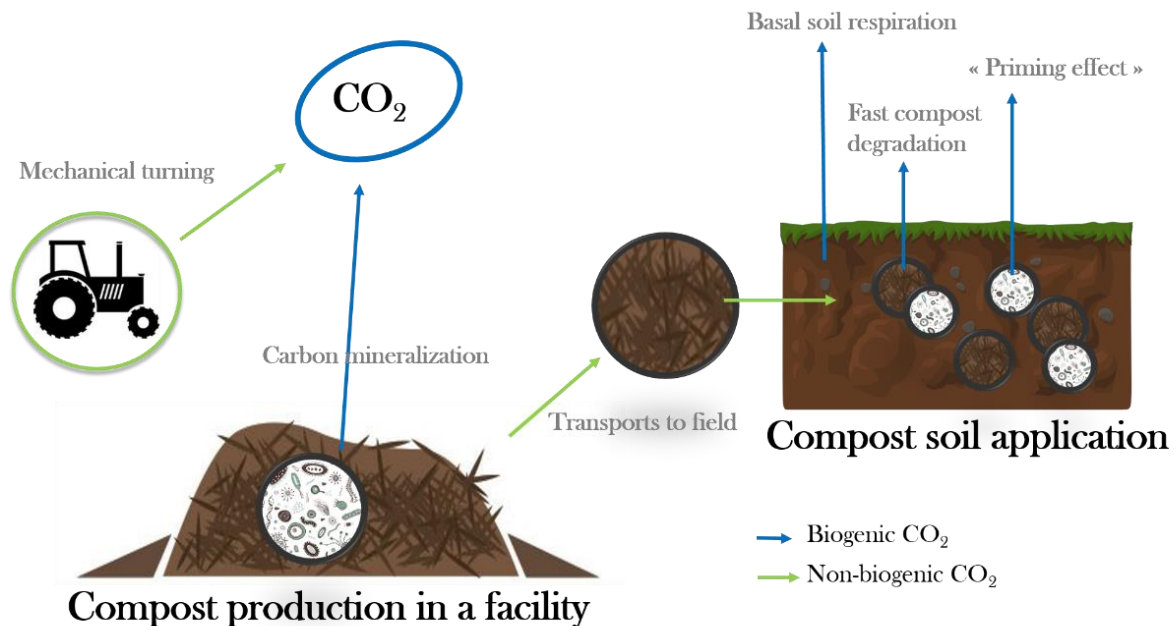


Figure 3 Biogenic and anthropic origins of CO₂ from the composting process, from the waste to the soil application

2. Optimizing composting processes by using additives

2.1. Different types and sources of additives

A wide variety of additives are used during composting and vermicomposting (**Fig.4**). Additives can be organic, mineral or only microbial (*i.e.* inoculum). The main purpose of their use in a compost pile or vermicompost bin, is to enhance the process and/or the finished product (Bernal *et al*, 2009; Onwosi *et al*, 2017). Additives which are used to increase the aeration and porosity during composting are usually named as bulking agent. Bulking agents are needed to provide structural support when the composting materials are too wet as sewage sludge. Other additives are used to equilibrate the initial parameters of the feedstock (pH, C: N ratio, nutrient

contents...), to absorb heavy metals or to limit GHG emissions and potential odours from

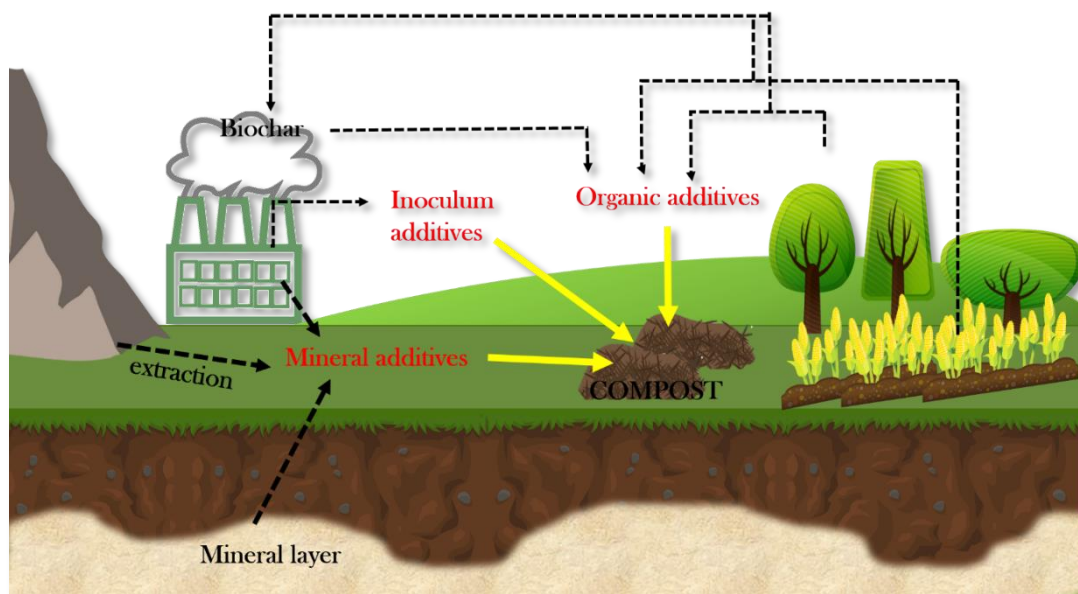


Figure 4 The different sources of the additives used for composting. Mineral additives can originate from mineral deep soil layer or quarries, organic additives can originate directly from agricultural residues or biological wastes or can be transformed into biochar and biological additives (inoculum) are generally industrial products.

composting.

A wide variety of additives, classified in three categories (mineral, organic or biological) may be used during composting and vermicomposting (**Fig.4**) in order to enhance the process and/or the quality of the final product (Bernal *et al*, 2009; Onwosi *et al*, 2017). A non-exhaustive list of the different additives used is given in **appendix 1**.

Biological additives refer to microorganisms, which are inoculated to a compost or vermicompost pile. These microorganisms are generally isolated from composts during the thermophilic phase, cultivated and sold as a commercial solution. According to Manu *et al* (2017), effective microbes (EM), and vertical transmitter (VT) bacteria are usual commercial additives. Few manufacturers reveal the identity, fate and functions of the microbes present in the commercial solutions. However, in most of the commercial microbiological additives, *Alcaligenes*, *Bacillus*, *Clostridium*, *Enterococcus* and *Lactobacillus* microorganisms are present (Sasaki *et al*, 2006; Wakase *et al*, 2008). These microorganisms are usually involved in the ammonia assimilation occurring during composting (Wakase *et al*, 2008).

Organic additives cover a large variety of products (**Fig.4**), from residual straws to mature composts: refuse from green waste compost screening, grass clippings (GC), crushed hardwood materials, crushed wood pallets, bark and corn stalks (Doublet *et al.*, 2011). When choosing

organic additives, attention must be paid to the C:N ratio of the initial mixtures, to ensure OM degradation and prevent N leaching during composting (Doublet et al., 2010). Furthermore, biochar has recently received great interest as soil amendment (Lehmann and Joseph., 2015) or as highly stabilised organic additives for composting (Dias *et al*, 2010). Biochar might enhance the soil health in terms of fertility and structure (Atkinson *et al*, 2010) and on the long term, biochar might sequester carbon (Lehman, 2006; 2007), thus mitigating climate change. Biochar is the by-product of biomass pyrolysis technologies used to produce energy. A great variety of biochars are produced, with different properties depending on the initial feedstock and pyrolysis temperature (Zhao *et al*, 2013).

Finally, inorganic or mineral additives mainly refer to lime, clays or industrial wastes, for example red mud or fly ash. Red mud is a by-product of the industrial alumina production (Wang et al, 2008) and fly ash is a waste product of clean-coal combustion used to mitigate gaseous emissions (*eg.* used in power plants). Therefore, the main advantages of these alkaline materials are their high availability (Gomes *et al*, 2016) and their low cost as industrial wastes. Minerals, such as zeolite became popular in the last decades due to their physical and chemical properties to adsorb heavy metals, in particular in soils and water purification systems (Lopez *et al*, 1998). Pure zeolite is easily synthesised by the slow crystallization of a silica-alumina matrix. Finally, pure minerals such as clay, extracted from quarries or soils, are increasingly used with the aim of reducing greenhouse gas emissions during composting (Bolan *et al* 2012; Barthod *et al*, 2016).

2.2. Effects of additives on the composting processes

During composting, the naturally developing microbial community can be affected by the addition of exogenous material, directly by adding inoculum or indirectly for other additives through their effects on the aeration, temperature, moisture content, pH, nutrient sources, etc.

2.2.1. Effects of additives on composting temperature profile

The temperature profile during composting, a good indicator of the microbial activity (Haug, 1993), can change due to the presence of additives. Additives have been shown to stimulate the microbial activity, leading to an earlier start and a longer duration of the thermophilic phase as compared to regular composting (Fang and Wong, 1999; Chen *et al*, 2010; Gabhane *et al*, 2012; Himanen and Hänninen, 2009; Jiang *et al*, 2014). Rapid temperature increases were observed following the addition of 6% of bamboo charcoal during pig manure composting (Chen *et al* 2010), 5 and 10% of biochar during poultry manure composting (Czekala *et al*, 2016), 1-2% of

zeolite during pig slurry composting (Venglovsky *et al*, 2005) or with jaggery (palm sugar) and polyethylene glycol during green waste composting (Gabhane *et al*, 2012). Direct microbial inoculation or addition of mature compost also led to similar temperature profiles (Manu *et al*, 2017) due to the increase of microbe amount and activity. However, some additives such as bentonite (Li *et al*, 2012), phosphogypsum and lime (Gabhane *et al*, 2012) do not have any effect on temperature profile, suggesting no stimulatory or inhibitory effect on microbial biomass.

The temperature profile is also important to assess the removal of pathogens from the finished compost, which requires high temperature. In this purpose, Gea *et al* (2007) preconize to add a 5 mm bulking agent in a 1:1 ratio to the sludge to ensure an optimal performance of the composting and remove pathogen.

2.2.2 Additives to stimulate microbial activity

The contents of the additives in nutrients and readily available forms of carbon influence the microbial activity. For example, adding jaggery increased the number of microorganisms and thus enhanced the enzymatic degradation of cellulose during composting of green wastes (Gabhane *et al*, 2012). Similar results were observed with the addition of fish pond sediment, spent mushroom substrate and biochar (Zhang and Sun, 2014; 2017). The effect of biochar on the microbial activity was probably due to their effect on microbial habitat and protection from grazers (Meng *et al*, 2013; Wei *et al*, 2014). Alkaline substrates such as fly ash and lime are also additives with high contents in nutrients, however they do not favour microbial activity due to their high pH (Fang *et al.*, 1999). At high rates, fly ash addition inhibited phosphatase, B-glucosidase or dehydrogenase but did not affect urease activity, indicating that some stages of N-compound degradation were unchanged (Wong *et al*, 1997).

2.2.3. Additives to improve aeration

An optimal aeration during composting is required as explained by Gao *et al* (2010). A low aeration rate might lead to anaerobic conditions while a high aeration rate might result in excessive cooling and thus preventing thermophilic conditions. The common way to enhance the aeration and thus favour the microbial activity during composting is through mechanically turning the composted material (Chen *et al*, 2015; Chowdhury *et al*, 2014; Manu *et al*, 2017) and forced aeration through pipes (Ogunwande and Osunade, 2011). These actions are not necessary in the case of vermicomposting due to worm activity. The costs of the pile turning or

forced aeration may be reduced by the use of bulking agents, such as biochar, residual straws, wood chip or sawdust, crushed branches, etc. since they enhance the natural aeration and porosity of the composting pile (Kulcu and Yaldiz, 2007; Czekala *et al*, 2016). Due to their numerous pores and low moisture content, these bulking agents support the formation of inter and intraparticles voids (Iqbal *et al*, 2010). Moreover, according to De Bertoldi *et al* (1983), the biological oxidation rate is directly related to the surface area exposed to the microbial attack.

2.2.4. Additives to regulate the moisture content

The moisture content during composting influences the oxygen uptake rate and thus, the microbial activity and the degradation rate. The optimal water content for organic matter biodegradation has been established within 50-70% (Richard *et al*, 2002). However, some organic wastes have a higher or lower moisture content; for example, sewage sludge, which has a moisture content ranging from 80% to 90%. Such high humidity might favour odour production during composting (Jolanun *et al*, 2008). The bulking agents commonly chosen to offset the high moisture content of these organic wastes are fibrous materials with low moisture content (Miner *et al.*, 2001, Eftoda and McCartney, 2004, Doublet *et al.*, 2011), which can absorb part of the leachate. The leachate absorption by cornstalk, sawdust or spent mushroom substrate was also observed by Yang *et al* (2013) during kitchen waste composting. Furthermore, Chang and Chen (2010) showed that increasing water absorption capacity by increasing sawdust addition during food waste composting also resulted in higher degradation rate due to more air flow through the particles.

On the contrary, water loss during the first days of composting might delay the composting process and necessitates water sprinkling. The addition of an additive with a capacity of water retention, such as clays, might limit water losses. Li *et al* (2012) showed that the initial moisture decrease was buffered by the presence of bentonite, due to its swelling performance. During composting of green wastes, the water holding capacity was also increased by the addition of ash (Belyaeva and Haynes, 2009), or phosphate rock (Zhang and Sun, 2017). However, some additives such as egg-shells had no influence on water holding capacity (Soares *et al*, 2017), and may even have a negative effect on the biological activity.

2.2.5. Additives to buffer pH

The pH varies during composting with a decrease during the early stages of composting and an increase during the later stages (Onwosi *et al*, 2017), which affects the microbial activities. Some additives are used to increase the pH and thus enhance the composting of acid feedstocks,

such as food waste (Wong *et al*, 2009). The addition of an inoculum consortium was reported to result in a pH increase from 4.3 to 6.3 during food waste composting (Manu *et al* 2017). This may be explained by the degradation of acids along with organic matter through enhanced biological activity. The use of bulking agents such as bagasse, paper, peanut shell and sawdust might also increase the pH during composting (Iqbal *et al* 2010). Fly ash or lime amendments also increased the initial pH of the co-compost (Fang and Wong, 1999; Gabhane *et al*, 2012), but such alkaline amendments might inhibit the metabolic activity. Wong *et al* (1997) observed less thermophilic bacteria in the initial phase when sludge was co-composted with 25% fly ash, demonstrating the adverse effect of alkaline amendments on bacteria.

The increase of pH during the thermophilic phase was offset by using bamboo charcoal, that adsorbed ammonia, and inhibited OH⁻ release (Chen *et al*, 2010). Similar results were observed by Venglosky *et al* (2005) with the addition of 2% zeolite to pig slurry composting. A lower pH may also decrease nitrogen loss, by avoiding ammonia volatilization, which occurs at high pH (Chen *et al* (2010). Finally, the addition of elemental sulphur considerably decreased the pH during poultry manure composting (Mahimairaja *et al*, 1994), mainly explained by the oxidation of elemental sulphur producing H₂SO₄, and thus increasing the H⁺ ion concentration.

4. Influence of the additives on gas emissions during composting

4.1 Odour emissions

NH₃ and sulphur are the main odorous gases emitted during composting. No reference has been found in the literature on vermicomposting and odour gas emissions, likely due to the fact that worm activities allow a continuous aeration of the composting pile, which limits formation of anaerobic zones responsible for odour emissions. By improving oxygen transfer inside the composting pile of municipal wastes with the addition of rice straw (1:5), Shao *et al* (2014) decreased the cumulative malodorous sulphur-containing gases. Koivula *et al* (2004) obtained similar results with ash addition and Steiner *et al* (2010), Hua *et al* (2009) and Khan *et al* (2014) with biochar addition. Another way to reduce the odour emissions is to trap the nitrogen excess and thus reduce the ammonia contents in the composting pile. The addition of natural zeolite as an adsorbent appears to reduce ammonia odours (Turan, 2008), Wang *et al* (2014), Lefcourt and Meisinger (2001), with increasing effect on odour reduction when more zeolite is added. Mahimairaja *et al* (1994) confirmed that carbonaceous materials with high cation exchange capacity reduced the cumulative loss of NH₃ due to the immobilization of NH₄⁺ and enhanced this process by acidifying compost with elemental sulphur. Bernal *et al* (1993) and Witter and Lopez-Real (1988) showed that zeolite placed on the top of the compost pile reduces ammonia

emissions. Zang *et al* (2017) observed that chemical additives (a nitrogen electron acceptor, sodium nitrate and sodium nitrite) efficiently controlled sulphur odours, by reducing the emissions of dimethyl sulphide and dimethyl disulphide by 92.3% and 82.3% respectively, without altering the composting process. Similarly, Yuan *et al* (2015) efficiently reduced H₂S emissions with a chemical product (FeCl₃). Ren *et al* (2010) and Jeong and Hwang (2005) observed a reduction of the ammonia loss during pig manure composting due to a chemical reaction (struvite precipitation) with an absorbent mixture of magnesium hydroxide and phosphoric acid. By contrast, sawdust, cornstalk and spent mushroom substrates did not influence ammonia emissions (Yang *et al*, 2013; Yuan *et al*, 2015), while bentonite even increased them during swine manure composting (Jiang *et al*, 2014).

4.2.GHG emissions

The amounts of GHG released during composting of different initial feedstock with different additives are shown in **Table 2**. GHG emissions from co-composting processes have been largely studied but few data are available on vermicomposting processes and let alone on co-vermicomposting. Moreover, among GHG gases, only CH₄ and N₂O emissions during composting are accounted for in national GHG inventories, but CO₂ of biogenic origin is not (IPCC 2014).

N₂O can be produced under aerobic conditions, during incomplete nitrification/denitrification and anaerobic conditions, when a lack of O₂ leads to nitrate accumulation. Additives affect differently the N₂O emissions according to the initial feedstock. For instance, adding mineral as phosphogypsum (Hao *et al*, 2005; Luo *et al*, 2013) significantly reduced N₂O emissions during manure composting, probably by increasing SO₄²⁻ content of the compost or affecting the nitrification process while there is no effect of the bulking agent (woodchip and polyethylene tube) on N₂O emissions during municipal wastes composting (Maulini-Duran *et al*, 2014). Finally, during kitchen waste composting, addition of sawdust significantly reduced N₂O emissions (Yang *et al*, 2013).

In a composting pile, CH₄ emissions generally occurred due to a lack of oxygen, an excessive moisture or the presence of anaerobic zones (Amlinger *et al*, 2008). Contrary to N₂O emissions, CH₄ emissions appear to be more dependent on the properties of the additives than those of the initial feedstock. Thus, to reduce CH₄ emissions, two types of additive might be applied, affecting directly or indirectly the carbon cycle: (1) inoculation of methanotrophic bacterium

(Luo *et al*, 2014) might enhance CH₄ oxidation and thus reduce CH₄ emissions; (2) addition of bulking agents might decrease anaerobic zones. For instance, recent studies (Yang *et al*, 2013; Maulini-Duran *et al*, 2014) observed that addition of bulking agents as cornstalk, sawdust, spent mushroom substrate or even polyethylene tube improved the compost structure, decreased the moisture content and thus the CH₄ emissions. Moreover, cornstalk appeared more efficient than sawdust and spent mushroom substrate due to its larger particle size (Yang *et al*, 2013). However, some organic bulking agents such as woodchip (Maulini-Duran *et al*, 2014) had the opposite effect, generating even more CH₄ than a regular compost. The authors explained these results by a rapid consumption of easily biodegradable matter, leading to the formation of compact zones.

Compared to regular composting, CO₂ emissions are generally increased by the addition of organic materials with readily decomposable carbon (paper, straw, peat materials, etc) (Mahimairaja *et al*, 1994), and decreased by the addition of organic materials rich in lignin or other slowly degrading organic compounds (Mahimairaja *et al*, 1994; Chowdhury *et al*, 2014). Biochar addition during composting has contradictory effects on CO₂ emissions compared to regular compost, either increasing (up to 8% in Czekala *et al* 2016), or decreasing them (Chowdhury *et al* 2014). Such contradictory effects were also observed for biochar addition during vermicomposting (Barthod *et al*, 2016). Addition of other relatively inert materials, such as plastic or pumice increased CO₂ emissions, through aeration improvement and microbial activity enhancement (Czekala *et al*, 2016; Wu *et al*, 2015). Finally, reducing CO₂ emissions from composting without altering the biodegradation process appears difficult. A possible method to limit CO₂ emissions is to trap emitted CO₂ with an adsorbent, such as red mud or to protect carbon from decomposition by associating it with clays or amorphous hydroxyl-Al (Bolan *et al*, 2012; Barthod *et al*, 2016; Haynes and Zhou, 2015).

Table 2 GHG emissions released during composting processes.

Initial feedstock	Process	Bulking agent	Duration (days)	Gaseous emissions (reported on 100 days)			Reference
				CO ₂ (g/kg)	N ₂ O (g/kg)	CH ₄ (g/kg)	
Straw-bedded cattle feedlot manure	composting	cereal straw	99	166,67	9,01	0,078	Hao <i>et al</i> , 2004
Straw-bedded cattle feedlot manure	composting	wood ship	99	147,07	9,02	0,085	
sewage sludge	composting	cotton waste	105	239,77	-	-	Bernal <i>et al</i> , 1998
poultry manure, olive-mill wastewater	composting	cotton waste	105	200,89	-	-	
sewage sludge, olive-mill wastewater	composting	cotton waste	140	196,62	-	-	
sewage sludge	composting	maize straw	112	269,36	-	-	
sewage sludge, olive-mill wastewater	composting	maize straw	119	260,19	-	-	
pig slurry, poultry manure	composting	sweet sorghum bagasse	112	210,86	-	-	
city refuse	composting	sweet sorghum bagasse	133	179,31	-	-	
Duck manure	pre-composting +vermicomposting	reed straw	77	400,91	0,12	<0,01	
Duck manure	pre-composting +vermicomposting	reed straw and zeolite	77	405,84	0,14	<0,01	
Cattle manure	composting	Barley straw	31	300	0,08	1,19	Chowdhury <i>et al</i> , 2014
Cattle manure	composting	Barley straw	31	274,19	0,08	2,41	
Hen manure	composting	Barley straw	31	254,84	0,09	0,42	
Hen manure	composting	Barley straw	31	225,81	0,10	0,41	
Hen manure	composting	Biochar+ barley straw	31	200,00	0,08	0,09	
Hen manure	composting	Biochar+ barley straw	31	174,19	0,08	0,07	
animal manure+ food waste (75/25)	composting	Plastic tube (3:1)	28	225,71	0,02	0,08	
animal manure+ food waste (75/25)	composting	Platic tube (6:1)	28	233,21	0,02	0,13	
animal manure+ food waste (75/25)	composting	Woodchip (3:1)	28	171,43	0,03	0,12	
animal manure+ food waste (75/25)	composting	Woodchip (6:1)	28	178,57	0,05	0,14	
animal manure+ food waste (75/25)	composting	Biochar (3:1)	28	147,14	0,01	0,02	
animal manure+ food waste (75/25)	composting	Biochar (6:1)	28	162,14	0,01	0,04	
animal manure+ food waste (75/25)	composting	Barley straw (3:1)	28	179,64	0,01	0,08	
animal manure+ food waste (75/25)	composting	Lupin residues (3:1)	28	218,57	0,01	0,68	
Kitchen waste	composting	Sawdust	28	-	0,78	0,15	Yang <i>et al</i> , 2013
Kitchen waste	composting	Cornstalk	28	-	0,40	-	
Kitchen waste	composting	Spent Mushroom substrate	28	-	1,04	0,05	
Municipal wastes	composting	wood chip	24	-	<0,01	0,01	Maulini-Duran <i>et al</i> , 2014
Municipal wastes	composting	polyethylene tube	24	-	<0,01	<0,01	
Pig manure	composting	mature compost + cornstalk	30	-	0,69	2,22	Luo <i>et al</i> , 2016

3. Effects of application of co-compost on soil properties and plant development

3.1. General soil definition and soil “health” definitions

Soil can be defined as the fine layer (from some cm to 3-4 m) which is at the interface between the atmosphere and the lithosphere. Soil is difficult to describe as it the assemblage of micro and macroaggregates, composed of water, air spaces, organic matter and minerals. These aggregates are mainly formed by microbial activity or micro and macrofauna (Bossuyt *et al*, 2005). Mineral compounds originate from the rock alteration while organic matter comes from vegetal or animal organisms (exudates or decomposition). Thus, the organic matter in soil is complex as it comprises different compounds, under various forms. Soils are classified according to their structure and mineral composition. The mineral composition is generally linked to the climate and localisation (**Fig. 5**). Therefore, there is not one soil but several different soil types.

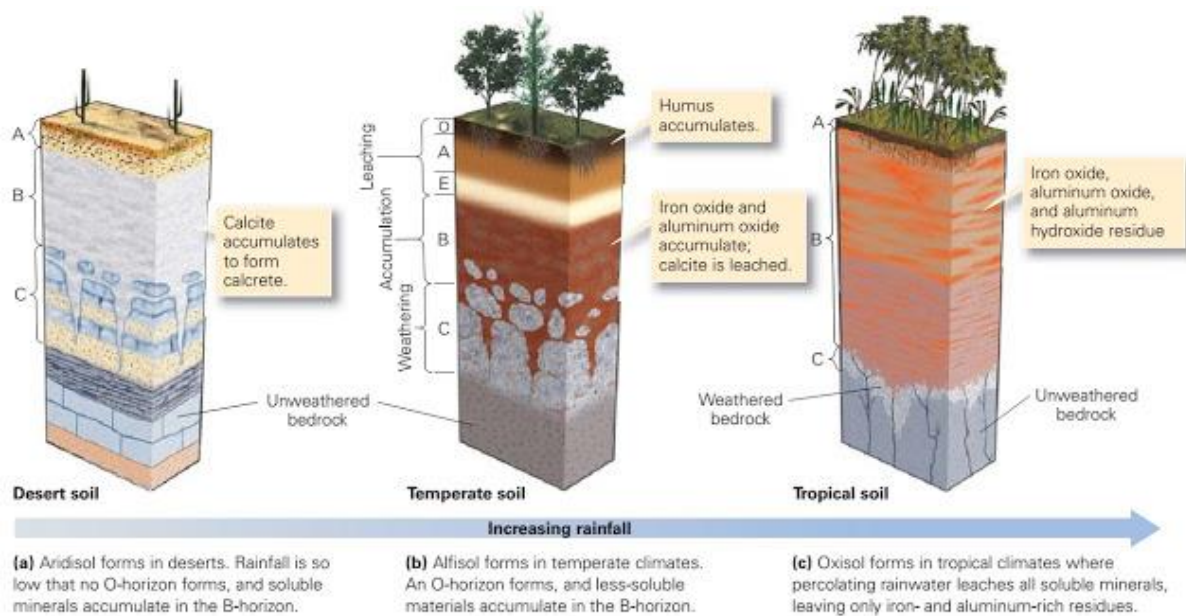


Figure 5 Different soil compositions, according to the localisation and climate on Earth. Figure from learninggeology.com

Soil health is defined as “the capacity of a specific kind of soil to function, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation” (Doran and Zeiss, 2000). Therefore, soil is at the heart of numerous ecosystem services and a “good” soil health is necessary to fulfil these services as shown by **Fig.6**.

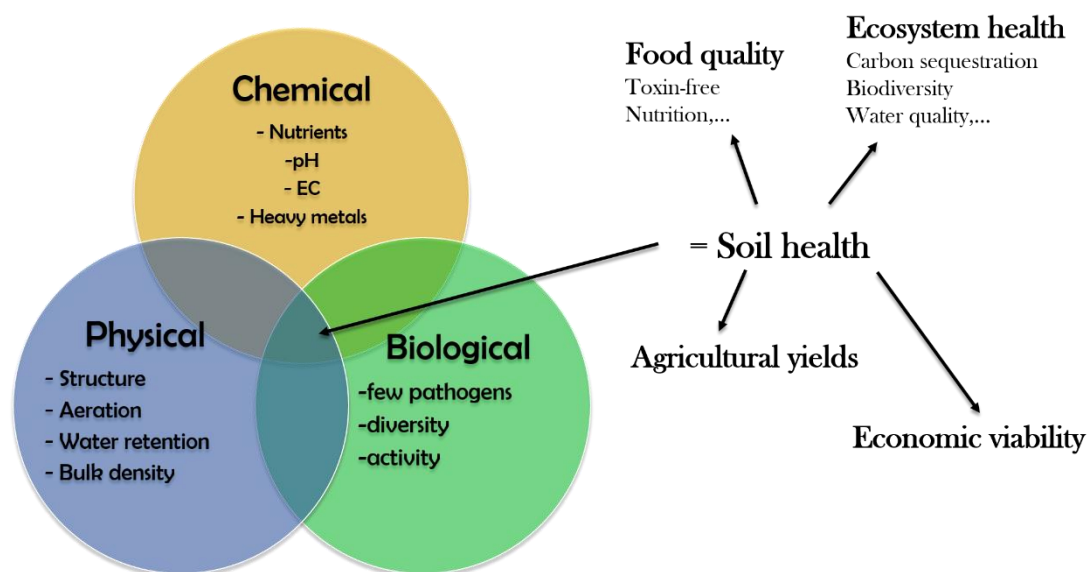


Figure 6 Definition of soil health

3.2. Qualities of co-compost as potting media or soil amendments: influences of additives on nutrient availability, metal availability and soil fertility.

Soil health and functions, such as fertility, can be improved by compost and vermicompost application, through their effects on soil structure and porosity, soil microbial biomass and soil nutrient contents (Thangarajan *et al*, 2013). In addition to soil application, compost and vermicompost are largely used as horticultural potting media (Zaller, 2007; Hashemimajd *et al*, 2004). The presence of heavy metals or high content of nutrients in composts might cause important adverse effects on animal and human health (Senesil *et al*, 1999). Therefore, the nutrient and heavy metal contents, as well as their respective availability and mobility in the compost are important parameters to control. Additives can be used to improve the agronomic qualities of compost related to their nutrient availability or metal mobility.

3.2.1. Reduction of environmental hazards due to heavy metals

Depending on the initial feedstock, heavy metals might be present in the composted material. During composting and vermicomposting, they can react with organic matter and their speciation might change. For instance, manure composts may contain high levels of Zn, Cd, Pb and Cu contents and their application on soils may lead to an excessive input of heavy metals to soil (Chen *et al*, 2010). Using additives during composting is a way to reduce heavy metal availability, resulting in a marketable, safe material. In this way, the addition of 9% of bamboo charcoal to pig manure reduced the mobility of Cu and Zn by 35% and 39% respectively (Chen

et al, 2010). Metal mobility was also decreased by chestnut/leaf litter addition to poultry manure (for Zn, Cd, Pb and Cu, Guerra-Rodriguez *et al* 2006) and by phosphate rock addition (5%) (Lu *et al*, 2014). Similarly, sewage sludge contains high amount of water-soluble heavy metals such as Cu, Pb and Mn, which may be transformed into poorly available forms (Wong *et al*, 2009; Fang and Wong, 1999) through addition of lime or zeolite during composting (Nissen *et al*, 2000; Zorpas *et al*, 2000; Villasenor *et al*, 2011). The decrease of the labile fractions of heavy metals in manure or sewage sludge is mainly due to the formation of organo-metal complexes between the additives and metals (Wang *et al*, 2013) or a complexation of the metal ions (Lu *et al*, 2014). Co-vermicomposting may be used to decrease heavy metal hazards, since worms act as bio-accumulators of heavy metals. During sewage sludge composting, worms improved the efficiency of additives such as fly ash or sawdust in increasing the exchangeable fraction of nutrients such as Zn and Cu and decreasing the soluble forms of non-essential elements such as Cr and Pb (Wang *et al.*, 2013).

The application of co-compost to soil may also reduce soil environmental hazards related to heavy metal polluted soils. In this purpose, Zhou *et al* (2017) recommended the application of co-compost (obtained with red mud) on polluted soils to decrease the ecological risk of heavy metals, by efficiently reducing soil heavy metal availability and also increasing soil microbial biomass.

3.2.2. Influence of additives on the nutrient contents and availability

The major possible negative effect of additives in composting or vermicomposting is a decrease of the nutrient content due to dilution (Banegas *et al*, 2007) or a decrease of nutrient availability due to adsorption on mineral additives. In some cases, the adsorption of excess nutrients from compost is beneficial to avoid environmental risks, such as eutrophication, after compost application to soil. Compost soluble P contents were decreased compared to regular composting by the addition of coal fly ash to sewage sludge, due to the formation of insoluble phosphate compounds (Fang *et al* 1999; Menon *et al* 1993) and by the addition of alum and zeolite to dairy slurry or green waste, due to adsorption and to the formation insoluble Al-phosphate intermediates (Lefcourt and Meisinger, 2001; Belyaeva and Haynes, 2012). Chen *et al*, 2010 observed a 65% reduction of nitrogen leaching from manure compost with biochar additives, but Iqbal *et al* (2015) observed no effect of this additive on nitrogen leaching from yard compost. Iqbal *et al* (2015) suggest that only biochar produced at low temperature may be suited for leachate retention.

Inversely, some additives, such as phosphate rock (Nishanth and Biswas, 2008; Billah and Bano, 2015), lime and fly ash (Zhang *et al.*, 2017, Gabhane *et al.*, 2012) are helpful to increase nutrient contents of the final compost made from nutrient poor feedstocks. Nitrogen content increased in the presence of 2.5% bentonite during composting (Li *et al.*, 2012) or with both bacteria (N₂-fixing and P-solubilizing) and phosphate rock during vermicomposting (Busato *et al.*, 2012; Unuofin and Mnkeni, 2014). The use of branches, pallets and corn stalks as additives during sludge composting had a positive effect on N availability in the final composts by enhancing the N organisation and limiting N losses by volatilisation during composting (Doublet *et al.*, 2011). Moreover, in order to optimize nutrient availability in co-vermicomposting with phosphate rock, Unuofin and Mnkeni (2014) showed that up to 22.5 g worms kg⁻¹ increased the extractable phosphorus fractions. Their results suggest that for maximizing phosphorus availability, a high worm density should be considered.

Therefore, composts used as potting media or soil amendment can improve fertility through two mechanisms: (1) because they are rich in nutrients when they are made with additives rich in available nutrients or/and (2) because they increase microbial biomass, which play a pivotal role in nutrient cycling. In this way, some authors observed that application to soil of co-composts obtained with phosphate rock and bacteria inoculum (Meena and Biswas, 2015) not only resulted in increasing soil microbial biomass but also increasing microbial biomass phosphorus, inducing a high amount of available phosphorus.

However, these observations are highly dependent on soil properties as the availability of nutrients such as P or N, and their leaching differ according to the soil and climate. For instance, in acidic soils, P ions may precipitate, decreasing P availability (Hinsinger, 2001) while nitrogen contents decrease if the pH falls below 5 (Lucas and Davis, 1961). Thus, to improve acid tropical soil, it is preconized to apply a co-compost of wood ash to retain Al and increase the bio-availability of exchangeable cation (Bougnom *et al.*, 2009). Therefore, application of co-compost (rate and additives) should be adapted to the soil properties to improve soil health and avoid environmental risks (Roca-Perez *et al.*, 2009).

3.2.3 Co-compost stability and carbon sequestration potential of soil amended with co-compost.

The degree of degradation of organic matter in composts is an important parameter to consider for their use on soil (Francou *et al.*, 2005). A stable organic matter applied to soil will release less carbon emissions than an instable one (Bernal *et al.*, 1998). As the stability is related to the

composition in organic compounds (Dignac *et al*, 2005), presence of stable phenolic compounds and complex aromatic compounds may be used as indicators of the stability (Fukushima *et al*, 2009). Therefore, Manu *et al* (2017) and Wei *et al* (2007) suggested that higher contents of aliphatic carbon chains and carboxyl and hydroxyl groups in the compost produced with the addition of inoculum consortia compared to regular compost point to a more stable product. They also observed decreased contributions of cellulose, hemicellulose and lignin in the co-compost compared to the regular compost, indicating that organic matter transformation was facilitated by the microbial inoculum addition. Beside biological additives, Zorpas and Loizidou (2008) observed that the addition of sawdust and clinoptilolite (zeolite) during sewage sludge composting increased the percentage of NaOH extractable compounds in the final products and thus the number of oxygen containing functional groups. Finally, the addition of biochar during poultry manure composting also increased the degree of transformation of the compost (Dias *et al* 2010).

Soil can retain carbon on the long term, thus avoiding its mineralization and release in the form of CO₂ or CH₄. Soil contains about three times more carbon than the atmosphere (Eglin *et al* 2010), and carbon storage in soils might be one of the solution to mitigate climate change (IPCC 2014; Lal *et al* 2004), that can be favoured by agricultural practices adapted to soil and climate conditions (Dignac *et al.*, 2017). One strategy to increase soil carbon stocks is to apply compost or vermicompost to soils (Ngo *et al*, 2012). However, these organic amendments show a very fast degradation in soil due to the presence of a large amount of labile organic matter, consumed by soil microbial biomass and thus, might increase carbon emissions. Hundred years after soil application of compost, 2-10% of initial C from the amendment was still present in the soil (Favoino and Hogg, 2008). This estimation depends largely on the type of soil (Busby *et al*, 2007), on the pedoclimatic conditions and on the type of compost (Peltre *et al.*, 2012). For instance, the mineralization process in temperate countries takes place rather slowly while in tropical countries, the mineralization generally leads to a rapid decline of the added compost. Based on the estimation of Favoino and Hogg (2008), the carbon remaining in soil after application of various co-composts to soil was estimated (**Table 3**).

Table 3 Potential carbon sequestration after application of different compost and vermicomposts. Calculation of the carbon potentially remaining in soil are based on Favoino and Hogg (2008) estimation as follows: $C_{\min} = C_{\text{content}} * 2\%$ and $C_{\max} = C_{\text{content}} * 10\%$

Initial waste	Composting process	Additive used	C content in the end-product (mg g ⁻¹)	C _{min} potentially still remained after 100 years (mg g ⁻¹)	C _{max} potentially still remained after 100 years (mg g ⁻¹)	References
Sewage sludge	composting	Shredded rice straw	227	4,5	31,8	Roca-Perez <i>et al</i> , 2009
	composting	non-shredded rice straw	184	3,7	25,8	
Biowaste	composting	sulphate, oxide iron, magnesium manganese + zinc mixed with clay	458	9,2	64,1	Himanen <i>et al</i> , 2009
		mixture of calcium hydroxide, peroxide and oxide	446	8,9	62,4	
Alperujo	composting	grape stalk	470	9,4	65,8	Alburquerque <i>et al</i> , 2006
		olive leaf	484	9,7	67,8	
Green-waste		degradable plastic	453	9,1	63,4	Unmar and Mohee, 2008
		biodegradable plastic	338	6,8	47,3	
Poultry manure	composting	wheat straw +5% biochar	377	7,5	52,8	Czevelka <i>et al</i> , 2016
		wheat straw +10% biochar	385	7,7	53,9	
poultry manure	composting	barley waste	401	8,0	56,1	Guerra-Rodriguez <i>et al</i> , 2006
		chesnut burr/leaf litter	410	8,2	57,4	
Kitchen waste	composting	Phosphogypsum	292	5,8	40,9	Yang <i>et al</i> , 2015
		Superphosphate	280	5,6	39,2	
Biowaste	composting	15% wood ash	229	4,6	32,1	Juarez <i>et al</i> , 2015
Green-waste	composting	Fly ash	391	7,8	54,7	Gabhane <i>et al</i> , 2012
		Phosphogypsum	390	7,8	54,6	
		Jaggery	424	8,5	59,4	
		Lime	411	8,2	57,5	
		Polyethylene glycol	434	8,7	60,8	
Duck manure	vermicomposting	reed straw	236	4,72	33,04	Wang <i>et al</i> , 2014
		reed straw + zeolite	233	4,66	32,62	

Therefore, reducing the amount of labile carbon through the carbon stabilization in compost and vermicompost has recently raised interest (Barthod *et al*, 2016; Bolan *et al*, 2012; Chowdhury *et al*, 2014). To improve the carbon stabilization of the organic amendments, addition of minerals as goethite, montmorillonite or industrial wastes as red mud is assessed. Some authors observed that the presence of these minerals during composting allows to increase the half-life of the organic amendment carbon and thus increase soil carbon stocks without altering soil properties (Bolan *et al*, 2012). However, little is known on the mechanisms occurring during composting with these minerals and even less on those occurring during vermicomposting (Barthod *et al*, 2016). The main hypothesis proposed in these studies is that

carbon from fresh organic matter used for composting and vermicomposting is stabilized through similar mechanisms as in soil.

4. Stabilization of organic matter: mechanisms occurring in soil

4.1. Physical and chemical stabilization mechanisms

Organic matter can be stabilized in soils due to its intrinsic chemical resistance; its associations with minerals or oxide and its low accessibility due to physical occlusion (Six *et al*, 2002; Sollins *et al*, 1996, von Lützow *et al*, 2006). These three mechanisms are classified as chemical, biochemical and physical mechanisms, respectively.

4.1.1. Biochemical protection through organo-mineral associations

Minerals, present in soil, originate from the degradation and erosion of rocks. Thus, there is a large variety of minerals, which are distinguished by their structure and ion composition. **Fig. 7** shows the main clay minerals found in soil and their macro-scale differences.

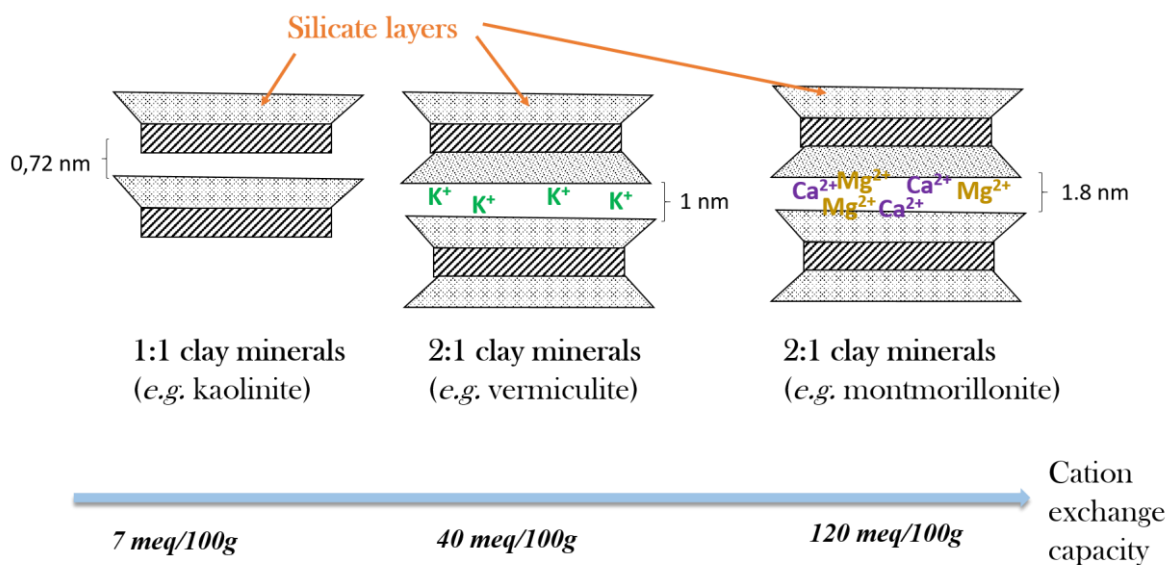


Figure 7 Example of clay minerals structures (kaolinite, vermiculite and montmorillonite) related to their cation exchange capacity.

Beside clays, iron or aluminium hydrous-oxides can also be found in soil. The specific surface area (SSA) is another parameter used to describe a mineral. This surface is more important in mineral which have two layers of silicate (vermiculite; montmorillonite) than those with one (kaolinite). The surface area of minerals is generally negatively charged and might interact with soil cations. In their study, Kleber *et al* (2007) proposed a conceptual model of organo-mineral interactions (**Fig.8**), showing that the influence of mineral surface on organic matter is not limited to the contact zone. In their model, the first molecules to sorb are thus the best

stabilized. Based on the SSA, previous studies (Mayer, 1994) have estimated that maximum carbon loaded onto mineral surface approximately represents 1mg of carbon per m². Therefore, it appears that, as 2:1 minerals offer a larger contact area for organic matter bonding, they create stronger bonds with organic matter than 1:1 minerals (Kleber *et al*, 2015). However, Sanderman *et al* (2014) investigated the importance of the mineralogy on organo-mineral associations and showed that the dominant driver of mineral retention and subsequent stability of organic matter is not linked to the SSA but to the mineralogy and surface chemistry. This result is in line with the observations of Kögel-Knaber *et al* (2008) suggesting that ligand exchange reactions are more stable than via cation/water bridging (Mikutta *et al*, 2007). Thus, the complexation of organic matter by Al and Fe is an important mechanism for the stabilization of organic matter (Kögel-Knaber *et al*, 2008; Mikutta *et al*, 2006). Finally, little is known on the relative effects of binary systems (hydrous oxides plus clay minerals) on organic matter bonding (Saidy *et al*, 2012).

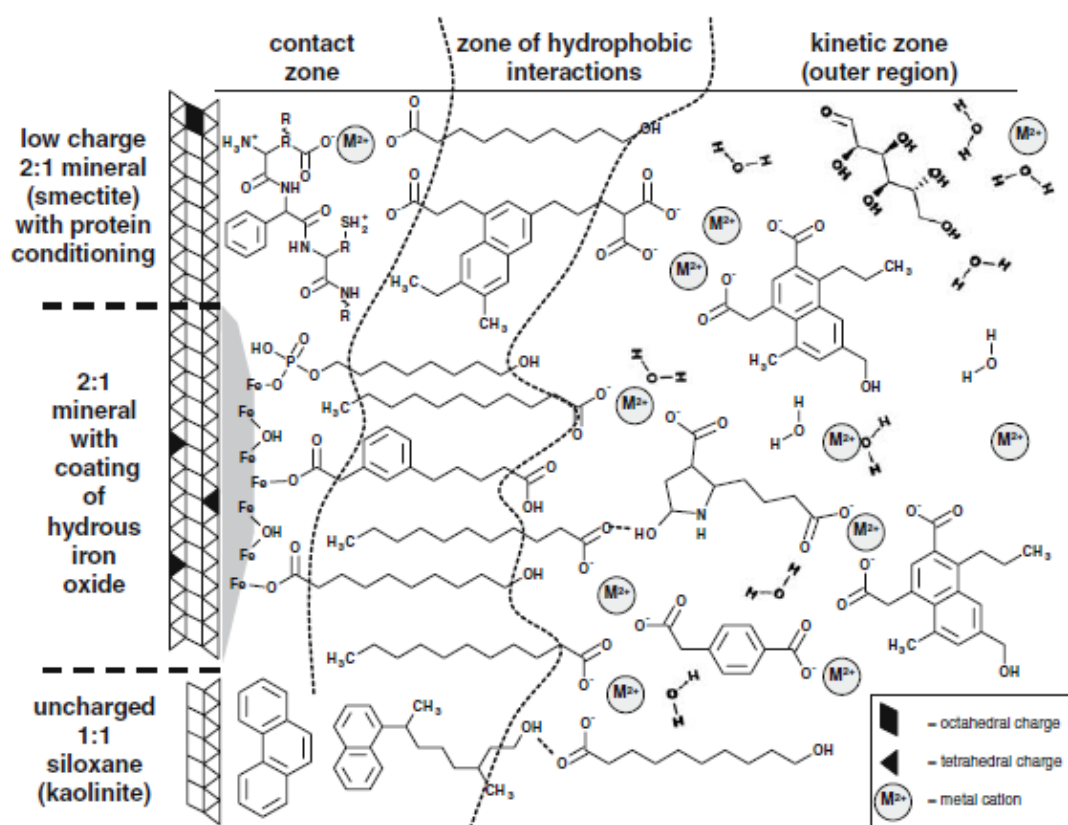


Figure 8 Zonal model of organo-mineral interactions from Kleber *et al* (2007): three types of minerals are represented: a low-charge smectite, a hydroxylated iron oxide and an uncharged mineral as kaolinite. In the contact zone, amphiphilic fragments are accumulated on charged surfaces through electrostatic interactions. Some hydrophobic organic compounds may also associate with noncharged mineral surfaces. The zone of hydrophobic interactions concerns the adsorbed organic molecules from the polar aqueous phase by a second layer of amphiphilic molecules. In the outer region or kinetic zone, further accumulation of organic molecular fragments is possible.

4.1.2. Physical protection through macro- and microaggregates

According to Sollins *et al* (1996), the accessibility is defined by “the location of organic substances as it influences their access by microbes and enzymes”. Soil aggregation is one of the parameters that directly impact the accessibility. Soil macro-aggregates are mainly formed due to root exudates, presence of binding agents as polysaccharides released by bacteria and fungi and mycorrhizal hyphae which tend to increase the aggregate stability (Beare *et al*, 1997). Macroaggregates is composed of micro-aggregates containing plant and fungal debris, clay microstructure, and mycorrhizal hyphae. A schematic structure of a soil macroaggregate is shown on **Fig.9**.

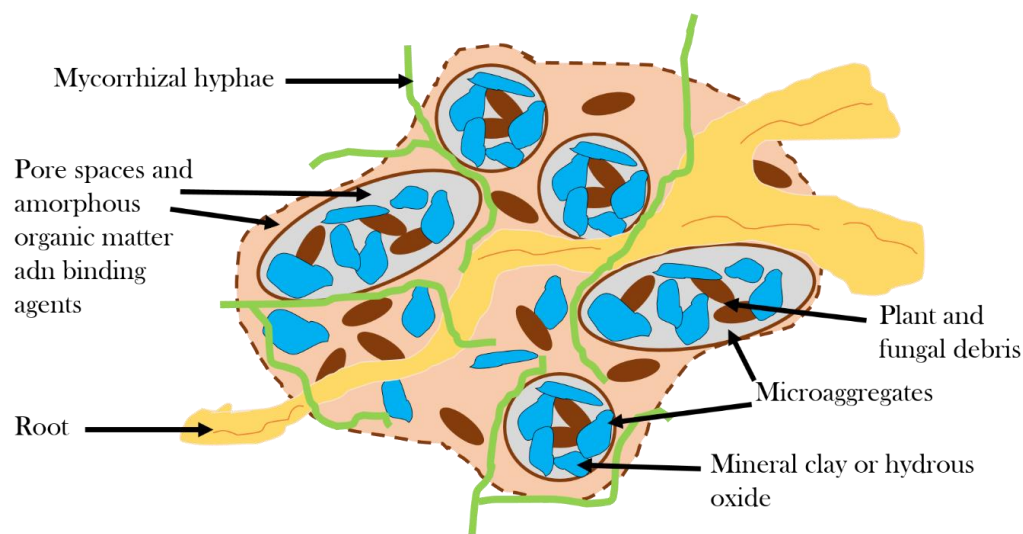


Figure 9 Schematic representation of a macroaggregate containing microaggregates.

Moreover, root growth causes a decrease in unstable macroaggregates (Denef *et al*, 2002). Therefore, soil aggregates involve a physical protection of the organic matter, difficult to access for microbial decomposers, due to occlusion, intercalation, hydrophobicity or encapsulation (von Lützow *et al*, 2006). The formation of soil aggregates is largely dependent on soil properties (clay proportion; microbial and diversity abundance; etc.) (Baldock and Skjemstad, 2000).

4.1.3. Chemical protection through recalcitrance

Chemical stabilization or recalcitrance concerns all the molecules which are difficult to degrade. For instance, von Lützow *et al* (2006) observed that lignin and suberin molecules are slowly degraded while monomers such as glucose, amino-acids and biopolymers as polysaccharides and proteins are considered as easily decomposable molecules. The main reason for this recalcitrance or fast degradation is the molecular structure: hydrolytic bindings present in easily decomposable molecules are decomposed by hydrolases, able to hydrolyse several C-N-bonds (von Lützow *et al*, 2006). A comparison of the molecular structure is shown on **Fig.10**. However, the chemical recalcitrance of organic-matter is not an intrinsic property and slow degradation rates can be caused by other stabilization mechanisms (Marschner *et al*, 2008).

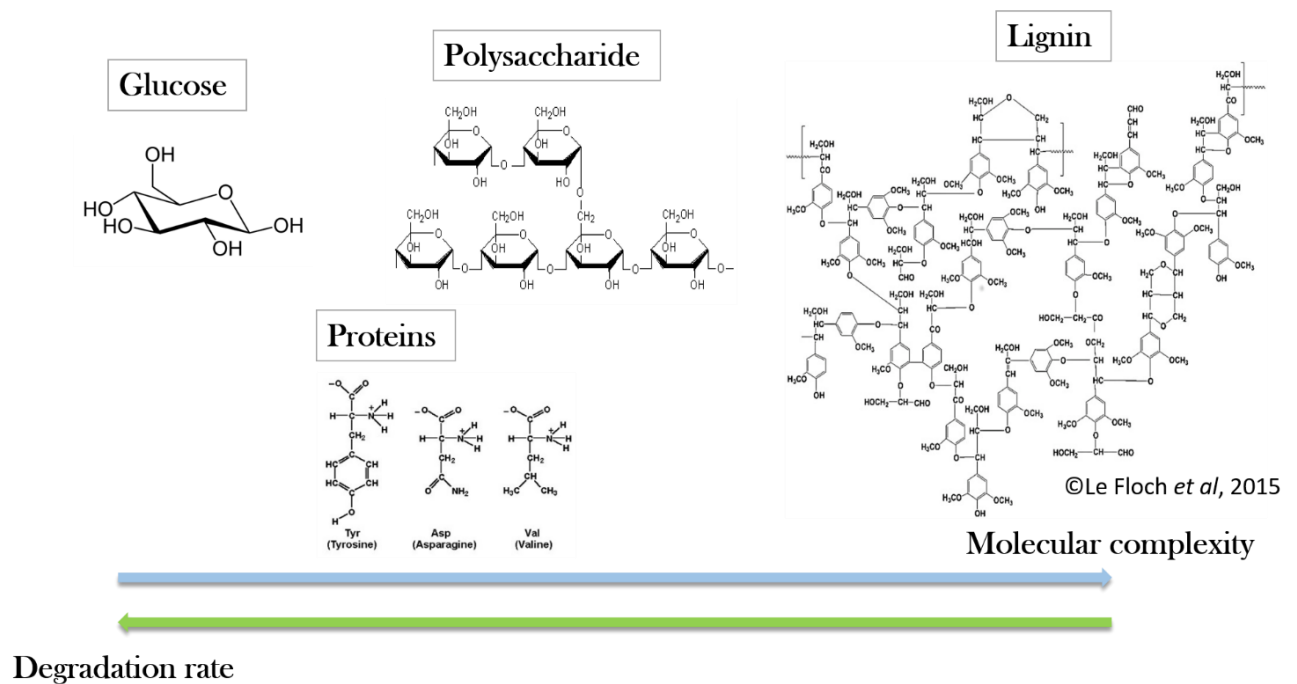


Figure 10 Example of molecules constituting organic matter, their molecular complexity and degradation rate.

4.2. Worm implications in stabilization of organic matter

Historically, the acknowledgment of the importance of worm activity on soil structure and formation dates back to the 19th century: Darwin wrote and intitled his last book “The formation of vegetable mould through the action of worms with observations and their habits”. Thanks to the success of this book, due to original experiments carried out to show if worms have a certain “intelligence”, the role of worms burrowing and casting in soil fertility and in the burial of organic matter with mineral elements was highlighted (Feller *et al*, 2003).

Worms are classified into three ecological categories, depending on their feeding habit and localisation in the soil (**Fig.11**).

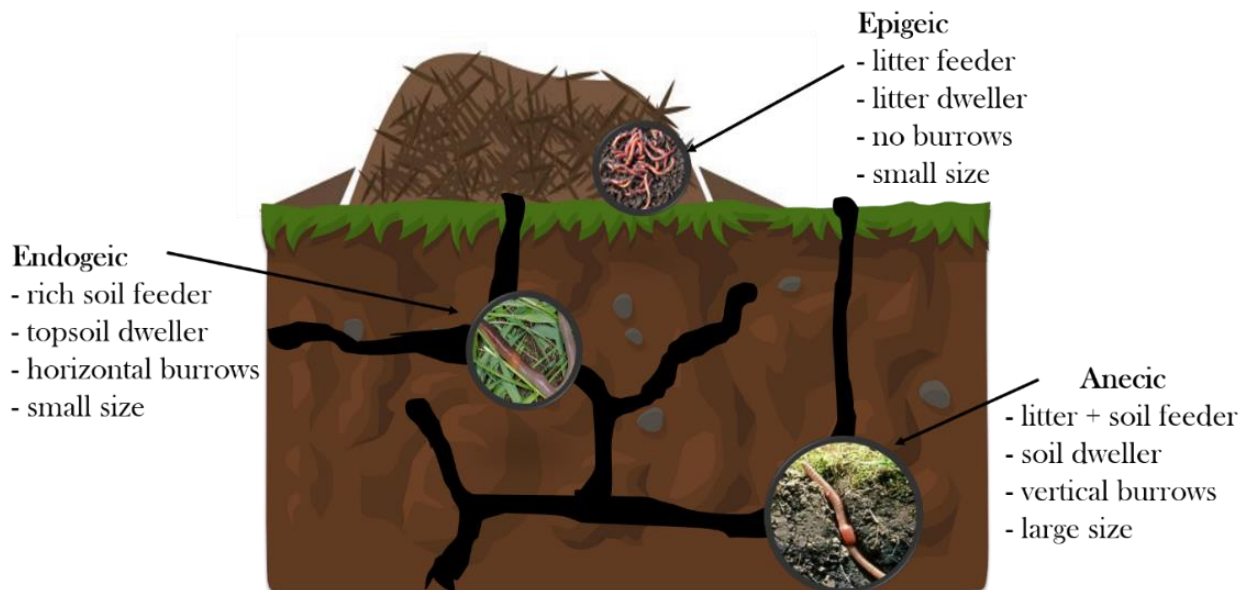


Figure 11 Worm classification according to their environment and behaviour.

Since Darwin's book, several studies have focused on the impact of worms on soil and in particular on protection of soil carbon (Bossuyt *et al*, 2005; Shipitalo and Protz, 1988; Bossuyt *et al*, 2004; Schrader and Zhang; 1993). Despite the fact that worms are considered as a key engineer and account for the main biomass in soil (Edwards, 2004), their influence on the carbon stabilization and formation of organo-mineral associations is still poorly understood.

Soil ingestion and destruction through worm gut might lead to destabilization of soil structure. However, cast or new aggregates formed by worms has been assessed to be more stable than initial soil aggregate (Shipitalo and Protz, 1988; Zhang and Schrader, 1993). According to Bossuyt *et al* (2004), *Aporrectodea caliginosa*, worms helped to form large macroaggregates, composed of more stable microaggregates than if they were formed without worms. Moreover, these new aggregates further enhanced the physical carbon protection from microbial mineralization on the long term, from years to decades (Brown *et al*, 2000). Besides, organo-mineral associations might also occur in worm's gut, as worms feed simultaneously on organic matter and soil minerals (Barois *et al*, 1993). Shipitalo and Protz (1989) observed that worm excreted Ca^{2+} , which can serve to bond clay to organic matter. These authors suggested also

that the mucilage secreted by worms, containing polysaccharides, might also bond organic matter to clay (**Fig.12**).

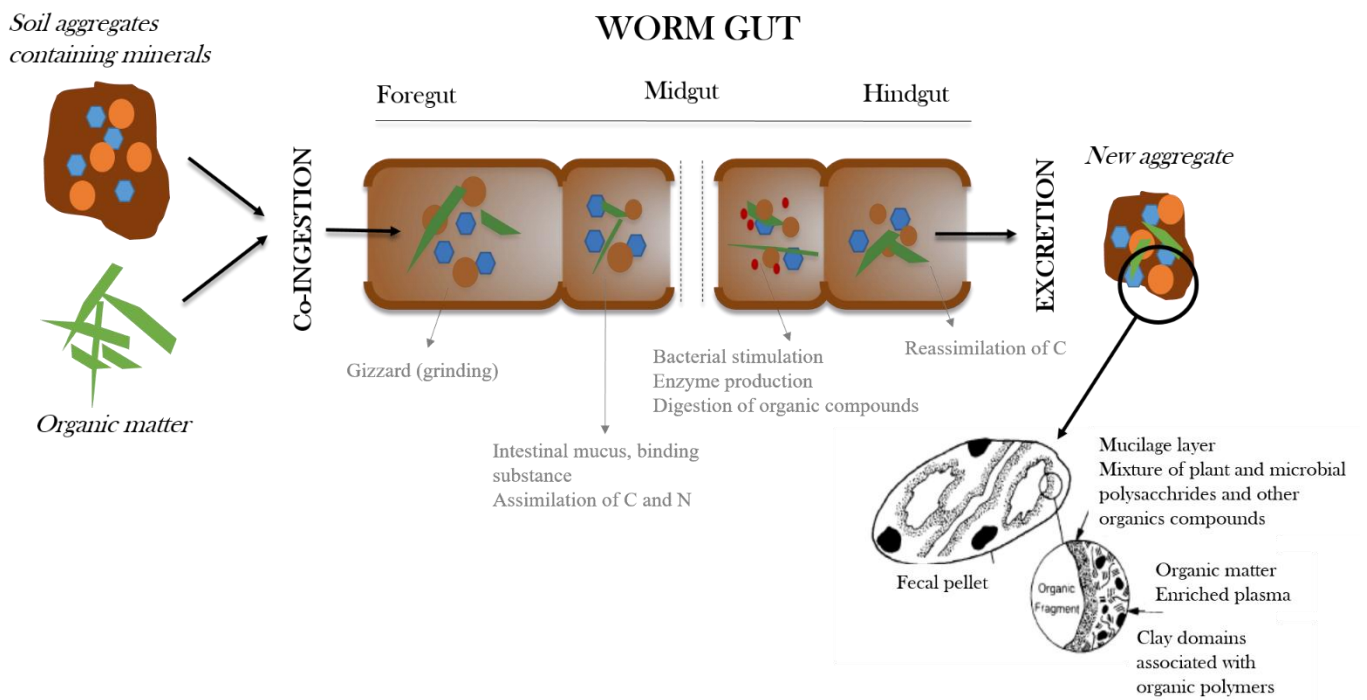


Figure 12 Schematic representation of aggregate formation in worm casts. Adapted from Shipitalo and Protz (1989) and Brown *et al*, 2000

Problematic and Objectives

In this state of knowledge review, we reported that composting process is based on the biological degradation of waste organic matter. Even if composting appears as a friendly process for the environment, GHG emissions occur during this process. Numerous studies have investigated and performed new composting strategies to reduce GHG emissions as N₂O and CH₄ by using bulking agents. Although during composting, more than 50% of the initial carbon is transformed into CO₂, little is done in order to reduce CO₂ emissions and stabilize carbon on the long-term. This is mainly due to the fact that biogenic CO₂ emissions are not accounted for in the total GHG emissions (IPCC, 2014).

In addition, there is a real challenge to increase carbon stabilization during composting in order to improve soil health. Indeed, even if compost or vermicompost organic matter showed a high degree of degradation, they are relatively rapidly degraded in soil, becoming more a source of carbon than a sink. In some world regions, soil organic carbon (SOC) is depleted by intensive agricultural practices or erosion (Lal, 2004). Applying a compost containing a high amount of stable carbon might increase the SOC on the long term (Ngo *et al*, 2012). Therefore, searching to stabilize carbon during composting and apply the finished product on soils might be a solution to restore soil health.

By imitating soil carbon stabilization mechanisms (Sollins *et al*, 1996), composting with minerals might allow the formation of stable aggregates on the long term. Beside minerals, using key engineers as worms, might have a significant impact on carbon stabilization, but also on compost structure and nutrient availability. Worms in soil form casts by ingesting both organic matter and minerals. In this way, worms might enhance organo-mineral associations, thus tending to stabilize and protect carbon from mineralization on the long term (Brown *et al*, 2000). Although these processes are known in soil, composting processes rarely take advantage of them and they have been rarely studied during composting. Moreover, there are still some knowledge gaps on the interaction between worms, minerals and fresh organic matter. Building a simple model of organic matter decomposition with minerals and worms based on composting may help to elucidate some of these knowledge gaps.

The aim of this work is to investigate the effect of worms on carbon stabilization, combined with different minerals, during the decomposition of fresh organic matter. A multidisciplinary approach was used to assess the worms and mineral effects, combining physical and chemical analysis with microscopic tools as TEM. Therefore, several composting units were implemented and used as “model” to describe carbon stabilization processes according to

minerals and presence/absence of worms. Beside carbon stabilization, and in a perspective of waste management, the quality and maturity of the finished composts were evaluated and performed through a plant growth experiment.

The following questions were used as guiding thread in this work:

- Which is the most relevant type and amount of mineral or combination of minerals to reduce carbon mineralization and thus enhance carbon protection? (**Chapter 2**)
- What impact do worms have on the carbon protection? (**Chapter 2**)
- Does the established carbon protection during composting last in an amended soil? And how mineral(s) and worm's casts will affect the soil properties? (**Chapter 4**)
- Will mineral and worm presence influence the quality and maturity of the finished compost? (**Chapter 3**)
- What influence do the co-compost or co-vermicompost has on the plant development? (**Chapter 5**)

The last part of this work (**Chapter 6**) will focus on the feasibility to perform composting or vermicomposting with minerals, particularly with two industrial mineral wastes (red mud and fly ash). The following **Figure 13** summarizes the global experimental design applied and the purpose of each chapter.

Chapter 2 and 6: Evaluation of the carbon mineralization and protection from composting and vermicomposting with different amounts and type of minerals. Assessment of worm and mineral effects.



Chapter 3 and 6: Nutrient availability assessment and microbial aspects in the end-products

Co-compost

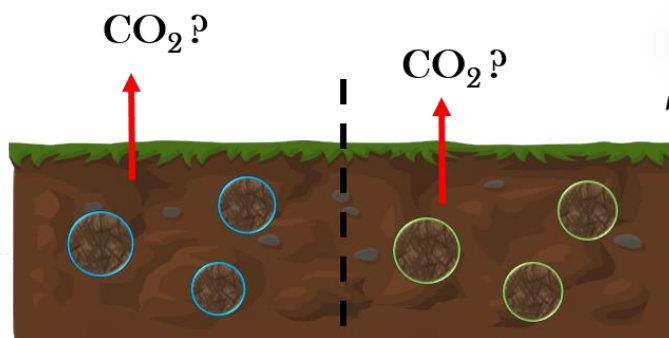


- Phosphorus fractionation
- Phospholipid acids (PLFA)
- Microbial biomass
- Nitrogen content
- Physico-chemical measurements, ...

Co-vermicompost

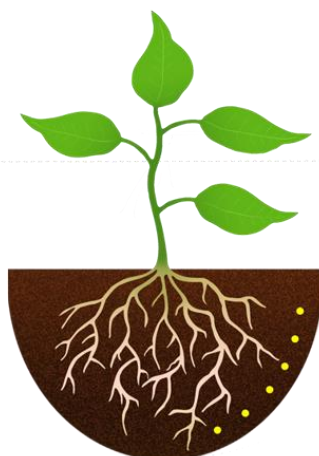


Chapter 4: soil carbon mineralization from amended soil and physico-chemical changes induce by organic amendments



Arenic cambisol

- Soil carbon mineralization
- Carbon distribution in soil (Zimmerman fractionation)
- Modelisation



- Follow of a plant growth experiment (*A. Thaliana*)
- Carbon and nitrogen accumulation in plant tissues

Chapter 5: Influence of co-compost and co-vermicompost on plant development

Figure 13 General approaches and methods used to fulfil this study. TEM: Transmission electron microscopy

Chapter 2

Influence of worms and minerals on fresh organic matter degradation and compost stabilization

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Introduction

Stabilizing and protecting organic carbon in soil might limit organic matter mineralization and thus the release of greenhouse gases such as CO₂. In addition to these beneficial effects, stable soil organic carbon may have a positive effect for agriculture due to the improvement of soil structure and fertility and the increase of carbon stocks (Smith, 2004). Organic matter can be stabilized in soils due to its associations with clays or oxides and its low accessibility due to physical occlusion (von Lützow *et al*, 2006; Schmidt *et al.*, 2011; Lehmann and Kleber, 2015). These two mechanisms can be classified as biochemical and physical mechanisms, respectively. The factors controlling these mechanisms mainly depend on the amounts and mineralogy of clay or iron oxide (Kleber *et al*, 2007). For instance, Mikutta *et al* (2007) observed that the binding mechanisms of organic matter with clay are different from those with iron oxide. Iron oxides, such as goethite, retain organic matter predominantly by ‘ligand exchange’ while sorption of organic matter onto clay, such as vermiculite, is due to the formation of Ca²⁺ bridges, depending on pH. Metal oxides can greatly interact with organic matter due to their permanent charges. Several studies have demonstrated that the presence of metal oxides decreased dissolved organic carbon in soil (Eusterhues *et al*, 2005) and that the carbon sorption on their surfaces was barely reversible (Kaiser and Guggenberger, 2007). In addition, the stabilization of carbon in soils due to sorption on mineral surfaces is largely dependent on the type of phyllosilicate (Kleber *et al*, 2007). Thus, 1:1 clays which have small specific surface area (SSA) and cation exchange capacity (CEC) will sorb less carbon than 2:1 clays, which present a high permanent surface charge, a high SSA and a large CEC.

Apart from these chemical and physical mechanisms dependant on the soil matrix, remaining and protected carbon in soil is also influenced by soil biota, for example worms. Worms are generally considered as key soil engineers due to their capacity to modify soil structure (Oades, 1993; Blanchart *et al*, 1997), improve soil fertility (Makeschin, 1997) and regulate soil microbial activity (Binet *et al*, 1998; Zhang *et al*, 2000). However, their implication in carbon stabilization is complex (Schrader and Zhang and, 1997): soil ingestion in worm gut might lead to destabilization of soil structure and thus carbon mineralization (Cole *et al*, 2000), while casts and new aggregates formed by worms have been suggested to be more stable than non-ingested soil aggregates (Shipitalo and Protz, 1989; Garvin *et al*, 2001). According to Bossuyt *et al* (2004), the worms *Aporrectodea caliginosa* helped to form large macroaggregates, composed of more stable microaggregates compared to those of the bulk soil. These new aggregates might further enhance the carbon physical protection from microbial mineralization on the long term,

from years to decades (Brown *et al.*, 2000). Moreover, organo-mineral associations can be formed in worm gut, since worms simultaneously feed on worms. The interaction of mineral, chemical and biological processes for the formation and stabilization of OM are poorly known yet (Dignac *et al.*, 2017).

Most studies of soil carbon stabilization focused on clay and oxide effects (Saidy *et al.*, 2012; Hassink, 1997; Feng *et al.*, 2013; Sanderman *et al.*, 2014) without taking into account the biological influence of worms and their potential interaction with various soil minerals. Due to soil matrix complexity and soil mineral diversity and heterogeneity, we recently proposed a conceptual approach of worm and mineral effects on carbon stabilization, through the study of model systems (Barthod *et al.*, 2016). In these model systems, the degradation of fresh organic matter during co-composting with pure minerals, with and without worms was monitored. Other authors proposed similar approaches with well-controlled mineral matrices (Saidy *et al.*, 2012; Mikutta *et al.*, 2007; Pronk *et al.*, 2012). However, in these studies, no worms were added and the organic matter originating from soil extraction might differ compared to native soil organic matter. Our approach enables to directly compare the influence of worms and clay and iron oxide amounts, mixed together or not, on the degradation of fresh organic matter and its stabilization through organo-mineral associations.

In the present study, in order to understand the mechanisms of organic matter stabilization through organo-mineral interactions related to worm activity, combined to the mineralogy and amount of clay and also to the presence of iron oxide, we used these model systems to investigate the effects of mineral amount and composition on (1) the kinetics of fresh organic matter degradation (2) the formation of organo-mineral associations and (3) the chemical composition of the final product. Moreover, we evaluated the influence of worms on these processes.

1. Materials and methods

2.1. Fresh organic matter and mineral properties

Fresh organic materials used for the incubation experiments were prepared by mixing of lettuces, flesh of apples, residual leaves of maize, ground spent coffee and pieces of cardboard (2x3cm) in controlled proportions (proportions in dry weigh: 1.2% of lettuce, 19.6% of apples, 39.1% of spent coffee ground, 10.1% of maize and 27.9% of cardboard). These proportions resulted in an initial C:N ratio of 40 for the organic mixture. Lettuces and apples, products of organic agriculture, were obtained from a general store, the spent coffee ground from a public cafeteria, and the maize from a field in Grignon (France). The main characteristics of these materials are listed in **Table 4**.

Table 4 Main characteristics of the initial organic materials used for composting. Weight are given in dry weight. The data are presented as means and standard error (n=4).

Material	Water content (%)	C (mg g ⁻¹)	N (mg g ⁻¹)	C:N ratio
Apple	86.4 ± 0.43	443.37 ± 27.5	2.86 ± 0.2	155 ± 1.2
Cardboard	<1%	387.92 ± 1.36	2.31 ± 0.01	168 ± 0.5
Lettuce	96.98 ± 0.24	375.92 ± 0.29	43.79 ± 0.11	8.6 ± 0.1
Residual maize	64.61 ± 0.66	393.54 ± 2.52	4.9 ± 0.04	80 ± 0.3
Spent coffee ground	71.97 ± 2.1	529.80 ± 2.03	21.36 ± 0.12	25 ± 0.2

Montmorillonite (2:1 clay), kaolinite (1:1 clay) and goethite (iron oxide) were purchased from Sigma-Aldrich in powder form (purity 99%; average particle size < 80 nm). Their respective specific surface areas are given in **Table 5**. Specific surface area of minerals was measured by the Brunauer-Emmett-Teller (BET)/N₂-adsorption method.

Table 5 Specific surface areas, pH and EC of the mineral used.

Mineral	Specific surface area (m ² g ⁻¹)	pH	EC (dS m ⁻¹)
Goethite	11	4.8	0.20
Kaolinite	7.5	7.6	0.06
Montmorillonite	232	3.9	0.13

2.2. Worm species

We selected epigeic worms able to develop in the fresh organic matter used for this experiment. *Eisenia andrei* and *Eisenia foetida* worms were initially purchased from La Ferme du Moutta, a worm farm in France and reproduced in the lab. 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 (Domínguez and Edwards, 2010).

Worms were raised in the same organic matter mixture as used in the experiment. Seven adult worms were chosen and cleaned to remove adhering soil/organic matter before estimating their body mass and adding them to the fresh organic material.

2.3. Model (compost and vermicompost) systems

In order to assess the effects of pure minerals and mineral mixtures and their combination with worms on carbon degradation and stabilization, different treatments with fresh organic matter (see above) were tested: (i) control with no minerals and no worms (C-control) and (ii) control without minerals but with worms (V-control); (iii and iv) 15% montmorillonite, with (V15M) and without worms (C15M); (v and vi) 30% montmorillonite, with (V30M) and without worms (C30M); (vii and viii) 15% kaolinite, with (V15K) and without worms (C15K); (ix and x) 30% kaolinite, with (V30K) and without worms (C30K) and (xi and xii) 15% goethite, with (V15G) and without worms (C15G); (xiii and xiv) 15% montmorillonite and 15% goethite, with (VMG) and without worms (CMG); (xv and xvi) 15% kaolinite and 15% goethite, with (VKG) and without worms (CKG). All treatments were set up with three replicates.

The experiments were carried out in 2L jars, closed with a tissue to avoid dust contamination and prevent worms to go outside the jar. Jars were arranged in a randomized block design. Minerals were thoroughly mixed with 50 g (dry weight) of the previously described organic mixture (C:N ratio of 40) with a moisture level of 70% (w/w) and then worms were added. Water was sprinkled on jars at the beginning of the experiment to reach this moisture level that was maintained throughout the experimental period for all treatments. These parameters were chosen in order to optimize the degradation of organic matter according to the worm requirements. The experiment ended after 196 days.

The carbon mineralisation was monitored using a MicroGC (Agilent, Santa Clara, USA) after the jars were closed during 3 hours. To avoid high CO₂ concentration inside the jars, they were kept open and closed only before CO₂ measurements.

2.4. Analysis of mineral and organic constituents

OC and N contents were measured using a CHN auto-analyser (CHN NA 1500, Carlo Erba). Dissolved organic carbon (DOC) contents were measured in 0.034 mol L⁻¹ K₂SO₄ extracts (1:5 w/v) using a total organic carbon analyzer (TOC 5050A, Shimadzu).

2.5. NMR spectroscopy

Solid-state ¹³C NMR spectra of compost samples were obtained on a Bruker DSX-200 NMR spectrometer. Cross polarization with magic angle spinning (CPMAS) (Schaefer and Stejskal, 1976) was applied at 6.8 kHz. The ¹³C chemical shifts were referenced to tetramethylsilane. A contact time of 1 ms was used and pulse delay was 800 ms. Solid-state ¹³C NMR signal was recorded as free induction decay (FID) and Fourier transformed to yield a NMR spectrum. The spectra were integrated using the integration routine of the spectrometer. The chemical shift regions 0-45 ppm, 45-110 ppm, 110-140 ppm, 140-160 ppm and 160-220 ppm were referred to alkyl C, O-alkyl C, C substituted aryl C, O substituted aryl C and carboxylic C, respectively (Wilson, 1987). The variation in integration due to the treatment of a well resolved FID (Fourier transformation, phasing and baseline correction) is less than 5 % (Knicker, 1993).

2.6. Transmission electron microscopy

Transmission electron microscopy (TEM) analysis was performed on four treatments: control with and without worms (C-control and V-control, respectively), and treatments with and without worms with a mixture of 15% kaolinite and 15% goethite (CKG and VKG, respectively). Samples were prepared in the LSE laboratory (Nancy, France) after specific sampling without drying or sieving, in order to take into account the heterogeneity of the size and nature of the various constituents of each treatment (Watteau and Villemin, 2011). Sub-samples of around 1 mm³ were prepared as follows: (i) chemical fixation of organic structures with 2% (w/v) osmium tetroxide in cacodylate buffer (pH 7) for 1 h, (ii) dehydration in graded acetone series, (iii) embedment in epoxy resin until complete polymerization and (iv) preparation of ultra-thin sections (80 nm) with a Leica Ultracut S ultra-microtome using a diamond knife. These sections were stained with uranyl acetate and lead citrate, then examined with an electronic microscope (JEOL, JEM EXII (80kV)) equipped with a camera. According to the heterogeneity of the organic mixture, 15 to 25 sub-samples were realized per treatment and 3 to 10 sub-samples were characterized by TEM. The origin (plant and microbial) and the degradation stages of the organic matter were characterized with the TEM images, as well as the presence of minerals. Numerous examinations were realized in order to compare the different samples in terms of relative observation frequency of the visualized microstructure. For this, 360 micrographs were analysed.

2.7. Calculation and statistical analysis

The amounts of CO₂ emitted during the degradation of fresh organic matter were fitted to a first-order model (Marquardt-Levenberg algorithm), using the following equation:

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

where C is the carbon mineralised (mg g⁻¹ TOC) at the time t (day), C₀ is the potentially mineralisable carbon (mg g⁻¹ TOC), and k is the carbon mineralization rate (day⁻¹).

All reported data are the arithmetic means of three (for NMR analysis) and four (for carbon mineralisation and elemental analysis) replicates. A Kruskal-Wallis test was performed to assess any significant differences between treatments. Significance was declared at the 0.05 level. A two-way ANOVA (I) procedure was used to compare the carbon pools and degradation rates. Statistical analyses were carried out using the R 3.12 statistical package for Windows (<http://www.r-project.org>).

2. Results

2.1. Macroscopic observations of organic residue degradation

Visual observation of the mixtures during the 196-day experiment (**Fig.14**) suggested different degradation states, depending on the presence of worms and minerals. After 7 days, easily decomposable fresh organic matter like lettuces and apples was no more recognizable when worms were present, while without worms, some leaves were still recognisable and intact. The volume occupied by the mixture at day 196 compared to initial volume of organic matter in the jar declined and this decrease was higher with worms than without worms (approximately 80% volume was lost with worms). At day 196, the aggregate size appeared substantially different in treatments with and without worms. With worms, aggregates were small, homogenised, and few initial organic materials were identifiable. Only maize residues were present on the top of the pile. By contrast, the regular composting process led to aggregates of different sizes, generally bigger than those obtained with worms. Maize residues and some cardboard residues were still recognizable. The presence of minerals did not seem to impact the visual aspect of the end-product except for the orange colour obtained with goethite.

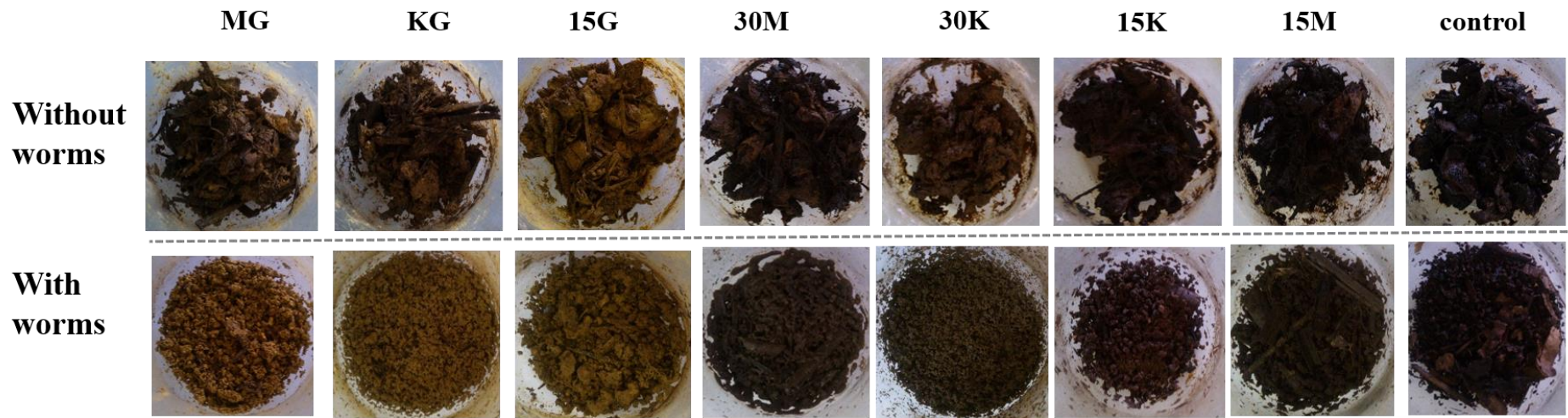


Figure 14 Morphology of organic matter after 196 days of composting in presence and absence of worms, with different minerals. Abbreviations refer to treatments: 15K: 15% kaolinite; 15M: 15% montmorillonite; 15G: 15% goethite; 30K: 30% kaolinite; 30M: 30% montmorillonite; KG: 15% kaolinite+ 15% goethite; MG: 15% kaolinite+ 15% montmorillonite.

2.2. Microscopic structure of the end-products: TEM analysis

The observation of C-control by TEM evidenced the presence of highly transformed organic matter, mainly as plant cell residues (walls and contents) of non-recognizable origin (**Fig. 15**). However, some residual spent coffee grounds were recognizable (**Fig 15.1**) and some fibres were still intact with partly degraded cell walls (**Fig 15.3 and 15.4**). TEM observations also showed some bacteria (**Fig 2.5**) and fungal residues as melanised cell walls (**Fig.15.6**). With kaolinite and goethite (CKG), the composted organic matter was also transformed (**Fig 16.1**), but compared to C-control, there were more large residues, such as fragments of plant tissues showing various states of degradation (**Fig. 16.2**). As a consequence of this lower degradation state of the organic matter, more micro-organisms in good physiological state were present compared to CKG. If minerals appeared dispersed among organic matter fragments (**Fig 16.1 and 16.2**), they were also grouped in some large areas (**Fig 16.3**). Minerals were also observed near poorly degraded organic matter (plant tissues, **Fig. 16.4**). By contrast, dispersed minerals appeared strongly associated with degraded plant cell walls, as they were intimately linked to fine size organic matter (**Fig. 16.5**). To a lesser extent, few minerals were found associated with bacterial cell walls. Next to poorly transformed organic matter, intact fungi and bacteria were observed. Minerals were dispersed but in some regions, they formed aggregates containing poorly degraded organic matter. By contrast, dispersed minerals were strongly associated with degrading vegetal cell walls. Few minerals were found associated with bacterial cells (**Fig. 16.5**).

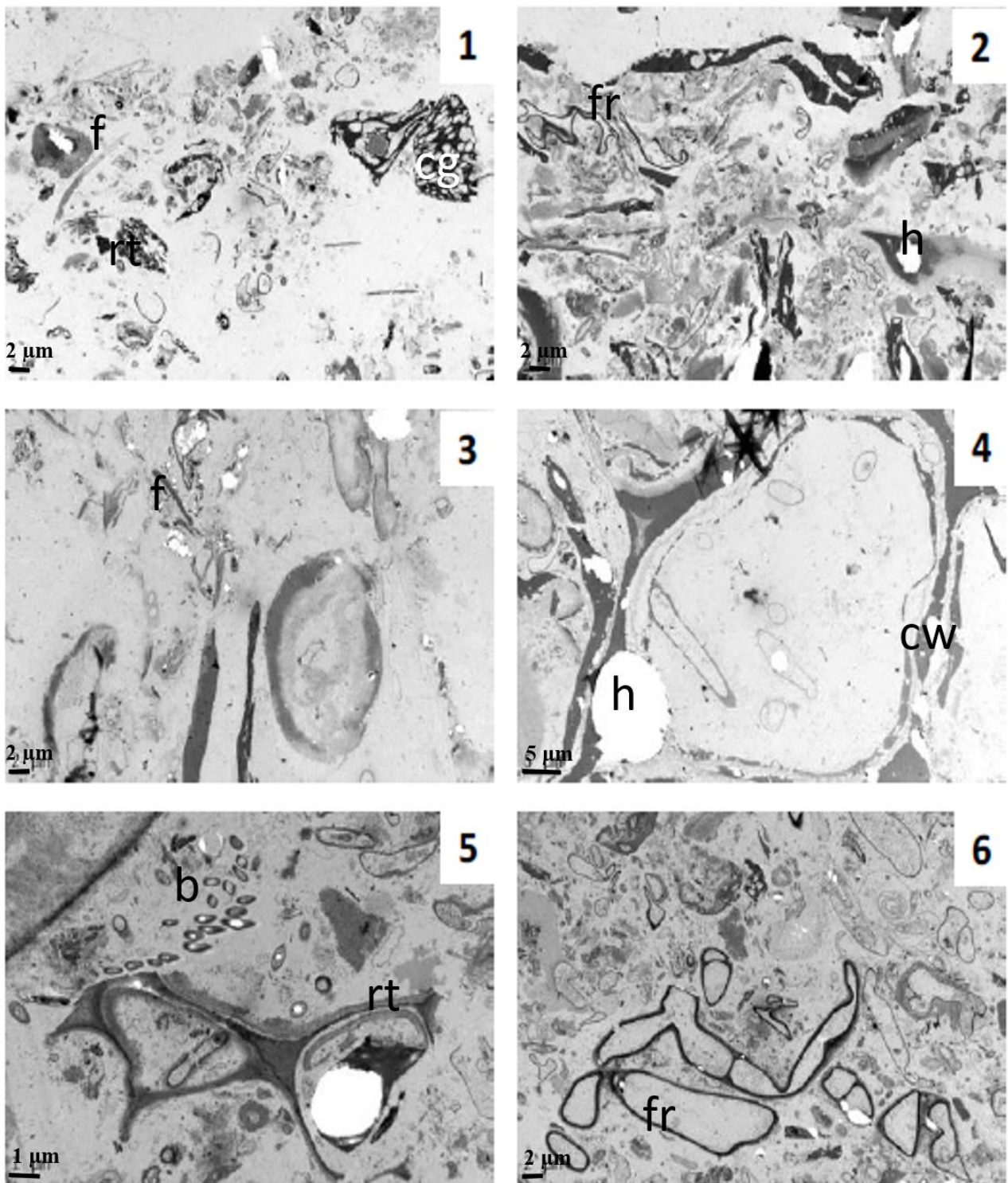


Figure 15 TEM images showing microstructures of organic matter produced after 196 days of incubation of fresh organic matter without worms and without minerals (C-control); 1 and 2] general view; 3] fibres; 4] residual tissues; 5] bacteria and residual tissues and 6] residual fungi. h: hole (artefact) in the resin due to the mechanical constraints during cutting; b: bacteria; cg: residual spent coffee ground; cw: cell wall; f: fibres; fr: fungal residue; rt: residual tissue.

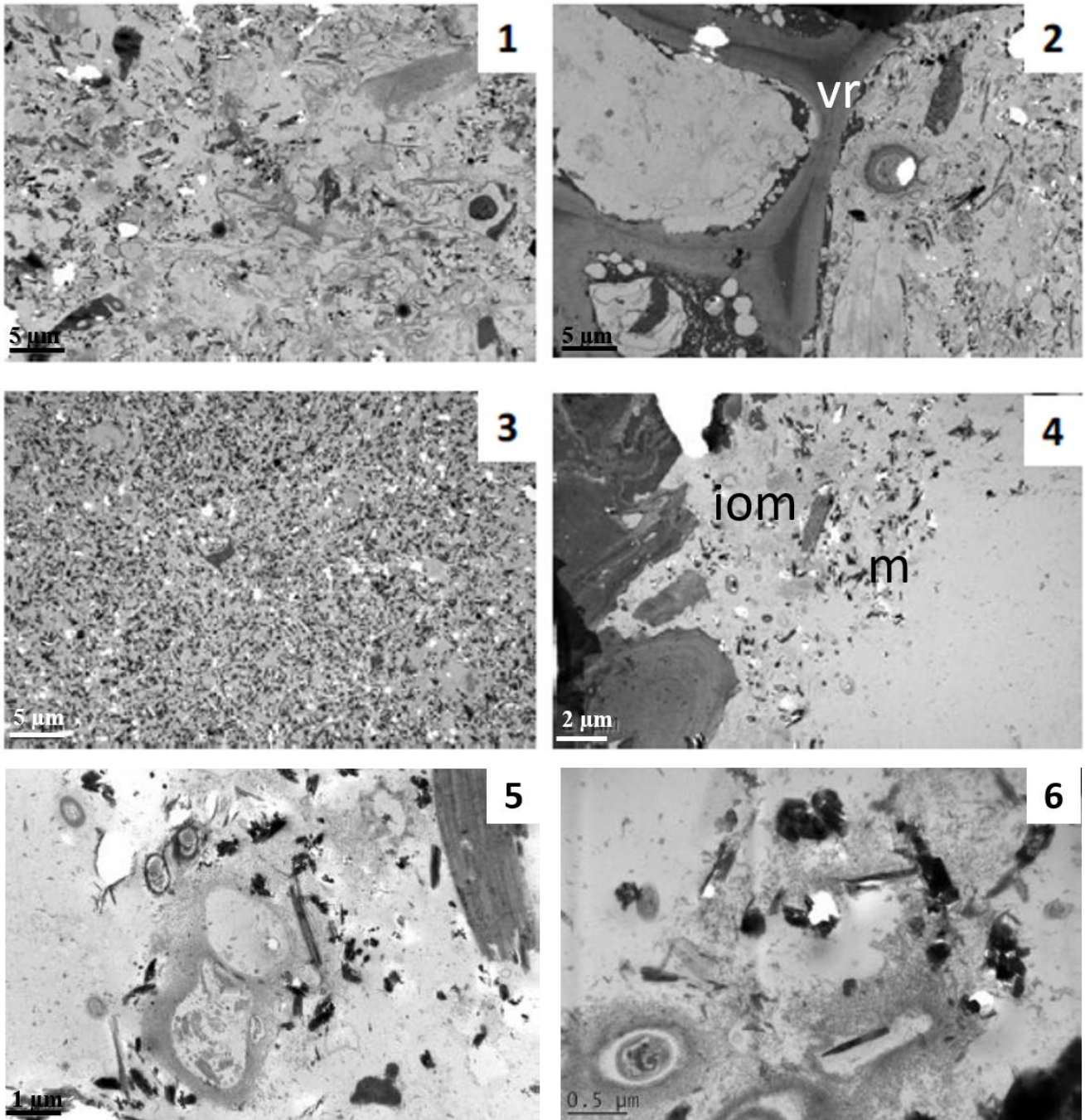


Figure 16 TEM images showing microstructures of the end-product obtained without worms and with goethite and kaolinite (CKG) 1] general view of degraded organic structures; 2] plant tissue residue; 3] large quantity of minerals not associated with organic matter; 4] minerals in the vicinity of intact organic particle; 5 and 6] mineral adhesion onto degraded vegetal membrane. iom: intact organic matter; m: mineral; vr: vegetal residue

In V-control treatment, no fibre fragment was sampled as the mixture was more homogeneous compared to C-control. TEM images of V-control treatment (**Fig.17**) revealed degraded plant residues similar to the ones in C-control (**Fig. 17.1 and 17.2**). As a result of worm mixing, organic matter mainly formed microaggregates (~10µm), i.e. association between organic matter of diverse origins (**Fig. 17.3**), whereas in C-control those structures were not observed. The intact microbial cells were more frequently observed in V-control than in C-control. Lytic activity of actinomycetes was highlighted in cell walls (**Fig. 17.4**) and also by some bacteria showing typical intracellular structures (**Fig. 17.5**). In VKG treatment, TEM images revealed organo-mineral associations between minerals and poorly degraded organic matter, i.e. cell wall and cardboard (**Fig. 18.1 and 18.2**) which were not observed in CKG. Degraded organic matter were frequently associated to minerals (**Fig. 18.3**) and minerals appeared more homogenously associated to organic particles in all the matrix than in C-control (**Fig. 18.4**). Microbial activity was more frequently highlighted in VKG compared to CKG as bacteria showing lytic activity and intact fungi were observed (**Fig. 18.5 and 18.6**).

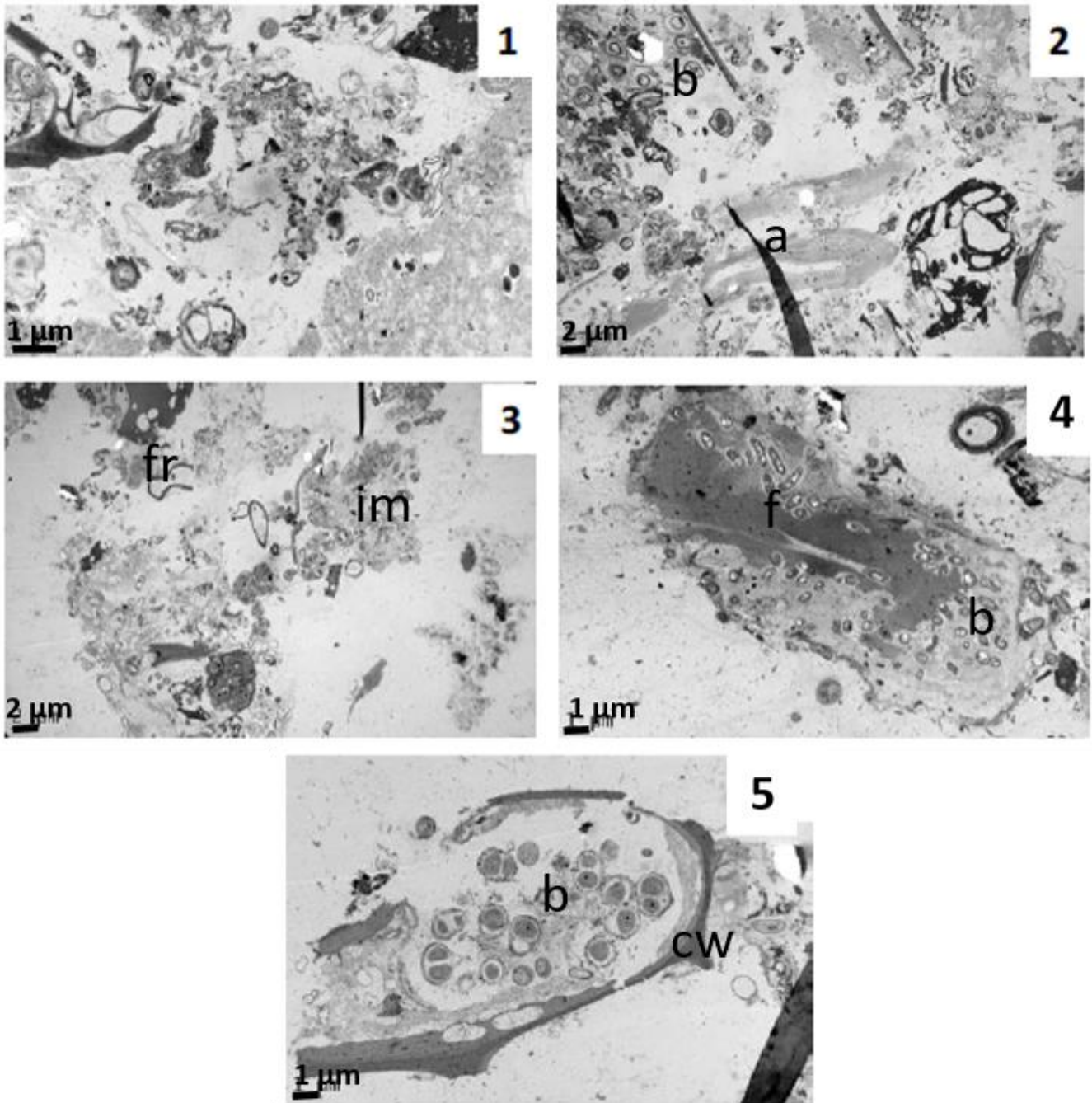


Figure 17 TEM images showing the microstructure of organic matter produced after 196 days of incubation of fresh organic matter with worms and without minerals (V-control). 1 and 2] general view; 3] individualized organic microaggregates; 4] fibres of cellulose degraded by bacteria; 5] bacteria in a residual cell. a: artefact (resin oversize); b: bacteria; cw: cell wall; f: fibres; fr: fungal residue; im: individualized microaggregates.

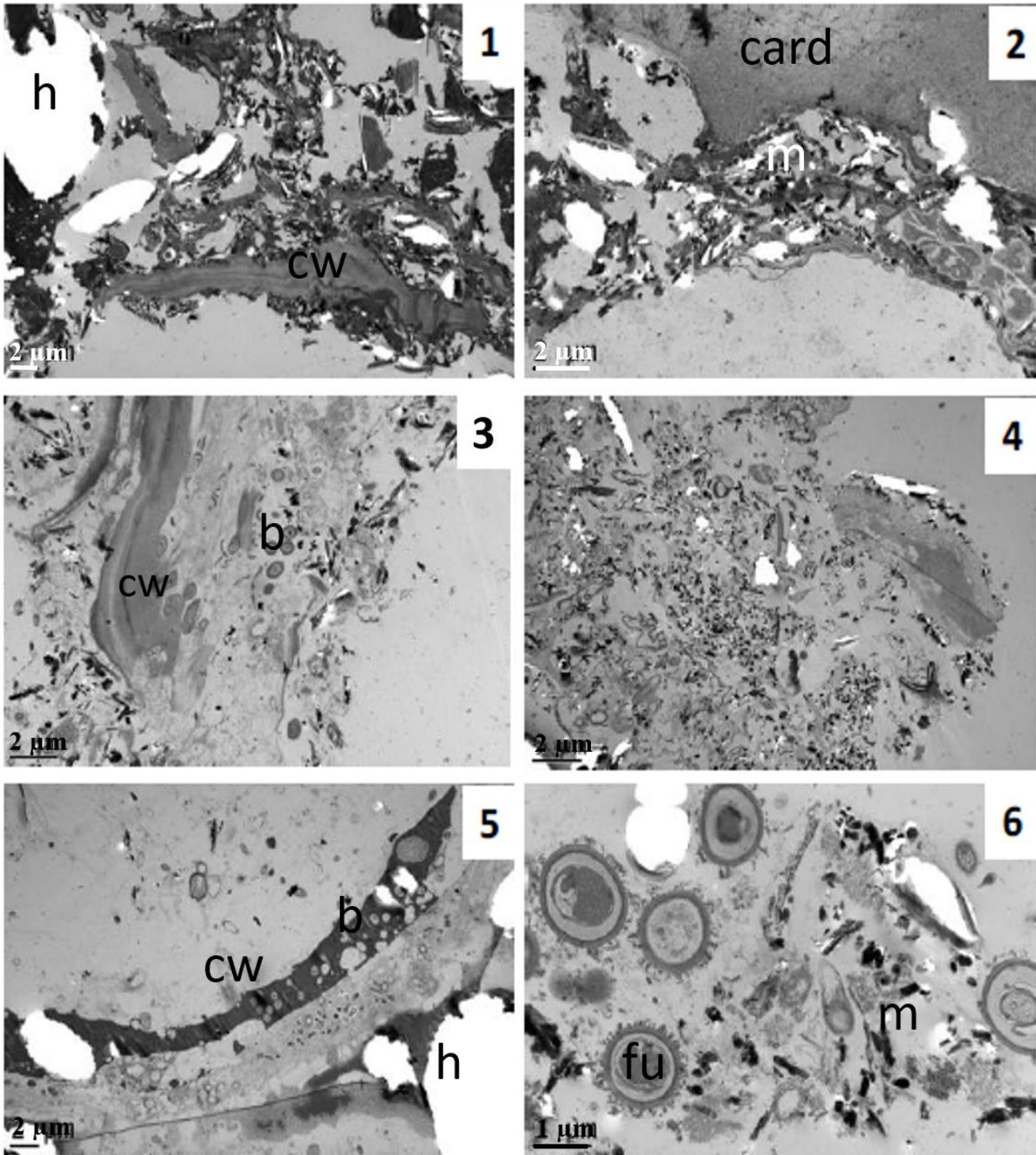


Figure 18 Observations of degraded organic matter in presence of worms with kaolinite and goethite. 1] mineral associations with poorly cell walls; 2] mineral associations with residual cardboards; 3] mineral associations with degraded organic matter; 4] general view showing the homogeneous repartition of minerals in the organic matrix; 5] degradation of cell walls by bacteria; 6] intact fungi. b: bacteria; card: cardboard; cw: cell wall; fu: fungi; h: hole; m: mineral.

2.3. Carbon dynamics during incubations

CO₂ emissions were monitored during the incubation experiments. The cumulative emissions ranged from 532 to 718 mg C-CO₂ g⁻¹ initial C (**Table 6**). For both processes (composting and vermicomposting), the lowest cumulative carbon emissions were recorded in the presence of 30% montmorillonite. The addition of 30% montmorillonite led to similar decrease in carbon emissions in the presence or absence of worms, approximately 12% compared to the corresponding control (**Table 6**).

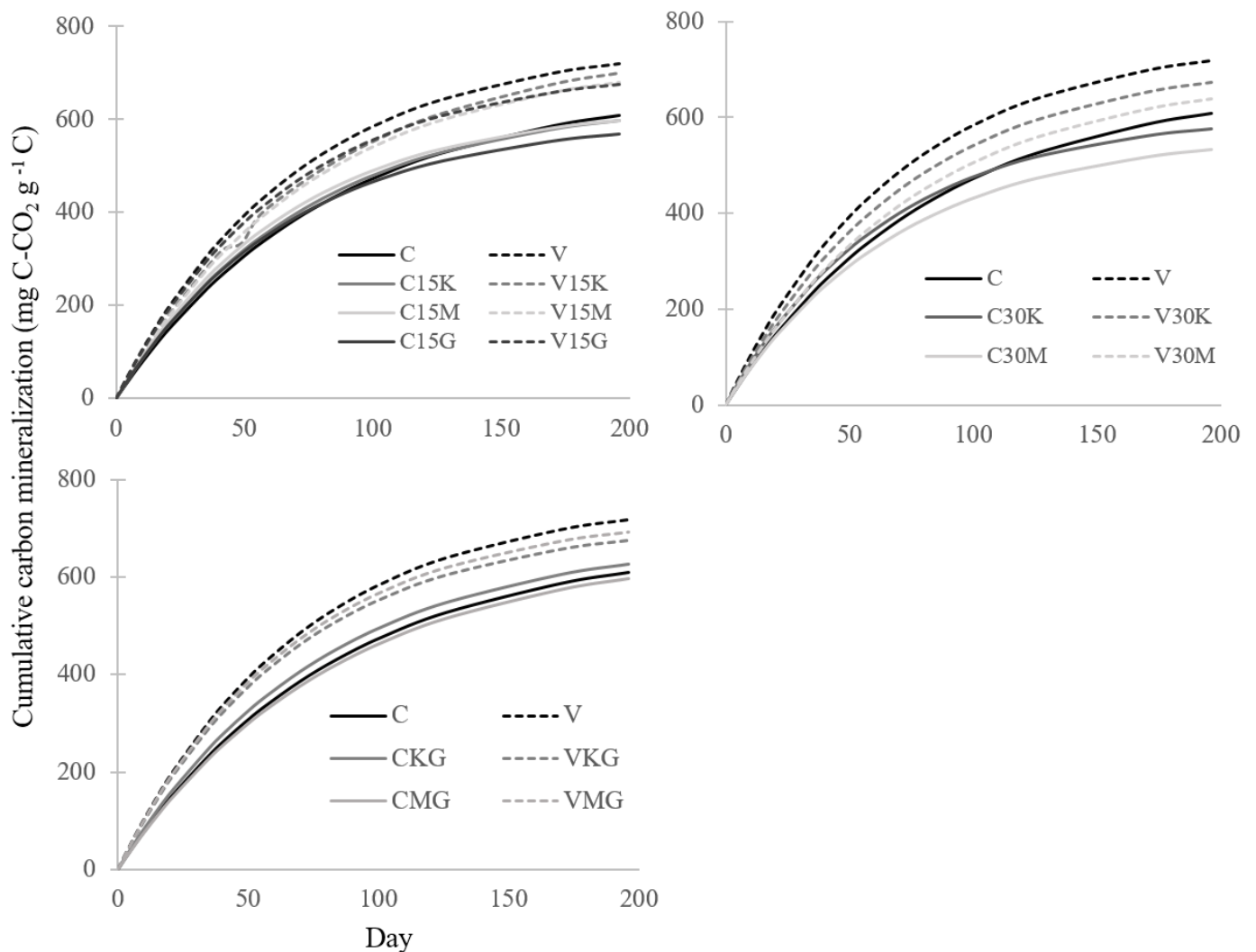


Figure 19 Cumulative carbon emissions released from fresh organic matter monitoring during 196 days with and without worms and, with and without minerals. C and V letters indicate respectively without worms and with worms. 15K: 15% kaolinite; 15M: 15% montmorillonite; 15G: 15% goethite; 30K: 30% kaolinite; 30M: 30% montmorillonite; KG: 15% kaolinite+ 15% goethite; MG: 15% kaolinite+ 15% montmorillonite.

The presence of worms induced higher cumulative emissions compared to treatments without worms. The highest emissions were recorded in the control with worms (V-control). Significant carbon emission differences between composts and vermicomposts were observed from day 15 onwards (**Fig. 19**). Most treatments showed significant decrease of carbon emissions in the presence of worms, except for kaolinite (15% and 30%) and the montmorillonite and goethite mixture (**Table 6**). By contrast, without worms, only the addition of 30% montmorillonite led to a significant decrease of carbon emissions (**Table 6**).

Table 6 Cumulative C-CO₂ emissions with and without worms and minerals and deficit in carbon mineralization compared to the control due to mineral addition. Different small letters indicate statistical difference between treatments from the same column; ‘*’ indicates a significant difference between similar compost and vermicompost treatments.

	Cumulative C-CO ₂ at day 196 (mg C-CO ₂ g ⁻¹ C)		Deficit in carbon mineralization compared to respective control (%)	
	No worm	Worm	No worm	Worm
control	608,92 ^{ab}	718,74 ^a *	-	-
15% kaolinite	597,35 ^{ab}	700,14 ^{ab} *	1,90	2,59
15% montmorillonite	597,17 ^{ab}	680,30 ^{bc} *	1,93	5,35
15% goethite	567,14 ^{bc}	674,90 ^{cd} *	6,86	6,10
30% kaolinite	576,33 ^{bc}	674,10 ^{abc} *	5,35	6,21
30% montmorillonite	532,03 ^c	638,89 ^d *	12,63	11,11
mixture kaolinite goethite	627,62 ^a	675,88 ^{cd} *	-3,07	5,96
mixture montmorillonite goethite	596,27 ^{ab}	693,01 ^{abc} *	2,08	3,58

These mineralisation data were fitted with a first order exponential model (**Table 7**). For composting with and without worms, the amount of potentially mineralisable carbon was significantly decreased with 15% goethite or 30% montmorillonite. In addition, in the presence of worms, 15% montmorillonite and kaolinite- goethite mixture reduced this amount. Without worms, montmorillonite (15% and 30%) and goethite (15%), significantly increased the degradation rate while reducing the cumulative carbon emissions.

Table 7 Amounts of potentially mineralizable carbon and degradation rates obtained from a first order model for the different treatments. Different small letters indicate statistical difference between treatments from the same column; ‘*’ indicates significant difference between similar compost and vermicompost mineral treatments.

	potentially mineralizable C (mg C g⁻¹ C)		Degradation rate <i>k</i> (day⁻¹)	
	<i>No worm</i>	<i>Worms</i>	<i>No worm</i>	<i>Worms</i>
Control	669,99 ^{ab}	764,01 ^{a*}	0,0123 ^{cd}	0,0145 ^{ab*}
15% kaolinite	642,12 ^{bc}	767,00 ^{a*}	0,0139 ^{abc}	0,0132 ^{ab}
15% montmorillonite	630,87 ^{bc}	733,65 ^{ab*}	0,0150 ^a	0,0134 ^b
15% goethite	599,37 ^c	711,75 ^{bc*}	0,0149 ^a	0,0152 ^a
30% kaolinite	606,94 ^{bc}	721,42 ^{ab*}	0,0156 ^{bcd}	0,0139 ^{ab}
30% montmorillonite	568,33 ^c	691,85 ^{c*}	0,0144 ^{ab}	0,0131 ^b
Kaolinite: goethite	681,23 ^a	715,82 ^{bc*}	0,0130 ^{bcd}	0,0149 ^{ab*}
Montmorillonite: goethite	657,59 ^{ab}	733,52 ^{ab*}	0,0121 ^d	0,0149 ^{ab*}

A two-way Anova on these data showed that the potentially mineralisable carbon pool is affected by the presence of worms, the amount of minerals and the type of mineral (2:1; 1:1 layer or iron oxide). No interaction between these factors was significant. By contrast, ANOVA performed on the degradation rates evidenced some interactions between worm presence/absence and mineral amount, and between worm and mineralogical properties.

2.4. Properties of the final products after 196 days of incubation

2.4.1. Carbon content and dissolved organic carbon

Organic carbon content in the end-products ranged from 145.2 to 381.2 mg g⁻¹ (**Fig. 20**). Remaining carbon contents were highest in the control treatments and were not statistically different in the control treatments with and without worms. Addition of minerals decreased carbon contents, mainly due to dilution effect (larger dilution effect for 30% addition). For a similar mineral treatment, the decrease of carbon content was larger when worms were present than when they were absent, except for 30% montmorillonite and for the goethite and montmorillonite mixture (C30M and CKG respectively larger than V30M and VKG).

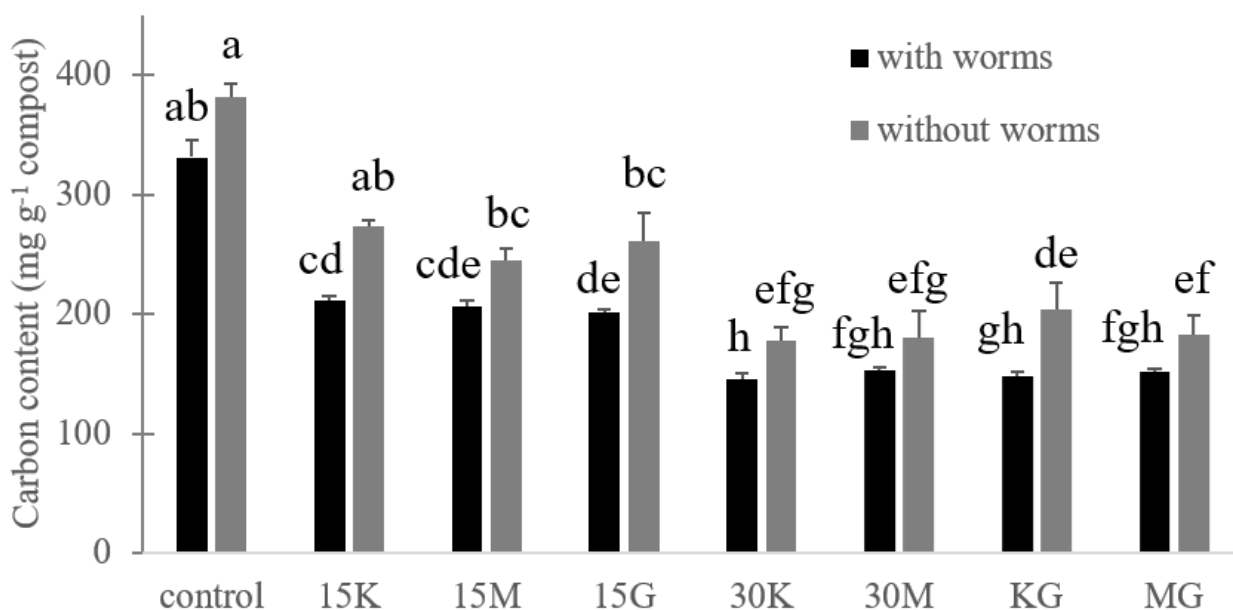


Figure 20 Final carbon content in the end-products obtained with and without worms, with and without minerals. 15K: 15% kaolinite; 15M: 15% montmorillonite; 15G: 15% goethite; 30K: 30% kaolinite; 30M: 30% montmorillonite; KG: 15% kaolinite+ 15% goethite; MG: 15% kaolinite+ 15% montmorillonite. Different small letters indicate statistical difference between treatments.

Dissolved organic carbon (DOC) can be considered as a labile carbon pool (**Fig. 21**). This carbon fraction ranged from 3.4 to 37 mg g⁻¹ end-product after 196 days. The end-product DOC was decreased in the presence of 30% minerals (kaolinite, montmorillonite or goethite) and of 15% montmorillonite. This is partly explained by a dilution effect. When treatments with minerals in similar amounts are compared, montmorillonite in treatments C15M, C30M and CMG led to the lowest amount of extracted DOC, which was up to six times lower compared to the control (C-control). With worms, end-product DOC was decreased only by the addition of montmorillonite (V15M, V30M and VMG). Other minerals did not change end-product DOC, or even increased it (15% kaolinite, V15K). Worm presence led to lower DOC in the end-products compared to the treatments without worms.

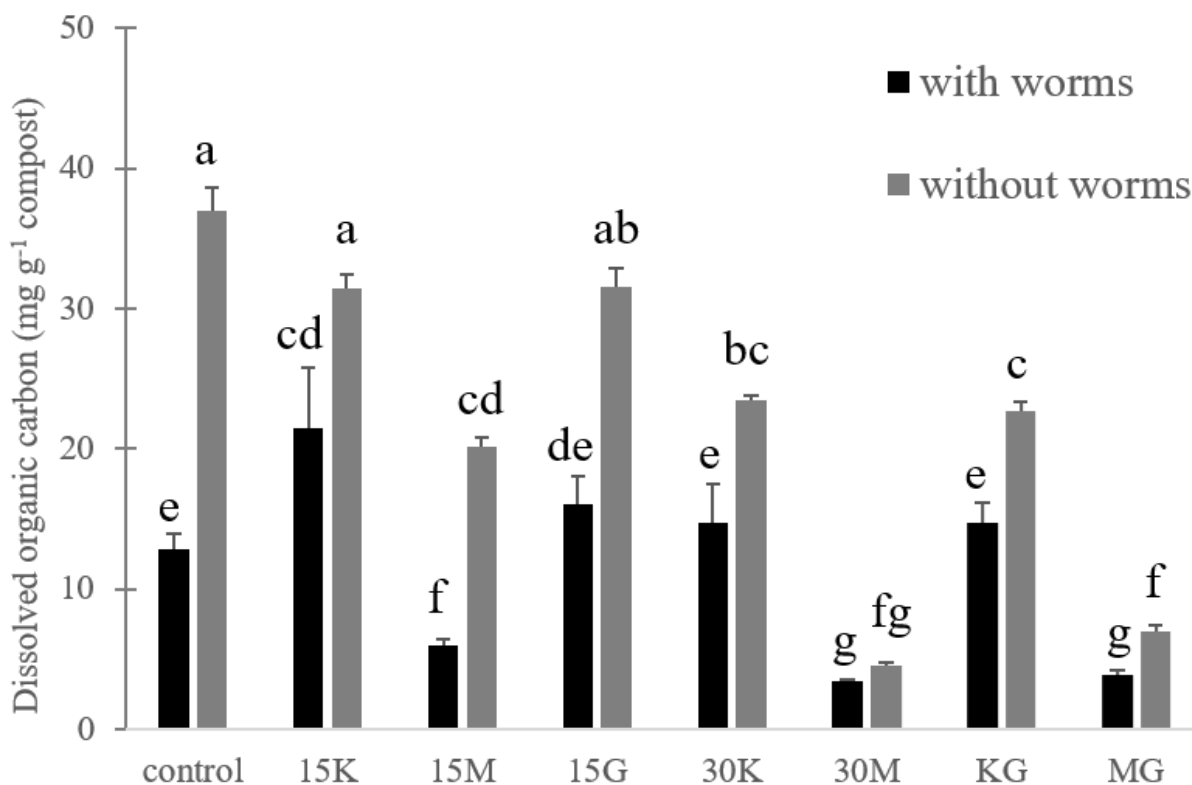


Figure 21 Final dissolved organic carbon in end-products obtained with and without worms, with and without minerals. 15K: 15% kaolinite; 15M: 15% montmorillonite; 15G: 15% goethite; 30K: 30% kaolinite; 30M: 30% montmorillonite; KG: 15% kaolinite+ 15% goethite; MG: 15% kaolinite+ 15% montmorillonite. Different small letters indicate statistical difference between treatments.

2.4.2. NMR spectroscopy

The global chemical composition of the end-products was characterized by solid-state ¹³C CPMAS NMR spectroscopy. The carbon distribution obtained for each treatment by integrating the NMR spectrum is described in **Table 8**. The O-alkyl C region, most probably related to alcoholic or ether structures in polysaccharides (Wilson, 1987) showed the greatest contribution to total signal intensity of all samples (>45%). In treatments without worms the relative contribution of O-alkyl structures increased up to 8% with minerals (C30M > CKG > C15M > C-control) while in treatments with worms this relative contribution was in the order V30M > Vcontrol > VKG > V15M. Regardless of worm presence, the alkyl-C region, most likely explained by the contribution of methylene C of plant or microbial origin, contributed from

17.23% to 28.03% to the total signal intensity, and decreased in the order: control > 15M > 30M > KG. The alkyl-C to O-alkyl-C ratio may give information on the organic matter degradation state. According to Baldock *et al* (1997), a high ratio may indicate a high degree of organic matter decomposition related to a degradation of plant derived polysaccharides and concomitantly to an increase of microbial derived methylene structures. In the absence of worms, this ratio significantly decreased in the following order: C-control > C15M > C30M > CKG, while in the presence of worms this ratio tended to decrease in the order V15M ≥ V-control > VKG > V30M.

C-substituted aryl carbon slightly increased in the presence of minerals in all treatments, except when 30% montmorillonite was added. The O-substituted aryl C showed the lowest contribution with less than 10% of the total signal intensity. Similar tendencies were observed for C-substituted aryl carbon. The relative contributions of both of these regions followed the order 30M > control > 15M > KG in treatments with and without worms. Carboxylic carbon showed the highest relative contributions (above 12%) for CKG, VKG and V15M treatments while the lowest contributions were observed for V-control and V30M treatments (8.3% and 8.2% respectively). Minerals had no significant influence on the carboxylic C contribution, except when worms were present (V15M and VKG) which increased their contributions.

Table 8 Relative areas of peaks of ¹³C CPMAS NMR spectrum of end-products obtained after 196 days of degradation, with and without worms (V and C respectively) and with and without minerals. 15M: 15% montmorillonite; 30M: 30% montmorillonite; KG: 15% kaolinite+ 15% goethite.

	Alkyl-C	O-Alkyl-C	C substituted aryl C	O-substituted aryl C	Carboxylic C	Alkyl/ O-Alkyl
C-control	28,0 ^a	47,2 ^c	10,4 ^{ef}	3,8 ^d	10,5 ^{abc}	0,59 ^a
V-control	24,4 ^{ab}	51,7 ^{abc}	11,2 ^{bc}	4,3 ^c	8,3 ^{bc}	0,47 ^{ab}
C15M	21,8 ^c	50,0 ^{bc}	11,9 ^{bc}	4,8 ^{bc}	11,4 ^{ab}	0,44 ^b
V15M	21,9 ^{bc}	46,1 ^c	13,0 ^{ab}	5,1 ^b	13,9 ^a	0,48 ^{ab}
C30M	21,5 ^c	55,8 ^{ab}	8,9 ^f	3,6 ^d	10,2 ^{bc}	0,39 ^{bc}
V30M	20,1 ^{cd}	57,0 ^a	10,5 ^{de}	4,2 ^c	8,2 ^c	0,35 ^c
CKG	17,2 ^d	54,2 ^{ab}	11,2 ^{cd}	5,2 ^b	12,2 ^a	0,32 ^c
VKG	18,7 ^d	46,1 ^c	15,1 ^a	7,8 ^a	12,2 ^a	0,41 ^{bc}

3. Discussion

3.1. Carbon mineralisation without worms: effect of clay and iron oxide addition

The decomposition rate of the fresh organic matter during composting increased in the presence of minerals (**Table 6**), which is in contrast to the common observation of the stabilisation of organic matter by soil minerals (von Lützwow *et al*, 2006; Oades, 1988). This may be explained by a stimulative effect of minerals on organic matter decomposition, which was observed by some authors for clays (Dilustro *et al*, 2005). For example, Wei *et al*, 2014 showed that high bentonite contents in artificial soils, rich in organic matter, may accelerate organic matter decomposition through increasing microbial biomass, likely due to the fact that clays provide a large surface area and also reduce the distance between microorganisms and their substrate. Thus, addition of minerals to fresh organic matter may induce a rapid decomposition of organic matter.

At low mineral contents (15%), our results showed that iron oxide is more efficient to reduce carbon emissions than clay. Similar results were observed in soil matrix (Rasmussen *et al*, 2005) suggesting that mineral properties (SSA, permanent charge) may control carbon dynamics. This is supported by recent studies showing that the properties of iron oxides are dominant drivers of carbon protection from mineralization (Sandermann *et al*, 2014; Saidy *et al*, 2012). A high content of minerals (30%) decreased carbon emissions more than did a low content, except when mineral mixtures were used (CKG and CMG). These observations are in line with the traditional view that a high amount of minerals may protect more carbon from mineralization than a low content of minerals, mainly related to the higher available specific surface area (Hassink *et al*, 1997; Kleber *et al*, 2015). Another potential explanation is the fact that the amount of available water may be low in the presence of montmorillonite, a swelling phyllosilicate, which decreased indirectly the CO₂ evolution through a limitation of microbial activity.

Previous studies suggested that 2:1 clay trap more carbon than 1:1, for similar amounts in the substrate (Saidy *et al*, 2012; Saggar *et al*, 1999) and that 1mg of carbon is trapped per m² of specific surface area (Feng *et al*, 2013). A linear relationship is thus expected between the remaining C and the amount of added mineral. However, the amount of carbon in our finished products did not linearly relate to mineral amounts (**Fig.22**) ($R^2=0.5194$), in particular when montmorillonite is present. Thus, our results on mineralization of fresh organic matter with minerals (1) confirm that the entire specific surface area of 2:1 mineral as montmorillonite is

not completely available to form organo-mineral associations; and (2) suggest that iron oxides alone are more efficient to stabilize carbon than montmorillonite or kaolinite, probably due to chemical interactions and physical protection, as suggested by Mikutta *et al* (2007), who demonstrated that goethite trapped up to 30.1 mg organic carbon per g, predominantly by ‘ligand exchange’ and that these interactions were resistant against mineralization; (3) the application of a binary system (iron oxide with clay) did not appear efficient to reduce carbon emissions from fresh organic matter degradation, while in soil these systems are able to limit carbon mineralization (Saidy *et al*, 2011; Wiseman and Puttman, 2006). In our case, mixing 2:1 or 1:1 clay minerals with an iron oxide may decrease the specific surface area available for organic matter interactions and even stimulate microbial activity. According to Wei *et al*, 2012, a binary system containing goethite and kaolinite may induce electrostatic interactions, anion ligand exchange or hydrogen bonds between both minerals. Moreover, Fusi *et al* (1989) suggested that iron oxide may be coated on clay without increasing the carbon sorption, even though the oxides increase the specific surface area. However, TEM observations did not confirm this hypothesis since goethite and kaolinite were not distinguishable (**Fig. 16**)

The use of fresh organic matter in our study might explain these differences compared to soil system as its composition is different from soil organic matter, in particular with regards to the contribution of easily decomposable carbon pools. For instance, the potentially mineralisable carbon comprises more than 50% of the initial organic matter in our study (**Table 7**) while in soil, this pool represents less than 25% (Gulde *et al*, 2008).

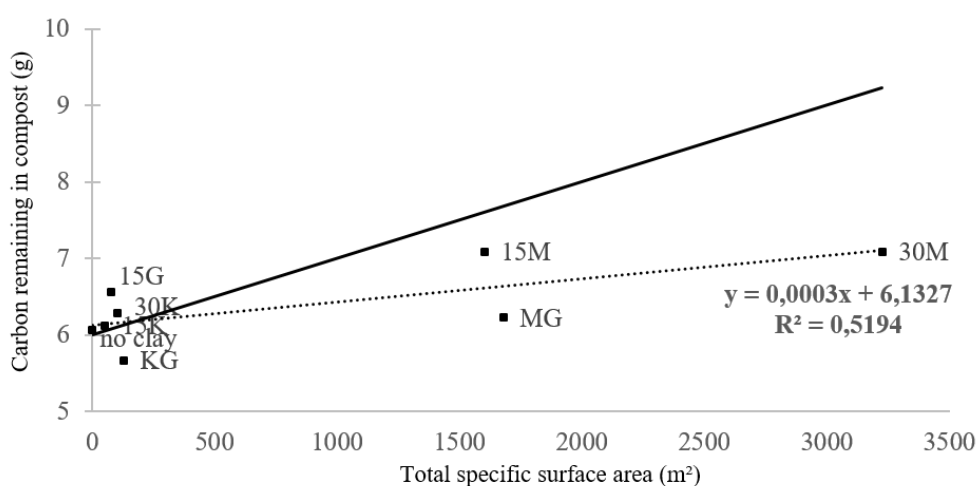


Figure 22 Relationship between carbon remaining after 196 days of decomposition without worms, with and without minerals and the total specific area. Solid line represents the estimation of remaining carbon, based on the hypothesis that 1 mg of carbon can be trapped per m² of mineral specific surface area (Feng *et al*, 2013).

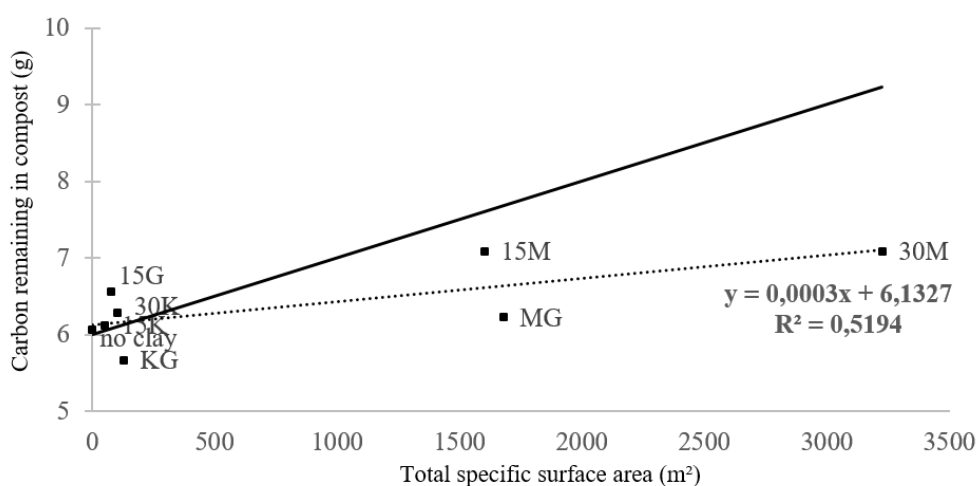


Figure 23 Relationship between carbon remaining after 196 days of decomposition without worms, with and without minerals and the total specific area. Solid line represents the estimation of remaining carbon, based on the hypothesis that 1 mg of carbon can be trapped per m² of mineral specific surface area (Feng *et al*, 2013).

3.2.Changes in compost chemical composition with mineral addition

Solid-state NMR spectroscopy showed that organic matter after the composting period contained a high proportion of O-alkyl-C, followed by alkyl-C, while aromatic, C-substituted and O-substituted structures represented approximately 25% of the total carbon. These results are similar to those of Lorenz *et al* (2000) who observed that decomposition of litter led to a relatively high amount of alkyl compared to aromatic C. Moreover, as mentioned by Schmidt *et al* (2000), the relative contribution of the C-substituted aryl C and O-substituted aromatic C is related to the presence of the complex molecules such as lignin, cutin or tannin. Thus, our results showed that, without worms, the presence of mineral increased the relative amount of these complex molecules, except with 30% montmorillonite. This suggests that complex molecules such as lignin and other phenolic compounds are actively involved in organo-mineral associations. In addition, their implication is relatively more important in a binary system (clay/iron oxide) than with clay alone. Gu *et al* (1995) demonstrated also that O-containing functional groups of soil organic matter are preferentially adsorbed to the iron oxide surface through complexation-ligand exchange. In addition to aromatic C contribution, the alkyl-C to O-alkyl-C ratio gives information on the organic matter degradation state (Baldock *et al*, 1997). In the absence of worms, this ratio significantly decreased as follows: C-control > C15M > C30M > CKG, suggesting that minerals limited organic matter decomposition.

Finally, the low carbon mineralization observed with 30% montmorillonite coupled with the low contribution of complex aromatic molecules may suggest that microorganisms breakdown organic matter into small molecules directly sorbed onto the clay surface (Chenu and Stotzky, 2002). By contrast, kaolinite-goethite treatments showed a high C mineralisation and a low decomposition degree of organic matter. Therefore, this system may protect complex molecules but not labile compounds from complete mineralization. Consequently, only a small proportion of carbon is preserved from mineralization in this system compared to the one with 30% montmorillonite. Similarly, Pronk *et al* (2013) showed that clay minerals were more important than metal oxides for the binding of organic carbon in artificial soil composed of manure and pure minerals. Our results on dissolved organic carbon at the end of the incubation supported this hypothesis (Fig.9), partly explained also by differences in specific surface area between clays (2:1 and 1:1) and iron oxides.

3.3. Influence of worms on the protection of fresh organic matter through organo-mineral associations

The carbon mineralization and degradation rate in the vermicompost (V-control) was higher than in the control treatment without worms (**Tables 6 and 7**). This is in line with the findings of Nigussie *et al* (2017), that worms accelerate the degradation rate of organic matter. Observations at the end of the experiment of C-control revealed that the organic matter formed aggregates of heterogeneous sizes including well degraded organic matter (**Fig.14**) while the images of V-control showed the formation of smaller aggregates (**Fig.14**), with less identifiable fibres and intact microflora (**Fig. 17**). These observations are in accordance with previous studies with anecic worms (Shipitalo and Protz, 1989; Vidal *et al*, 2017), who explained that worms accelerate the decomposition of fresh organic matter, because the residues are ingested by worms and thus fragmented by the grazing activity and grinded during the transit. Nevertheless, remaining carbon compounds in vermicomposts, as characterized by ¹³C CPMAS NMR (**Table 8**), showed a higher proportion of aromatic compounds (O-substituted carbon) compared to composts. Vincelas-Akpa and Loquet (1997) obtained similar results, which they explained by the production of new aromatic compounds at the end of vermicomposting. In addition, the contribution of the melanised walls of fungi should be taken into account, as they are more numerous in the presence of worms (**Fig. 17**). Other explanation may be done as initial aromatic compounds are difficult to degrade (Thevenot *et al*, 2010) and may concentrate due

to the rapid degradation of the other organic matter. However, these results, coupled with a low dissolved organic carbon content in V-control (**Fig. 21**), support the hypothesis that organic matter ingested by worms is more stable than in a regular compost (Ngussie *et al*, 2017; Ngo *et al.*, 2013).

When worms were combined with minerals, carbon mineralization decreased to reach similar proportions as in no-worm treatments (**Table 6**). Therefore, the decrease of carbon mineralization is more likely due to the mineral presence than to the combination of worms and minerals. In a previous study (Barthod *et al*, 2016) we found on the contrary that with worms which co-ingested both organic matter and minerals, carbon mineralisation was more efficiently decreased than with minerals alone. The use of a different initial organic matter (fresh *vs.* pre-composted) may explain these differences, underlining the importance of a given environment including organic matter quality, the mineral matrix and biological components for C stabilisation (Mikutta *et al*, 2007).

Worm presence decreased the degradation rate k , except with goethite, while without worms, clays accelerated organic matter degradation. Thus, worms seem to play a crucial role in the regulation of the degradation process of a mineral-organic mixture. Moreover, the decomposition degree of organic matter in vermicomposts seems to be also influenced by the mineral presence (**Table 7**). NMR results indicate that the relative amount of aromatic compounds increased when minerals were present, suggesting that they may protect organic matter, similarly to co-compost treatments and/or they favour the neo-formation of organic compounds by worms. However, our observations with TEM did not confirm this hypothesis since we observed minerals sorbed on degraded cell wall, generally composed with pecto-cellulosic molecules, mainly from plant and microbial origin (**Fig. 18**).

Mechanisms of organic matter protection by minerals appeared altered when worms were present. The relationship between the theoretical remaining carbon (Feng *et al*, 2013) and the real amount of remaining carbon is less pertinent with worms than without ($y= 0.0002$ with worms and $y=0.0003$ without worms instead of $y=0.001$ for theoretical value) (**Fig. 23**). As in regular compost, predictions of carbon remaining with 2:1 mineral lead to higher values than the observed carbon remaining, underlining that carbon loading onto 2:1 mineral surfaces was overestimated by this relationship and suggesting that the specific surface area of 2:1 mineral was not completely available for organo-mineral associations, even if worms were present. However, with worms, the relationship between the specific surface area and the remaining carbon is stronger than without worms ($R^2= 0.62$), which may indicate that worms improve

organo-mineral associations. Moreover, the lowest dissolved organic carbon pools were found in presence of montmorillonite and worms (**Fig. 21**), suggesting that worms promote the decrease of labile carbon through adsorption onto clay. This supports the idea that worms may accelerate the breakdown of organic matter into labile compounds (Cole *et al*, 2002) and further enhance their stabilization by incorporation within microaggregates (Bossuyt *et al*, 2005). This hypothesis is also supported by TEM observations showing homogeneous distribution of organic matter onto minerals and important degradation of organic matter by worms (**Fig. 18**).

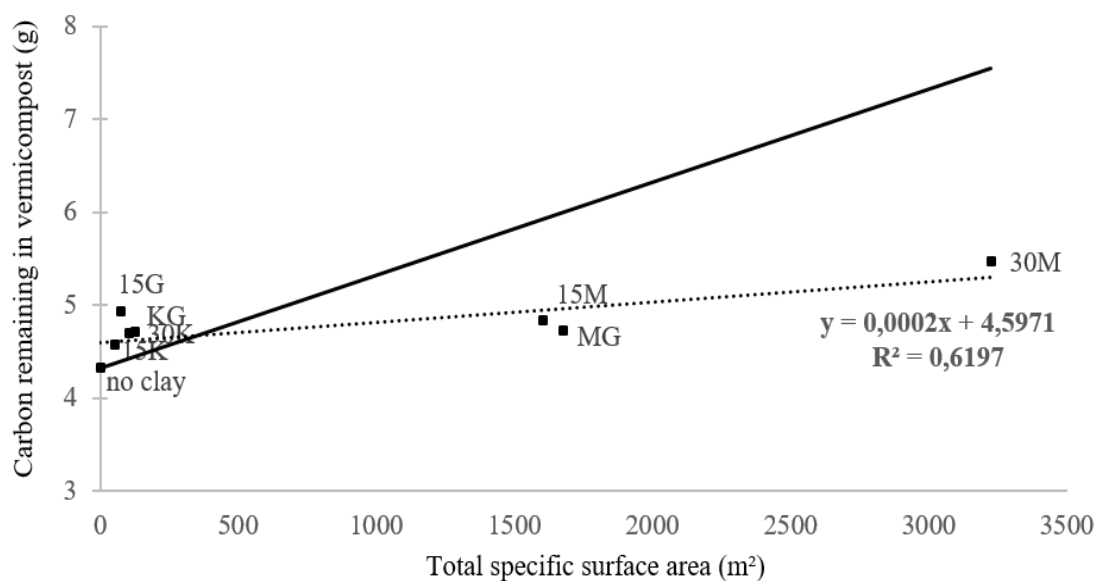


Figure 24 Relationship between remaining carbon in vermicompost, with and without minerals at day 196 and the specific surface area of added minerals. Solid line represents the estimated remaining carbon, based on the hypothesis that 1 mg of carbon might be retained per m² of mineral specific surface area (Feng *et al*, 2013).

In kaolinite-goethite treatments, similar calculations revealed that in the presence of worms 0.94 ± 0.04 mg carbon per m² of mineral surface remained in the final product, while in the absence of worms, only 0.83 ± 0.03 g C was bond to minerals. The higher carbon retention with worms may be explained by the fact that degraded and intact organic matter seemed to be associated to the mineral matrix (**Fig. 17 and 18**), while in the absence of worms, only degraded organic matter is associated to mineral surfaces (**Fig. 16**). Moreover, adding worms along with kaolinite-goethite minerals led to a greater dispersion of minerals due to worm mixing (**Fig. 18**), further promoting the mineral adhesion on residual cells. The interactions of OM and minerals in worm treatments may also be favoured by the secretion of Ca²⁺-rich worm mucus, most likely acting as bonding agent (Shipitalo and Protz, 1989). Moreover, vermicompost has

a pH of 7-8 (data not shown, **Chapter 3**), which may favour this type of associations. Thus, intact organic matter is likely associated with minerals in the presence of worms (**Fig. 24**).

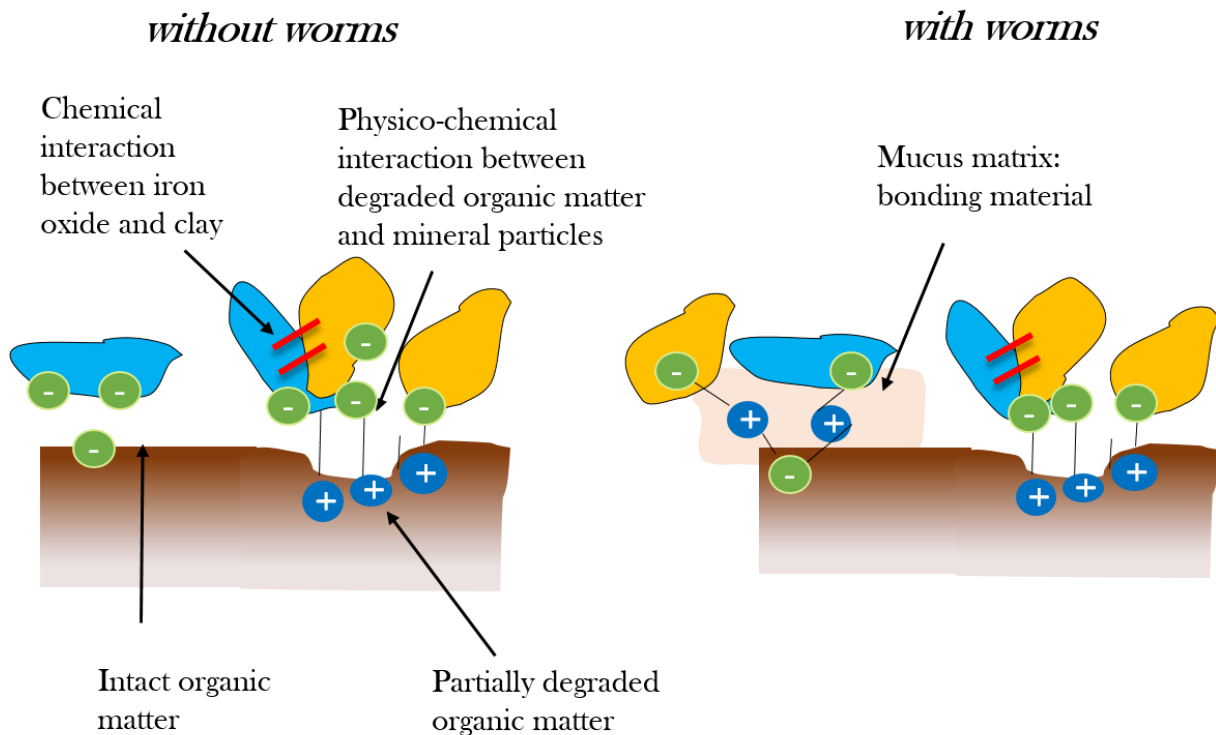


Figure 25 Conceptual view of the potential associations between organic matter and clay (1:1 kaolinite) mixed with iron oxide (goethite), with and without worms. Blue forms represent montmorillonite minerals while yellow forms represent goethite minerals.

4. Conclusion

To conclude, co-composting model systems allowed to investigate the role of minerals and worms during C stabilization. Our results indicate that the capacity of minerals to protect carbon from mineralisation depends on their mineralogy and chemical properties. The sorption of labile carbon onto iron oxide, a strong mechanism of carbon stabilization in soil, was not evidenced in this study, probably due to morphological and chemical differences of the initial organic material compared to soil organic matter. Moreover, the nature of the mineral matrix plays a crucial role for carbon stabilization, as the combination of iron oxide and clay (2:1 and 1:1) did

not improve the carbon stabilization compared to clay alone, suggesting that both minerals interacted. In addition, only a small proportion of the surface of 2:1 minerals appeared to be available to interact with fresh organic matter. In order to enhance organo-mineral associations, worms were added to our model systems. With worms, clay addition delayed the decomposition of organic matter and seemed to maximize organo-mineral associations between labile carbon and 2:1 clay. Finally, our results thus indicate that worms play a crucial role in the stabilization process of organic matter through mineral interactions. Worm presence enhanced the adhesion of both degraded and intact organic matter on minerals, while without worms only degraded organic matter appeared to be protected from further decomposition through organo-mineral associations.

Chapter 3

Addition of clay, iron oxide and worms during composting affects N, P nutrients, microbes and physicochemical properties of composts.

Article in preparation

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1. Introduction

The steady increase of world human population requires to increase food production and thus, agricultural yields (Wirsenius *et al*, 2010). To meet this objective, intensive agricultural practices involving the use of chemical fertilizers are often applied (Stewart *et al*, 2005). Although this management was efficient on the short-term, it had led to environmental issues such as eutrophication, erosion, loss of soil organic matter, etc. Moreover, the production and use of chemical fertilizer are expensive and may have harmful effects on human health (Savci, 2012). Therefore, there is a need to find a sustainable and environmentally friendly way to increase agricultural yields.

Composting and vermicomposting are both sustainable, cost-effective and ecological approaches for effective recycling of organic wastes into organic fertilizer and amendment, improving agricultural yields (Butler and Muir, 2006) or horticultural plant growth (Arancon *et al*, 2008). Although both are bio oxidative processes, they display major differences (Tognetti *et al*, 2005), usually leading to different end-products. Composting is divided into 3 phases, commonly named initial activation, thermophilic phase and maturation phase. During these phases, organic carbon compounds such as sugars or lignin are mineralised through microbial activities, producing CO₂, NH₃, organic acids and heat (Bernal *et al*, 2009). During composting, a succession of microorganisms (Mehta *et al*, 2014) develop, according to the temperature of the composting pile. For instance, fungi are not present above 60°C when bacteria predominate (Klamer and Bååth, 1998). Thus, the relative abundance of microbial groups as well as the temperature might be good indicators of the compost advancement.

The vermicomposting process, similarly to composting, is a bio-oxidative process. But in addition, worms intensively interact with microorganisms thus accelerating the degradation of organic matter (Edwards *et al*, 2004; Dominguez *et al*, 2010). Vermicomposting can be divided into 2 phases: worms enhance first microbial action by physically altering the organic matter leading to a rapid decomposition of the OM by microorganisms (Lores *et al*, 2006; Aira *et al*, 2007). When readily assimilable compounds have decreased, worms feed on microorganisms, decreasing OM mineralization and thus stabilizing the end-product. The presence of worms changes the microbial abundance compared to composting: at the beginning of vermicomposting, fungal and bacterial communities are abundant (Lazcano *et al*, 2008) and decrease with time (Gomez-Brandon *et al*, 2011, 2013; Huang *et al*, 2013). Due to its low cost, the use of vermicomposting has considerably developed, and more and more organic wastes are

treated by this process (Dominguez and Edwards, 2010). Moreover, the end-product is often defined as more stabilized (Lazcano *et al*, 2008) and richer in nutrients in forms that are readily taken up by plants compared to a regular compost (Gosh *et al*, 1999).

Although both recycling methods produce organic fertilizers and amendments, which can be an alternative to synthetic fertilizers, they release considerable amounts of greenhouse gases (Beck-Friis *et al*, 2001; Chan *et al*, 2011). Recent studies suggested to add minerals during composting and vermicomposting to reduce these emissions (Bolan *et al*, 2012; Chowdhury *et al*, 2014; Barthod *et al*, 2016). However, the major concern when adding a substrate to a compost is the decrease of nutrient availability, due to dilution (Banegas *et al*, 2007) or adsorption onto mineral additives. For instance, Wong *et al* (1999) and Menon *et al* (1993) observed that, compared to a regular sewage sludge compost, the soluble P contents decreased after addition of coal fly ash due to the formation of insoluble phosphate compounds. However, some mineral such as phosphate rock or lime and fly ash might increase the nutrient contents of the final compost (Zhang *et al*, 2017, Lu *et al*, 2008; Gabhane *et al*, 2012).

Several studies investigated the impact of mineral additives on compost quality in terms of nutrient availability, but the impact of these additives on vermicomposts and on microbial activity are unclear. To close this knowledge gap, we produced organic amendments with minerals, through composting and vermicomposting processes (**Chapter 2**), and assessed their quality and microbial properties. Our aim was to investigate the effects of the addition of different amounts of clay minerals and iron oxide, separately or in combination on (1) the physicochemical properties of the compost obtained from fresh organic wastes and (2) the microbial communities present in the end-products. We also investigated the effects on these parameters of worms, in association with minerals or not. We hypothesized that 1) due to nutrient adsorption, the quality of the end-product is decreased in the presence of minerals; 2) the type and amount of mineral might influence the microbial abundance and communities; 3) due to worm activities, vermicomposting process enhances the nitrogen and phosphorus bio-availability of the end-product compared to composting, also in the presence of minerals.

2. Materials and Methods

2.1. Experimental set-up

Fresh organic materials (lettuces, apples, residual maize, ground spent coffee and pieces of cardboard) were incubated in small composters (2L) as described in **Chapter 2**. Briefly, fresh organic materials were composted in controlled conditions during 196 days (in the dark at 20°C). Three minerals were added and thoroughly mixed with the fresh organic materials at the beginning of the experiment in different proportions and combinations. These treatments are summarised in **Table 9**. All treatments were established in four replicates. Vermicompost treatments were done with *Eisenia andrei* and *foetida* species, from La ferme du Moutta. Minerals were purchased from Sigma-Aldrich in powder form. Their main characteristics are mentioned in **Chapter 2**.

Table 9 Summary of the different treatments performed with the corresponding mineral types and amounts.

	Minerals	Mineral amount (% of dry weight of initial fresh organic materials)	Worm presence	Treatment name
No mineral	-	-	yes	V _{control}
			no	C _{control}
Pure minerals	Kaolinite	15%	yes	V15K
			no	C15K
		30%	yes	V30K
			no	C30K
	Montmorillonite	15%	yes	V15M
			no	C15M
		30%	yes	V30M
			no	C30M
	Goethite	15%	yes	VG
			no	CG
Mixture of minerals	Montmorillonite + Goethite	15%-15%	yes	VMG
			no	CMG
	Kaolinite + Goethite	15%-15%	yes	VKG
			no	CKG

2.2. Analytical methods

2.2.1. Elemental analysis

OC and N contents were measured using a CHN auto-analyser (CHN NA 1500, Carlo Erba). Dissolved organic carbon (DOC) contents were measured in 0.034 mol L⁻¹ K₂SO₄ extracts (1:5 w/v) using a total organic carbon analyzer (TOC 5050A, Shimadzu). NO₃⁻ and NH₄⁺ contents were measured in 1M KCl extracts (1: 5 w/v). The extracts were filtered then analyzed by colorimetry on a continuous flow analyzer (Skalar, The Netherlands). A glass electrode (METLER instruments) was used to measure pH and electrical conductivity (EC) in water extracts of (vermi)-composts (1:5).

2.2.2. Phosphorus fractionation

Final composts and vermicomposts with and without additives were subjected to the Hedley P fractionation (Hedley et al, 1982). Triplicate samples (0.5g, air-dried and ground) were put in polyethylene tubes and successively extracted with 30 mL distilled water, 0.5 M NaHCO₃ at pH 8.5, 0.1 M NaOH and finally with 1M HCl by shaking the samples for 16h. For each extraction, the solution was filtered (0.45 µm) and stored at 4°C until the P quantification. The residual P was extracted with a NaBrO solution from the HCl fraction. The inorganic P concentrations for each extracted solution, including the residual fraction were evaluated by colorimetry and the total P was estimated after an oxidation with a NaBrO solution. The concentration of organic P (Po) was calculated as the difference between total P (Pt) and inorganic P (Pi).

The H₂O and NaHCO₃ fractions represent the most readily soluble P pool, usually available to microbes and plants on the short term. The NaOH fraction is considered as a moderately labile pool and the HCl as non-labile inorganic P (Cross and Schlesinger, 1995; Ivanoff *et al*, 1998). Remaining residual P is highly resistant to chemical attack and contains both organic and inorganic P (Velasquez *et al*, 2016).

2.2.3. Microbial biomass

Microbial biomass of the end-products was quantified using the chloroform-fumigation extraction method (Vance *et al*, 1987). Briefly, 5 g of fresh material were fumigated with CHCl₃ in a desiccator during 16h, which allows lysis of microbial cells. The fumigated and non-

fumigated samples were extracted using 0.034 M K₂SO₄ (1:4 w/v) and the extract was filtered and analyzed for DOC using a TOC analyzer (Shimadzu). Microbial biomass C was calculated using the following equation:

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

Where MBC is the microbial biomass carbon, MB is the organic carbon extracted from fumigated and non-fumigated compost. 2.22 is a constant taking into account the efficiency of the extraction.

2.2.4. Phospholipids fatty acids (PLFA)

Lipid profiles were analysed after extraction of 1g (freeze-dried) of materials according to the method described by Frostegård *et al* (1991). To summarize, lipids were first extracted with a chloroform/methanol/citrate buffer solution, then separated by using a silicic acid column, and finally methanolysed. The final extract was analyzed with GC-FID. The following n-alcanoic acids were considered as originating from Gram-positive bacteria: iso C15:0, anteiso C15:0, iso C16:0 and iso C17:0 (ref). The Gram negative bacteria specific compounds were: C17:0, cis C18:1 ω 9, trans C18:1 ω 9 and C19:0. We considered C18:2 ω 6,9 acids as fungal marker.

2.3. Statistical analysis

All reported data are the arithmetic means of three or four replicates, according to the analysis done. A Kruskal-Wallis test was performed to assess the significant difference among treatments. 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. Physico-chemical characteristics of the composts and vermicomposts

Elemental composition and general characteristics of the end-products are given in **Table 10**. Nitrogen content in composts ranged from 15.4 to 34.8 mg g⁻¹ compost, and decreased with increasing amount of added mineral due to a dilution effect. Although differences were not

significant, the addition of 15% goethite tended to increase nitrogen in the finished product compared to the addition of 15% montmorillonite or 15% kaolinite. Similarly, when treatments with 30% minerals are compared, goethite increased the final nitrogen content compared to other minerals. In vermicomposts, the nitrogen contents were in the same range as in composts (from 15.5 to 35.2 mg g⁻¹ compost). Contrary to composting, when treatments with 15% and 30% minerals are compared, the amount of total nitrogen was not different when goethite was used during vermicomposting.

Similarly to nitrogen contents, carbon contents in composts and vermicomposts were also decreased due to addition of mineral. Carbon contents in vermicomposts were significantly lower than in composts. Therefore, lower C:N ratio were recorded for vermicomposts (ranging from 8.6 to 9.5) compared to composts (from 11.1 to 13.6). Vermicomposts had pH between 7.5-8.3, while composts had slightly alkaline pH (8.2-9.2). Kaolinite and goethite significantly increased compost pH, while with montmorillonite, no change was observed compared to regular compost. Regardless of the type and amount of clay added, the electrical conductivity (EC) was higher in vermicomposts (1.3 to 3.1 dS m⁻¹) than in composts (0.8 to 2.0 dS m⁻¹). Moreover, in both processes, the presence of clay significantly decreased EC values, up to 40%, compared to the respective regular compost and vermicompost. Finally, dissolved organic carbon (DOC) represented less than 14% of the total OC for all treatments. For both vermicomposts and composts, the addition of kaolinite or goethite led to the highest DOC contents, larger than 10%, while the addition of montmorillonite drastically decreased DOC proportions. Vermicomposts showed lower proportions of DOC than composts.

Table 10 Physico-chemical characteristics of the co-compost and co-vermicompost obtained with minerals and oxide.

Treatment	Total N (mg g ⁻¹ compost)	Total OC (mg g ⁻¹ compost)	C:N	pH	EC (dS m ⁻¹)	DOC (% OC)
C_{control}	34,8 ^a	381,2 ^a	11,1 ^{abcd}	8,3 ^c	2,0 ^{bc}	9,7 ^{bc}
C15K	20,3 ^b	273,1 ^{ab}	13,6 ^a	9,2 ^a	1,6 ^{cd}	11,5 ^{ab}
C15M	20,2 ^{bc}	244,5 ^{bc}	12,2 ^{ab}	8,2 ^{cd}	1,2 ^{def}	8,3 ^c
C30K	15,9 ^{def}	177,5 ^{efg}	11,3 ^{ab}	9,0 ^a	1,1 ^{ef}	13,3 ^a
C30M	15,4 ^f	179,9 ^{efg}	11,8 ^{bcde}	8,2 ^{cd}	1,2 ^{ef}	2,8 ^{de}
CG	23,1 ^b	261,0 ^{bc}	11,3 ^{ab}	8,7 ^{ab}	1,2 ^{def}	12,1 ^{ab}
CMG	16,5 ^{def}	182,7 ^{ef}	11,1 ^{abc}	8,2 ^c	0,8 ^f	4,1 ^{de}
CKG	17,3 ^{de}	204,5 ^{de}	11,8 ^{ab}	8,8 ^a	1,0 ^f	11,4 ^{ab}
V_{control}	35,2 ^a	331,2 ^{ab}	9,4 ^{ef}	7,6 ^{ef}	3,1 ^a	3,9 ^d
V15K	22,2 ^b	211,1 ^{cd}	9,5 ^{cdef}	7,6 ^f	1,9 ^{abc}	10,3 ^{bc}
V15M	23,1 ^{ab}	206,5 ^{cde}	9,0 ^{fg}	7,8 ^{ef}	1,9 ^c	2,9 ^{de}
V30K	15,5 ^{ef}	145,2 ^h	9,4 ^{def}	7,9 ^{de}	1,8 ^c	10,0 ^{bc}
V30M	16,5 ^{def}	152,5 ^{fgh}	9,3 ^{efg}	7,6 ^f	1,3 ^{def}	2,3 ^e
VG	22,8 ^b	201,7 ^{de}	8,9 ^{fg}	7,8 ^{ef}	2,9 ^{ab}	7,9 ^c
VMG	17,6 ^{cd}	151,8 ^{fgh}	8,6 ^g	7,5 ^{ef}	2,1 ^{abc}	2,6 ^{de}
VKG	16,4 ^{def}	147,7 ^{gh}	9,0 ^{fg}	8,3 ^{bc}	1,6 ^{cde}	10,0 ^{bc}

3.2. Microbial abundance and community structure in composts and vermicomposts

Microbial carbon biomass, measured in all treatments the last day of composting and vermicomposting (**Table 11**), ranged from 2.36 to 80.62 mg g⁻¹ compost, representing 1.85-20.85% of the total carbon in the organic material. Microbial biomass amount decreased when worms were present. For vermicomposts, the largest decrease was observed with montmorillonite (2.91, 2.36 and 2.82 mg g⁻¹ compost for V15M, V30M and VMG respectively). A similar trend was observed in composts (C15M, C30M and CMG) but was not significant compared to the addition of other minerals.

Table 11 Microbial carbon biomass and relative biomass carbon abundance in co-compost and co-vermicompost.

Treatment	Biomass-C (mg g ⁻¹ compost)	Biomass (%OC)
C _{control}	80,62 ^a	20,85 ^a
C15K	21,86 ^{ab}	7,90 ^{bcd}
C15M	14,47 ^{bc}	5,93 ^{bcde}
C30K	9,68 ^{cd}	5,35 ^{cdef}
C30M	12,07 ^{bc}	6,66 ^{bcd}
CG	20,28 ^b	8,43 ^{bcd}
CMG	14,29 ^{bc}	7,60 ^{bcd}
CKG	20,30 ^{ab}	9,98 ^{ab}
V _{control}	12,37 ^{bc}	3,88 ^{def}
V15K	17,91 ^{bc}	8,52 ^{bc}
V15M	2,91 ^d	1,40 ^f
V30K	15,77 ^{bc}	10,97 ^{ab}
V30M	2,36 ^d	1,55 ^{ef}
VG	19,87 ^b	9,85 ^{ab}
VMG	2,82 ^d	1,85 ^{ef}
VKG	15,44 ^{bc}	10,36 ^{ab}

To further characterize the microbial biomass, the relative abundance of Gram +, Gram – bacteria and fungi were evaluated through an analysis of phospholipid fatty acids (**Fig. 25**, **Fig.26** and **Fig. 27**) on some treatments (regular compost and vermicompost, C15M and V15M, C30M and V30M, CKG and VKG). These treatments were chosen based on previous results (*i.e.* carbon mineralization, **chapter 2**) and because these mixtures allowed to assess the effect of the amount of mineral and the presence of an iron oxide. Fungi markers represented 5.9-22.3 % of the total biomarker amount, the lowest proportion among specific biomarkers (**Fig. 27**). The relative abundance of fungi markers was the lowest with vermicomposting and was decreased by the addition of minerals. By contrast, mineral addition in compost significantly increased the abundance of fungi markers. The proportion of Gram + bacteria markers was not significantly different in composts compared to vermicomposts, with and without minerals. The proportion of gram – biomarkers tended to be higher in vermicomposts than in compost, regardless of the type and amount of minerals.

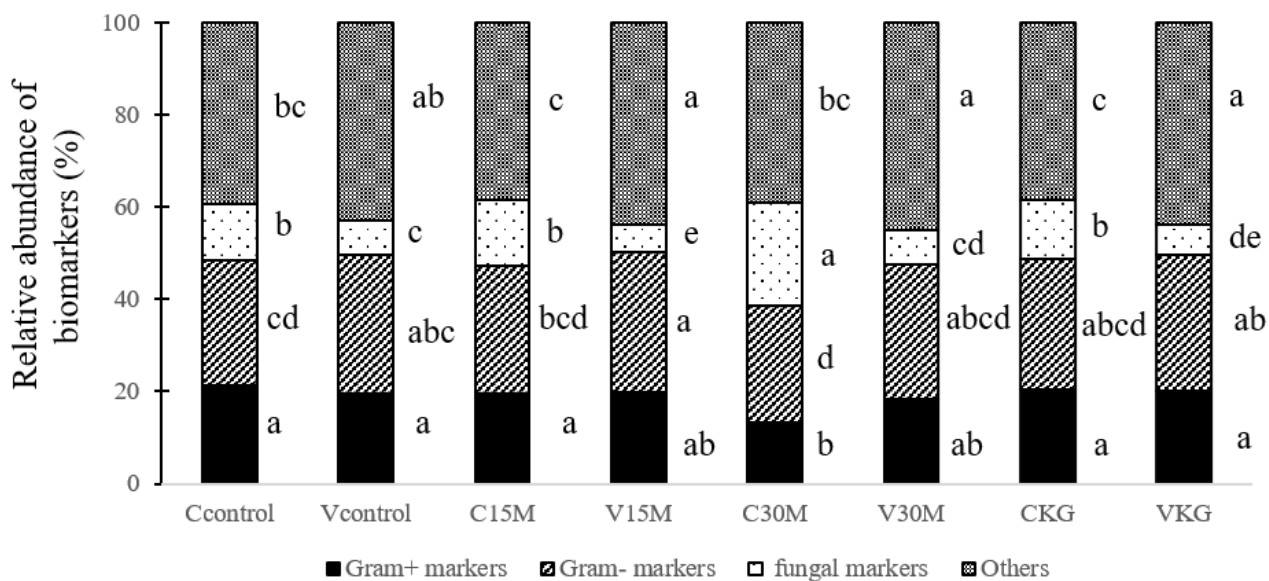


Figure 26 Relative abundance of biomarkers of the co-composts and co-vermicomposts. Different small letters indicate significant difference between similar markers.

The Gram⁺: Gram⁻ ratio (**Fig. 26**) was significantly lower in V_{control}, V15M, C30M and VKG compared to the regular compost. However, among vermicomposts and among composts, this ratio remained the same. The fungal: bacterial ratio ranged from 0.25 to 0.59 for composts, and from 0.12 to 0.16 for vermicomposts. The presence of minerals significantly increased this ratio in composts, while it was decreased or unchanged by the addition of minerals to vermicomposts.

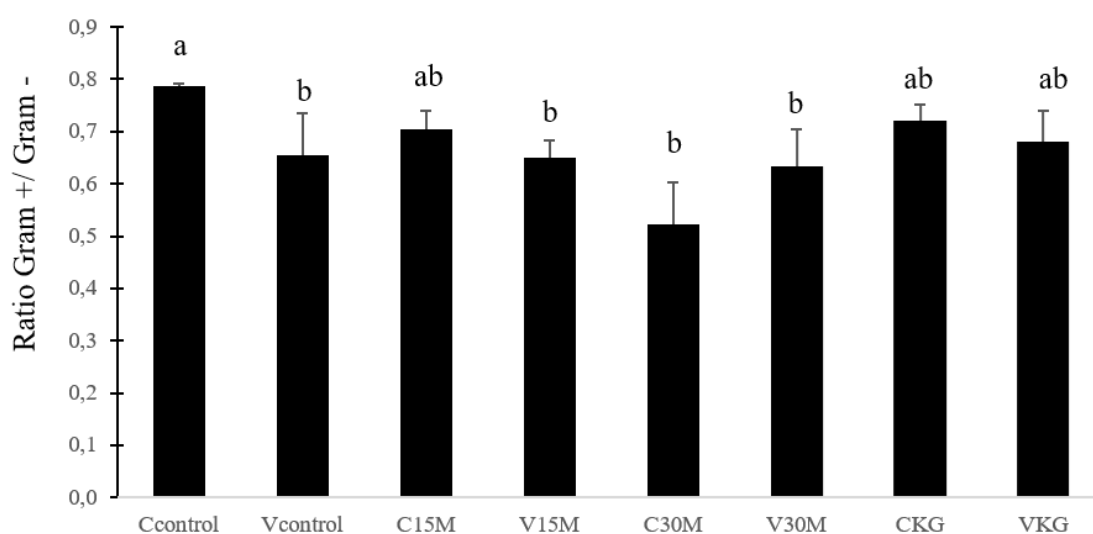


Figure 27 Ratio of Gram⁺: Gram⁻ biomarkers in the co-composts and co-vermicomposts. Error bars represent the standard error (n=3). Different small letters indicate significant differences.

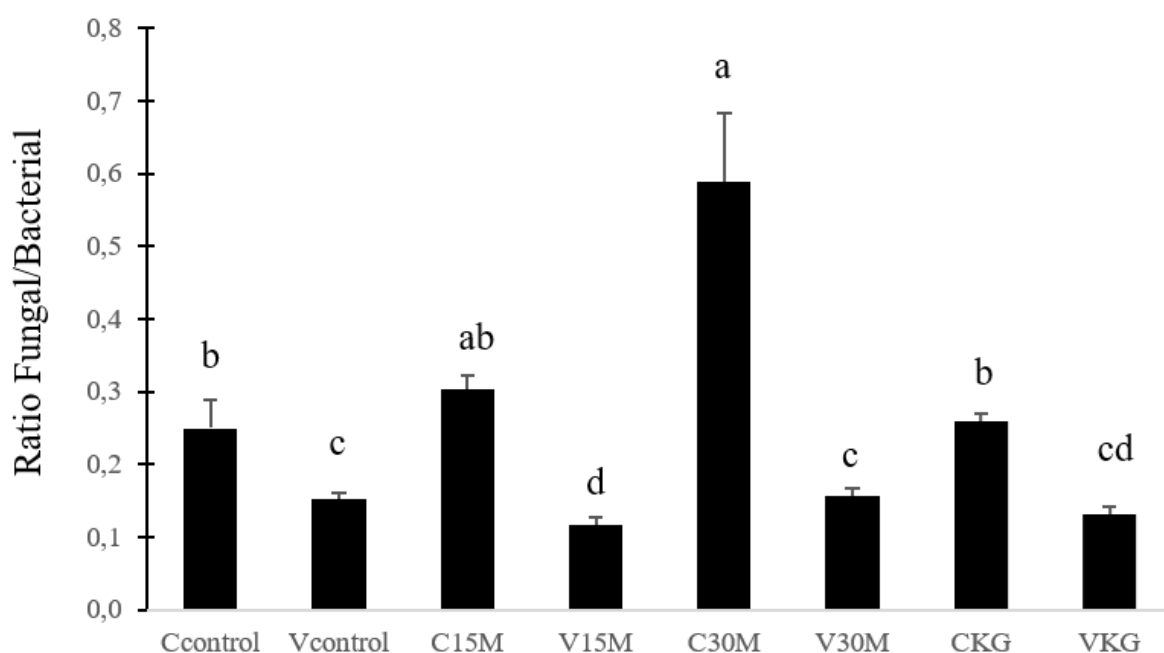


Figure 28 Ratio of fungal : bacterial biomarkers in the co-compost and co-vermicomposts. Error bars represent the standard error (n=3). Different small letters indicate significant differences.

3.3. Phosphorus and nitrogen availability

The organic and inorganic forms of phosphorus were quantified using the Hedley fractionation (Table 12). For P analysis, the regular compost and vermicompost, C30M and V30M, CKG and VKG were chosen based on their carbon mineralization data (Chapter 2). Due to a dilution effect, the presence of minerals decreased the total P in composts and vermicomposts. For a similar amount of added minerals, in 30M and KG treatments, the mixture of kaolinite + goethite led to higher amounts of total P than 30% montmorillonite, regardless of the composting process. Inorganic P was drastically decreased by the addition of minerals during composting, from 50% of total P without minerals ($C_{control}$) to 35.8% and 23.2% of total P for C30M and CKG, respectively. In vermicomposts, inorganic P, representing 50% of total P, tended to be increased due to worm presence, even with mineral additives. The organic P was the highest in CKG (75.4%), followed by C30M (63.2%) then by $C_{control}$ (49.6%), while it represented 48% in co-vermicomposts V30M and VKG and 42.8% in the regular vermicompost.

Table 12 Phosphorus distribution in co-composts and co-vermicomposts. IP: inorganic phosphorus; OP: organic phosphorus. Different small letters indicate a significant difference in the same column (n=3).

Treatment	Total P (mg kg ⁻¹)	IP (mg kg ⁻¹)	OP (mg kg ⁻¹)	IP (%)	OP (%)
C_{control}	2468 ^a	1232 ^a	1223 ^a	49,92 ^{ab}	49,57 ^{bc}
V_{control}	2356 ^a	1340 ^a	1009 ^b	56,87 ^a	42,82 ^c
C30M	1083 ^c	387 ^c	684 ^c	35,77 ^{bc}	63,16 ^{ab}
V30M	1233 ^{bc}	625 ^b	600 ^c	50,72 ^a	48,69 ^c
CKG	1353 ^b	313 ^c	1020 ^{ab}	23,17 ^c	75,38 ^a
VKG	1355 ^b	686 ^b	662 ^c	50,65 ^{ab}	48,89 ^c

For regular compost and vermicompost, the contributions of inorganic P forms (**Fig. 28**) decreased in this order H₂O > NaHCO₃ > HCl > NaOH. No significant difference between compost and vermicompost were noted among these fractions. In the presence of 30% montmorillonite, opposite trends were observed between compost and vermicompost. In V30M, the contribution of the H₂O fraction significantly decreased compared to regular vermicompost while NaHCO₃ fraction increased. HCl and NaOH fractions were unchanged. In C30M, H₂O fraction increased by 14% and the contribution of NaHCO₃ and HCl fractions decreased, respectively by 3% and 7%. Finally, the addition of kaolinite and goethite led to a 39% decrease of the H₂O fraction for both VKG and CKG compared to V_{control} and C_{control}, while NaHCO₃ and NaOH fractions increased.

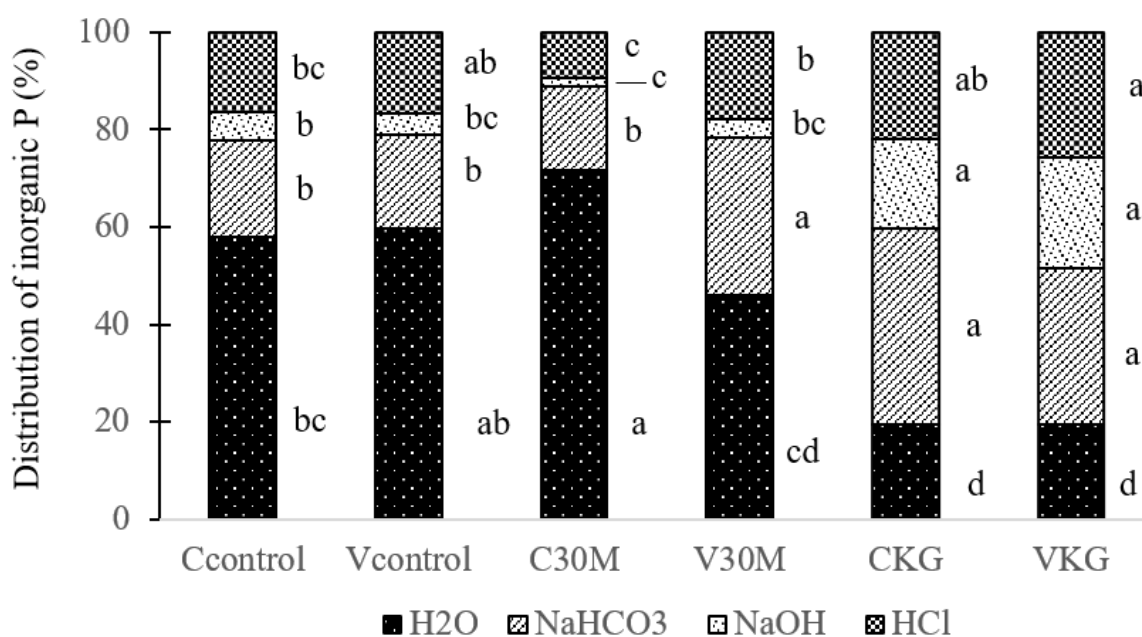


Figure 29 Inorganic phosphorus distribution in co-composts and co-vermicomposts. Different small letters indicate a significant difference for the same fraction (n=3).

The distribution of organic P is shown in **Fig. 29**. For all treatments, residual P represented the largest fraction of the total organic P, up to 69% for C30M. No consistent difference was observed between regular compost and vermicompost, except for HCl fractions, which is lower in V_{control} than C_{control} . The addition of 30% montmorillonite during composting decreased the NaHCO_3 and HCl fractions of compost, while it increased the HCl fraction in vermicompost. Finally, the presence of kaolinite and goethite significantly decreased NaOH fractions in compost (CKG), while in vermicompost (VKG), H_2O and NaOH fractions were decreased and HCl fractions largely increased compared to regular vermicompost.

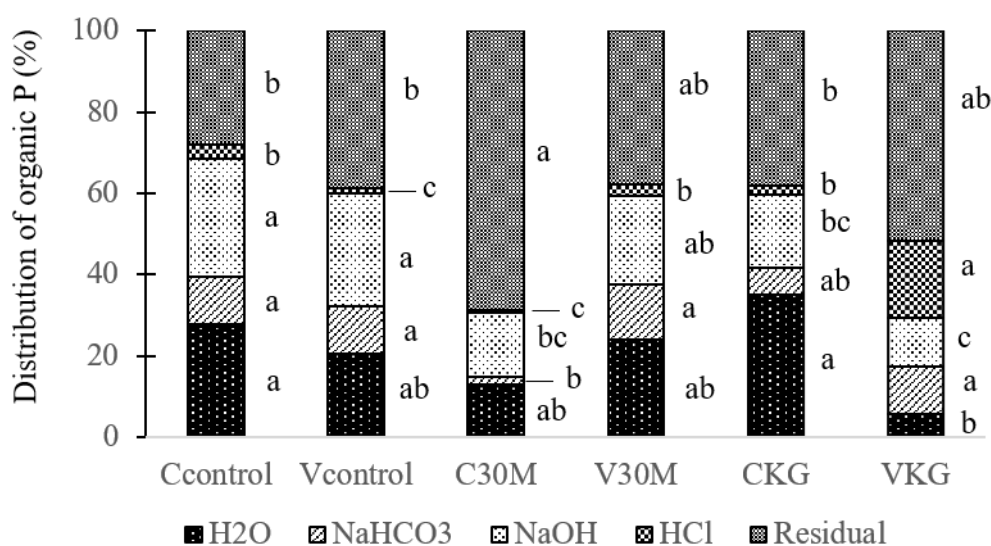


Figure 30 Organic phosphorus distribution in co-composts and co-vermicomposts. Different small letters indicate a significant difference for the same fraction (n=3).

NO_3^- and NH_4^+ forms of nitrogen were measured in all treatments (**Table 13**) and expressed per g of total N. NO_3^- content was largely higher in vermicomposts than in composts, ranging from 4.9 to 16.5 mg g⁻¹N. In compost treatments, addition of large amounts of minerals tended to decrease the NO_3^- content, except when a mixture of minerals was added (CKG and CMG). Although no significant difference was obtained within vermicomposts, the addition of kaolinite, alone or with goethite, decreased the NO_3^- content. NH_4^+ contents were the highest in composts, and consequently, $\text{NH}_4^+:\text{NO}_3^-$ ratio were higher in composts than in vermicomposts. For both processes, addition of minerals generally led to an increase of NH_4^+ content, especially in treatments with 30% kaolinite and with goethite. Finally, all ratios for composts were above 19 while for vermicomposts, they were below 0.30.

Table 13 NH_4^+ and NO_3^- contents, and their ratio, in co-composts and co-vermicomposts. Different small letters indicate a significant difference in the same column (n=4).

	Composts			Vermicomposts		
	NO_3^- (mg g^{-1} N)	NH_4^+ (mg g^{-1} N)	$\text{NH}_4^+:\text{NO}_3^-$	NO_3^- (mg g^{-1} N)	NH_4^+ (mg g^{-1} N)	$\text{NH}_4^+:\text{NO}_3^-$
control	0.08 ^{de}	1.51 ^{abc}	19.44 ^c	16.46 ^b	0.92 ^a	0.06 ^b
15K	0.08 ^{bcd}	1.71 ^{abc}	20.76 ^c	8.65 ^{ab}	1.17 ^{abc}	0.14 ^{ab}
15M	0.09 ^{cde}	1.56 ^{abc}	17.84 ^c	10.44 ^{ab}	1.09 ^{abc}	0.10 ^{ab}
30K	0.02 ^{ab}	2.46 ^{cd}	106.76 ^a	6.27 ^a	1.75 ^{bc}	0.28 ^a
30M	0.01 ^c	1.12 ^d	104.70 ^{ab}	10.40 ^{ab}	1.08 ^{abc}	0.10 ^{ab}
G	0.08 ^{abcd}	2.39 ^{bc}	30.52 ^{abc}	13.42 ^{ab}	1.29 ^{ab}	0.10 ^{ab}
KG	0.18 ^a	3.20 ^a	17.57 ^c	4.90 ^{ab}	1.09 ^c	0.22 ^a
MG	0.11 ^{abc}	2.55 ^{ab}	23.71 ^{bc}	13.46 ^{ab}	1.19 ^{abc}	0.09 ^{ab}

4. Discussion

4.1. Influence of minerals on the compost physico-chemical characteristics

The pH value is often used to characterize compost and a range of pH 6-8.5 is suggested as a standard to ensure compatibility with plants (Campitelli and Ceppi, 2006; Hogg *et al*, 2002). Based on these criteria, the composts produced in this study with and without montmorillonite are suitable organic amendments. However, the pH value of co-composts with kaolinite and/or goethite exceed 8.5, suggesting they further require a pH correction before their application or a longer composting time than a regular compost, since pH decreases during composting due to organic acid production and CO_2 emissions (Onwosi *et al*, 2017). Another parameter widely used to characterize the end-products of composting is the electrical conductivity (Bernal *et al*, 2009; Lasaridi *et al*, 2006) as it reflects the salt concentration. An organic amendment with a value above 4 dS m^{-1} is generally considered as a source of stress for plants and a seed germination inhibitor (Iannotti *et al*, 1994). All composts and co-composts were below this threshold. Moreover, EC was decreased by the addition of minerals, likely due to their low EC. In addition to pH and EC, C:N ratio is often considered as a good indicator of the stability and decomposition degree of the compost (Bernal *et al*, 2009). Generally, a C:N ratio below 25 guarantees the maturity of the compost (TMECC, 2002) but some authors recommend a C:N ratio lower than 12 (Bernal *et al*, 1998). With C:N ratios ranging from 11.1 to 13.6, all composts from our study can be considered as mature, with a high degree of OM transformation. To further decrease this ratio to reach a value below 12 for co-composts, increasing the composting duration may be a strategy. Finally, DOC content of composts is a good indicator to predict the

amount of CO₂ that might evolve from amended soils (Mondini *et al*, 2007). Indeed, DOC represents the labile carbon pool, an easy source of energy for soil microbial biomass. Since the addition of montmorillonite decreased DOC contents, almost proportionally to its amount, while kaolinite and goethite minerals increased DOC content, soil application of co-compost with montmorillonite may be preconized to limit CO₂ emissions from amended soil.

4.2. Influence of minerals on the microbial parameters of the composts

A high quality of composts is required for their use as potting media or soil conditioners. Generally, this quality evaluation is based on the physico-chemical parameters discussed above but, some authors take into account microbial parameters (Klamer and Bååth, 1998) to assess it. The value of microbial biomass carbon found in the control (80.6 mg g⁻¹ compost) is higher than for a municipal waste compost (Khan and Joergensen, 2009) and a biogenic household waste compost (Ayed *et al*, 2007). However, the initial feedstock and thus initial C:N may explain this difference as the nutrient availabilities for microorganisms are different (Eiland *et al*, 2001). Although few statistical difference were observed, in the presence of mineral this microbial biomass carbon drastically decreases likely due to a physical change and a lower amount of labile carbon as an energy source.

Compost PLFA can be used to characterize their viable microbial biomass (Vestal and White, 1989) and also contain information on physiological biomarkers of some microbes (Zelles *et al*, 1995). According to Klamer and Baath (1998), Gram – bacteria and fungi appear to grow only below 50°C while Gram + bacteria are considered as thermophilic. Therefore, PLFA analysis may provide a reliable tool for evaluating the compost maturity (Amir *et al*, 2010). The Gram+: Gram – ratios below 1 of our compost and co-composts indicate a high maturity degree, which is consistent with the physico-chemical parameters already discussed (**Fig. 26**). The presence of minerals during composting, and particularly of montmorillonite, tends to decrease this ratio, while increasing the fungal: bacterial ratio (**Fig.26**). These observations suggest that microbial communities are driven by the amount and clay mineralogy of compost additives. The microbial and physico-chemical characteristics together thus indicate that montmorillonite addition may allow to increase the maturity of the compost.

4.3. Influence of minerals on the compost nutrient availability

Compost is often used as potting media or soil conditioner (Zaller, 2007) in order to increase soil carbon stocks (Ngo *et al*, 2012) and improve plant growth and yields (Hashemimadjd *et al*, 2004). Therefore, compost nutrient availability is an important parameter since a low

availability will not be beneficial for plants and require further application of chemical fertilizer while a high availability, particularly for nitrogen, may have negative effects on seed germination (Osuna *et al*, 2015). According to Bernal *et al* (2009), the optimal $\text{NO}_3^-:\text{NH}_4^+$ ratio for compost is below 0.16 for very mature composts or below 3 for mature composts. With this indicator, our compost and co-composts cannot be considered as mature as they have large amounts of ammonium. Furthermore, clay minerals tended to increase the $\text{NO}_3^-:\text{NH}_4^+$ ratio, suggesting that they affect nitrification possibly through (1) a retention/protection from microbial attack of ammonium onto clay minerals (Strong *et al*, 1999) or (2) a decrease of the activity/abundance of nitrifying bacteria.

In soils, the proportions of the labile phosphorus fractions ($\text{H}_2\text{O-P}$ and $\text{NaHCO}_3\text{-P}$) and of inorganic P rarely exceed 18% of total P (Negassa and Leinweber, 2009). In composts, these fractions represent nearly 30% of total P (**Table 12**), which is consistent with previous studies (Khan and Joergensen, 2009). However, the sum of the proportions of the least labile fractions, HCl-P and residual P, generally associated with Ca, Fe or Al (Tiessen and Moir, 1993) reached approximately 50% of total P in the regular compost and were even higher in the co-compost with 30% montmorillonite. We can thus conclude that the presence of clay minerals decreases the phosphorus availability, likely due to the formation of associations between phosphorus and mineral specific surfaces, comparable to those evidenced in soils (Shen *et al*, 2011; Hinsinger *et al*, 2001).

4.4. Worms may counterbalance the negative effects of minerals

Without minerals, vermicomposting significantly modified the physico-chemical properties of the end-products compared to composts. The lower pH recorded in vermicomposts compared to composts might be attributed to a larger microbial production of organic acids and CO_2 than during composting (Gunadi and Edwards, 2003; Garg *et al*, 2006). Our pH values were slightly lower compared to those of Garg *et al* (2006) for a kitchen waste feedstock (7.5 vs. 8.3) but the duration of vermicomposting was different (196 days vs. 100 days). As mentioned by Elvira *et al* (1997), a decrease in pH may have beneficial effects on nitrogen retention, since nitrogen may be lost as volatile ammonia at high pH. Moreover, the pH close to neutral suggests that the vermicomposts might be useful for restoring acidic soils (Huang *et al*, 2014). The addition of minerals, despite their low pH, increased the vermicompost pH, which nevertheless remained lower than that of similar composts. Increase of vermicompost pH with minerals might be due

to a lower production of CO₂ (**Chapter 2**) and organic acids, related to a decrease of microbial biomass (**Table 11**). Electrical conductivity values were in the same range as those obtained by Garg *et al* (2006). Similarly to pH values, electrical conductivity increased in vermicompost compared to the corresponding compost, almost by a factor 2. This increase might be due to the generation of free ions during ingestion and excretion of organic matter by worms. A high EC (above 4 dS m⁻¹) may negatively influence plant growth as it reflects the salt concentration in the organic amendment (Lasaradi *et al*, 2006). The presence of minerals within vermicompost, in particular montmorillonite allows to decrease the EC and potentially avoid ecophysiological risks for plants. In addition, the presence of worms decreased the C: N ratio (Table X) below the value of 12 recommended by Bernal *et al* (1998), suggesting a higher decomposition degree of organic matter than without worms (Elvira *et al*, 1997). This trend was previously observed since worms, combined with microorganism activities, drastically reduce the initial OC content to produce CO₂ (Nigussie *et al*, 2017).

Similar proportions of bacterial markers were measured in regular vermicompost and compost. Fungal biomarker proportion decreased in vermicompost, as previously observed (Gomez-Brandon *et al*, 2013; Huang *et al*, 2013). Microbial biomass might be directly affected by the gut transit process, worms feeding on microorganisms preferentially to fungi (Curry and Schmidt, 2007; Shan *et al*, 2013). This hypothesis is further supported by the low amount of microbial biomass carbon in vermicompost (**Table. 25**). However, other studies obtained the opposite trend, an increase in microbial biomass with vermicomposting compared to composting of olive-mill waste (Vivas *et al*, 2009), municipal solid waste (Paul *et al*, 2011) or fresh fruit and vegetable waste (Huang *et al*, 2014) probably due to different substrates and environmental conditions used.

Compared to the regular vermicompost, microbial biomass amounts in co-vermicomposts only decreased with montmorillonite, regardless of its amount while with other minerals it increased. This suggests that montmorillonite decreases the growth of microorganisms, likely due to a retention of energy sources as carbon, nutrients or water (Chenu *et al*, 2002). By contrast, kaolinite and goethite with worms may enhance microorganism development due to (1) a small proportion of nutrients retained on their surface and a rapid desorption of these elements and/or (2) a fast colonization of microorganisms on their surface after their ingestion by worms. However, the carbon mineralized during vermicomposting (**Chapter 2**) suggested a low microbial activity when kaolinite and goethite are mixed with organic matter. These hypotheses

have to be tested in the future by measuring microbial parameters in the course of vermicomposting.

Although total N and P were in similar amounts in vermicomposts and composts, their bio-availabilities were drastically modified by worm presence. NO_3^- content increased with worms (**Table 13**) probably due to the organic N mineralization and/or the reduction of N_2 losses through denitrification (Nigussie *et al*, 2017; Lazcano *et al*, 2008). Therefore, the $\text{NH}_4^+ : \text{NO}_3^-$ ratio of vermicomposts can be considered as optimum as it is under the preconized threshold proposed by Bernal *et al* (2009), suggesting that worms improve compost quality. Finally, the presence of worms changed the inorganic and organic phosphorus distribution (**Table 12**). Worms significantly decreased the HCl-P fraction. Gosh *et al* (1999) previously observed that worms may increase soluble P fractions due to the rapid decomposition of organic matter and thus the conversion of insoluble P to more soluble forms. Although minerals tended to increase NH_4^+ , decrease NO_3^- content and increase the recalcitrant P fractions, vermicompost with minerals showed higher nitrate and soluble P proportions than co-composts (**Table 13**), suggesting that worm presence might counterbalance N immobilisation and P adsorption on the inorganic matrix.

5. Conclusion

The information presented here should provide a sound basis for the management of kitchen wastes mixed with minerals since the main effects of minerals on the physicochemical properties of composts were described. The benefices observed with composting with minerals were a reduction of carbon mineralized during the process and of electrical conductivity of the final product, and a low DOC content suggesting a higher stability in soil. However, similarly to what happens in soils, minerals in compost retain nutrients and affect the nitrogen cycle, leading to a compost potentially immature for plant growth. This negative effect appears dependent on the clay mineralogy, montmorillonite involving the highest retention of nutrients. In order to use these co-composts as potting media or soil conditioner without environmental risk, composting duration should be increased until nutrient availability, in particular nitrogen, increases above usual standard value for a compost. Our study showed that using worms during co-composting is another efficient strategy. Although more CO_2 was released in the presence of worms compared to regular co-composting, worms appeared to play a key role in nutrient cycle as they increased the nutrient availability and compost stability even if minerals were

present. However, the interaction between microorganisms, worms and minerals requires further work to understand the nutrient cycles, the capacity of minerals to adsorb/desorb energy sources or the potential shift of microbial activities and communities due to worms and clay mineralogy.

Chapter 4

Influence of application to soil of co-compost and co-vermicompost produced with clay minerals and iron oxides on carbon mineralization and distribution

Article in preparation

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1. Introduction

The increase in world human population since the last century have induced an important change in the land-use management and the agricultural practices in order to support the increasing demand for goods, foods and services. However, the change of land-use coupled with the intensive agricultural practices have led to a depletion of soil organic matter (Guo and Gifford, 2002; Lal, 2006), inducing major adverse effects such as soil erosion (Gregorich *et al*, 1998), decrease of soil fertility (Tiessen *et al*, 1994; Kononova, 2013) and indirectly a negative impact on the environment and climate change through an increase of greenhouse gas emissions due to the land-use change (Fearnside, 2000; Foley *et al*, 2005; Houghton *et al*, 2012) and the use of chemical fertilizer to restore soil fertility (Hillier *et al*, 2009). Therefore, this situation generates a serious environmental issue, calling to find clean and sustainable agricultural practices. Several management practices have been suggested in order to improve degraded soil and/or to avoid the depletion of soil organic matter, such as the application of organic amendments as compost or vermicompost to soil (Ngo *et al*, 2014; Annabi *et al*, 2011, D'Hose *et al*, 2016). This practice has two main advantages, the environmentally favourable management of wastes and the improvement of soil organic carbon stocks.

Composting and vermicomposting are two processes based on the bio-oxidation of organic matter into a stable product (Zuconi and Bertoldi, 1987; Lazcano *et al*, 2008). Applying these organic materials improves the soil health and fertility, enhancing plant growth (Donn *et al*, 2014), reduces soil erosion and nutrient leaching (Doan *et al*, 2015), and also increases soil carbon storage (Ngo *et al*, 2014). However, some studies underline that these organic materials have a fast degradation rate in soil (Fabrizio *et al*, 2009), inducing an increase of GHG emissions from soil due to the priming effect (Thangarajan *et al*, 2013). This degradation rate of composted organic matter is related to the initial feedstock composition, the composting process and the degree of stabilisation of the compost (Mondini *et al*, 2007; Bernal *et al*, 1998; Cambardella *et al*, 2003; Bustamante *et al*, 2010). Therefore, application of some organic materials may become more a source of carbon in the atmosphere than a sink (Thangarajan *et al*, 2013). New strategies to limit the fast degradation of exogenous organic materials have been recently explored, such as their transformation into biochar (Van Zwieten *et al*, 2010; Agegnehu *et al*, 2015; Bolan *et al*, 2012). Biochar can also be added during composting process as a co-substrate (Steiner *et al*, 2010; Dias *et al*, 2010). Beside biochar, organic carbon stabilization during composting may be improved by the addition of inert material such as clay minerals (Bolan *et al*, 2012; Barthod *et al*, 2016) or industrial residues as fly ash and red mud

(Chowdhury *et al*, 2014). However, little is known on the stabilization of organic matter during vermicomposting with minerals and on its stability after soil application.

In soil, worms are considered as key engineers, involved in the protection of soil carbon (Bossuyt *et al*, 2005; Shipitalo and Protz, 1988; Bossuyt *et al*, 2004; Schrader and Zhang, 1997). However, their influence on the carbon stabilization and formation of organo-mineral associations is still poorly understood. Soil ingestion and destruction through worm gut might lead to destabilization of soil structure. Some studies showed that casts or new aggregates formed by worms are more stable than initial soil aggregates (Shipitalo and Protz, 1988; Zhang and Schrader, 1993). According to Bossuyt *et al* (2004), *Aporrectodea caliginosa* worms helped to form large macroaggregates, composed of more stable microaggregates than if they were formed without worms. Moreover, these new aggregates might further enhance the physical protection of carbon from microbial mineralization on the long term, from years to decades (Brown *et al*, 2000). By analogy with the mechanisms studied in soil, worm activity during vermicomposting may lead to a more stable organic matter than with composting (Lazcano *et al*, 2008; Gomez-Brandon *et al*, 2011; Sierra *et al*, 2013) and this stability might be increased by the presence of minerals during the process.

Few studies have investigated the impact of the application to soil of co-composts produced with minerals on soil properties and soil carbon stocks (Bolan *et al*, 2012; Chowdhury *et al*, 2014) and, to the best of our knowledge, no study has investigated the impact of the application of co-vermicomposts produced with minerals. To address this knowledge gap, the effects on soil properties and carbon emissions of different organic amendments produced with minerals, through composting and vermicomposting processes were assessed (**Chapter 2**). In order to investigate the effects of the application of these co-composts and co-vermicomposts on an arable arenic cambisol, poor in organic matter and clay minerals, we evaluated (1) the carbon mineralized from the soil amended with compost and vermicompost and (2) the amended soil properties and the soil carbon distribution changes induced by the addition of these organic materials. We hypothesize that (1) the presence of minerals during composting leads to more stable organic matter than with traditional composting, which may increase carbon storage and limit carbon emissions after compost application on soil; (2) the amended soil properties and carbon mineralization from soil may be influenced by the type of mineral and its amount; (3) vermicomposting process with minerals enhances organic matter stability compared to composting, due to worm activities.

2. Materials and Methods

2.1. Soil sampling and preparation

The soil was collected from an agricultural plot near Crespières (Yvelines, France, 571° 053E 131° 535N). It is classified as an arenic cambisol (Noirot-Cosson *et al*, 2017), cropped with wheat and its characteristics are presented in **Table 14**. The upper layer of the soil (0-10 cm) was sampled before seedling in December 2015. After sampling, the soil was air-dried, sieved at 2 mm and plant residues and mesofauna were removed. The soil pF was determined in the laboratory: the soil humidity was 26.6% at pF 2.5.

Table 14 Characteristics of the soil used in this experiment.

Characteristics	Values
EC (dS m ⁻¹)	0.055
pH	6.6
Bulk density (g cm ³)	1.29
N (mg g ⁻¹)	1.03
Organic carbon (mg g ⁻¹)	10.45
C:N	10.16
Clay g kg ⁻¹	159
Fine silt g kg ⁻¹	135
Coarse silt g kg ⁻¹	245
Fine sand g kg ⁻¹	411
Coarse sand g kg ⁻¹	51

2.2. Composts and vermicomposts materials

The organic materials were obtained from a new series of composting and vermicomposting experiments in the same conditions as those described in **Chapters 2** and **3**. Fresh organic materials contained fixed contributions of lettuces, apples, residual maize, spent coffee ground and pieces of cardboard (2x3cm). Lettuces and apples, products of organic agriculture, were obtained from a general store, the spent coffee ground from a public cafeteria, and the maize from a field in Grignon (France). These organic materials were co-composted or co-vermicomposted (with worms *Eisenia andrei* and *foetida*) during 196 days with

montmorillonite (a 2:1 clay), kaolinite (a 1:1 clay) and goethite, an iron oxide. These minerals were purchased from Sigma-Aldrich in the powder form. Their main characteristics are given in **Table 15**.

Table 15 Main characteristics of the mineral materials used for composting.

Mineral	Specific surface area (m ² g ⁻¹)	pH	EC (dS m ⁻¹)
Goethite	11	4.77	0.19
Kaolinite	7.5	7.57	0.06
Montmorillonite	232	3.93	0.13

The different treatments produced were: (i) regular compost (C_{control}) and vermicompost (V_{control}); (ii) compost and vermicompost with 15% montmorillonite (C15M and V15M); (iii) compost and vermicompost with 30% montmorillonite (C30M and V30M) and (iv) compost and vermicompost with 15% kaolinite and 15% goethite (CKG and VKG). The main characteristics of these co-composts and co-vermicomposts are summarized in **Table 16**.

Table 16 Main characteristics of the co-composts and co-vermicomposts used in this experiment. Small different letters indicate statistical differences among organic amendments. C_{min}: Carbon mineralized during composting process (mg C-CO₂ g⁻¹ initial waste C); DOC: dissolved organic carbon; EC: electrical conductivity.

	NO ₃ ⁻ (µg N-NO ₃ ⁻ g ⁻¹ compost)	NH ₄ ⁺ (µg N-NH ₄ ⁺ g ⁻¹ compost)	C _{min} (mg C-CO ₂ g ⁻¹ C)	DOC (mg C g ⁻¹ compost)	Microbial biomass (mg C g ⁻¹ compost)	N (mg g ⁻¹ compost)	C (mg g ⁻¹ compost)	C/N	pH	EC (dS m ⁻¹)
V _{control}	670,4 ^a	29,0 ^b	719,8 ^a	12,2 ^c	9,6 ^e	34,6 ^a	325,4 ^{ab}	9,4 ^{bc}	7,6 ^e	3,3 ^a
C _{control}	1,7 ^d	50,6 ^a	614,8 ^{bc}	39,8 ^a	92,0 ^a	35,6 ^a	390,0 ^a	11,1 ^{abc}	8,5 ^a	1,8 ^{abc}
V15M	231,2 ^b	27,5 ^{bc}	687,3 ^a	6,3 ^d	2,9 ^f	22,9 ^{ab}	205,0 ^c	9,0 ^c	7,8 ^d	2,0 ^{ab}
C15M	1,0 ^d	33,3 ^{ab}	589,0 ^{cd}	20,8 ^b	18,4 ^{bc}	19,3 ^{bc}	249,4 ^{bc}	13,0 ^a	8,1 ^c	1,3 ^{cd}
V30M	206,7 ^b	14,4 ^d	635,9 ^b	3,5 ^e	2,9 ^f	16,5 ^d	150,8 ^d	9,1 ^c	7,4 ^e	1,4 ^{bc}
C30M	0,2 ^e	18,0 ^{cd}	540,2 ^d	4,7 ^e	14,3 ^{cd}	15,7 ^d	164,9 ^d	10,5 ^{abc}	8,2 ^c	1,2 ^{cd}
VKG	20,5 ^c	17,7 ^d	676,3 ^a	14,5 ^c	13,6 ^{de}	16,1 ^d	145,5 ^d	9,1 ^c	8,3 ^b	1,6 ^{bc}
CKG	3,9 ^c	48,9 ^a	632,0 ^{bc}	24,1 ^a	24,4 ^{ab}	17,8 ^{cd}	211,9 ^c	11,9 ^{ab}	8,8 ^a	0,8 ^d

2.3. Experimental design

The carbon mineralized from non-amended soil and soils amended with the different co-compost and co-vermicompost obtained with minerals was monitored under controlled conditions. Before incubation, the soil was re-humidified at pF 2.5 during one week. The soil was incubated at this pF in three replicates, each one containing three pots of 23 g soil (dry

weight) and one of 45 g, in 1L jar at 20°C in the dark during 7 days (pre-incubation) plus 79 days (incubation). For amended soils, the application rate of organic material was 20 mg g⁻¹ of soil (dry weight). Treatments carried out are the following: (i) soil control (no amendment), (ii) soil + C_{control}, (iii) soil+ V_{control}, (iv) soil + C15M, (v) soil + V15M, (vi) soil + C30M, (vii) soil + V30M, (viii) soil + CKG and, (ix) soil + VKG. The incubated soil was collected at 2, 15, 30 and 79 days after pre-incubation for further analysis.

2.4. Microbial biomass

Microbial biomass of non-amended and amended soils was quantified using the chloroform-fumigation extraction method (Vance *et al*, 1987). Microbial biomass amounts were measured on days 2, 15, 30 and 79. Briefly, 5 g of fresh material were fumigated with CHCl₃ in a desiccator during 16h, which allows lysis of microbial cells. The fumigated and non-fumigated samples were extracted using 0.034 M K₂SO₄ (1:4 w/v) and the extract was filtered and analyzed for DOC using a TOC analyzer (Shimadzu). Microbial biomass C was calculated using the following equation:

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

Where MBC is the microbial biomass carbon, MB is the organic carbon extracted from fumigated and non-fumigated compost. 2.22 is the coefficient used for calculation to take into account the efficiency of extraction.

2.5. Carbon fractionation

Soils were fractionated by combining physical and chemical methods (Zimmerman *et al*, 2007; Poeplau *et al*, 2013), in order to isolate five carbon pools: two slow carbon pools (silt and clay fraction and stable aggregates fraction, S+C and S+A respectively), two labile carbon pools (dissolved organic matter and particulate organic matter, DOC and POM respectively) and one recalcitrant carbon pool (oxidation resistant rSOC).

Briefly, 15g of dried soil were ultrasonically dispersed in 80 mL of deionised water (energy output: 22j mL⁻¹) to disperse labile microaggregates. Then the soil suspension was sieved on a 63 µm sieve to separate the coarse, the fine (S+C) and the dissolved (DOC) fraction. The

fraction > 63 μm was freeze-dried and separated into POM and S+A fractions by using a sodium polytungstate solution with a density of 1.8 g cm^{-3} . The recalcitrant fraction (rSOC) was quantified after sodium hypochlorite oxidation of the S+C fraction (1 g).

2.6. Analytical methods

Emitted CO_2 was regularly measured during the incubation with a MicroGC (Agilent, Santa Clara, USA). Jars were maintained closed between each measurement. When the CO_2 concentration was above 20 000 ppm, a free of CO_2 atmosphere was flushed during 20 min to avoid anaerobic conditions.

OC and N contents were measured using a CHN auto-analyser (CHN NA 1500, Carlo Erba). Dissolved organic carbon (DOC) contents were measured in 0.034 mol L^{-1} K_2SO_4 extracts (1:5 w/v) using a total organic carbon analyzer (TOC 5050A, Shimadzu). NO_3^- and NH_4^+ contents were determined in 1M KCl extracts (1: 5 w/v). The extracts were filtered then analyzed by colorimetry on a continuous flow analyzer (Skalar, The Netherlands).

2.7. Statistical analysis

The CO_2 emitted after organic material application to soil were fitted to two first-order models (Marquardt-Levenberg algorithm), using the following equations:

$$C_{\text{mineralized}} = C(1 - e^{-k}), \quad \text{equation 1}$$

$$C_{\text{mineralized}} = C_1(1 - e^{-k_1 t}) + C_2(1 - e^{-k_2 t}) \quad \text{equation 2}$$

where $C_{\text{mineralized}}$ is the carbon mineralized as CO_2 ($\text{mg C-CO}_2 \text{ g}^{-1} \text{ soil}$) at the time t (day). For the *equation 1*, C is the potentially mineralisable carbon ($\text{mg C-CO}_2 \text{ g}^{-1} \text{ soil}$), and k is the rate constant of carbon mineralization (day^{-1}). For the *equation 2*, C_1 ($\text{mg C-CO}_2 \text{ g}^{-1} \text{ soil}$) is the labile/fast mineralisable carbon pool, associated with the rate constant k_1 (day^{-1}) and C_2 ($\text{mg C-CO}_2 \text{ g}^{-1} \text{ soil}$) is the slow mineralisable carbon pool, associated with the rate constant k_2 (day^{-1}).

All reported data are the arithmetic means of three replicates. A Kruskal-Wallis test was performed to assess the significance of differences among treatments. Significance was declared significant at the 0.05 level. A principal component analysis (PCA) was carried out to differentiate the treatments based on the physical and chemical characteristics of organic materials.

Statistical analyses were carried out using the R 3.12 statistical package for Windows

(FactoMineR package for the PCA). (<http://www.r-project.org>).

3. Results

3.1. Characteristics of the organic amendments

Composts and vermicomposts have contrasting characteristics (**Table 16**). The presence of additives, montmorillonite, kaolinite and goethite, generally decreased their N and C contents, mainly due to a dilution effect. Composts had higher NH_4^+ contents than vermicomposts, and inversely for NO_3^- contents. The C: N ratios were similar among composts (ranging from 10.5 to 13) and among vermicomposts (from 9.0 to 9.4). Dissolved organic carbon contents were lower in vermicomposts than in composts. The lowest DOC content was recorded in treatments with 30% montmorillonite (3.5 and 4.7 mg C g⁻¹ compost for V30M and C30M, respectively). Similar trends were observed for the microbial biomass carbon in amendments. Lower pH values were also found in vermicomposts (7.4-8.3) compared to composts (8.1-8.8) and the contrary was observed for the electrical conductivity values. The study (**Chapter 2**) of carbon mineralization during the composting process, which produced similar amendments (described in **Chapter 3**), showed that the presence of clay and iron oxide decreased carbon mineralization. Moreover, the addition of 30% montmorillonite induced a significant 12% decrease of mineralized carbon for both composting and vermicomposting processes, leading to the lowest values of mineralized carbon for amendments produced with montmorillonite (635.8 and 540.2 mg C-CO₂ g⁻¹ initial C for V30M and C30M respectively).

3.2. Amended soil characteristics

After 79 days of incubation, the chemical and physical characteristics of non-amended soil remained unchanged, except DOC content which tended to decrease (**Table 17**). As expected, the addition of organic materials to soil increased their total organic C and total N contents, and also their DOC contents. DOC contents ranged from 32.53 (VKG) to 79.92 (CKG) µg g⁻¹ soil at day 2 and from 24.32 (V30M) to 49.02 (C_{control}) mg g⁻¹ soil at day 79. At day 2, the total organic C in amended soil ranged from 13.14 to 17.83 mg g⁻¹ soil with the highest value found in soil amended with C15M while at day 79, the highest values were recorded after addition of regular compost and vermicompost (15.72 and 15.75 mg g⁻¹ soil respectively). The C, N and DOC contents decreased during the incubation and thus, they were lower at day 79 than at day 2, except for N content which tended to increase. Therefore, C: N ratios also decreased during the incubation of composts and vermicomposts with soils. At day 2, C: N ratio were similar

among soils amended with co-vermicomposts (C: N<10) and among soils amended with co-composts (C: N> 10). Finally, NH₄⁺ and NO₃⁻ contents were measured at day 79 in non-amended and amended soils. Addition of organic amendments to soil increased both parameters, except when C_{control} was applied. Compared to non-amended soil, the greatest increase of NO₃⁻ content was recorded in soil amended with V_{control} while the largest NH₄⁺ content was found in V30M. The addition to soil of organic amendments obtained with vermicomposting generally led to higher contents of NH₄⁺ and NO₃⁻ than the addition of composts, except with CKG compost.

Table 17 Main characteristics of the amended soils at day 2 and at the end of the incubation (day 79) with co-composts and co-vermicomposts. Small different letters indicate statistical differences among organic amendments (n=3) and * indicates significant differences between day 2 and day 79.

	Day 2				Day 79					
	DOC (µg g ⁻¹ soil)	C (mg g ⁻¹ soil)	N (mg g ⁻¹ soil)	C:N	DOC (µg g ⁻¹ soil)	C (mg g ⁻¹ soil)	N (mg g ⁻¹ soil)	C:N	NO ₃ ⁻ (µg N-NO ₃ ⁻ g ⁻¹ soil)	NH ₄ ⁺ (µg N-NH ₄ ⁺ g ⁻¹ soil)
Soil	28,04 ^e	10,35 ^d	1,01 ^d	10,27 ^{abc}	15,49 ^e	10,36 ^d	1,02 ^c	10,17 ^a	12,21 ^{ef}	5,33 ^f
V _{control}	65,88 ^a	16,38 ^a	1,60 ^a	10,21 ^{abcd}	34,89 ^{ab*}	15,72 ^a	1,81 ^a	8,67 ^{cde*}	33,70 ^a	7,70 ^{ef}
C _{control}	60,54 ^{ab}	15,59 ^{ab}	1,47 ^{abc}	10,61 ^a	49,02 ^a	15,75 ^a	1,64 ^{ab}	9,62 ^{ab*}	10,15 ^f	5,47 ^f
V15M	47,64 ^{bcd}	14,18 ^{abc}	1,43 ^{abc}	9,92 ^{cd}	36,88 ^{bc}	14,36 ^{ab}	1,62 ^{ab}	8,86 ^{cd*}	26,36 ^{ab}	25,62 ^{ab}
C15M	47,16 ^{bcd}	17,83 ^a	1,71 ^a	10,46 ^{ab}	33,88 ^{ab}	14,65 ^{ab}	1,66 ^{ab}	8,80 ^{bc*}	19,91 ^{cd}	10,58 ^{ef}
V30M	37,17 ^{cde}	13,14 ^{cd}	1,33 ^{cd}	9,92 ^{bcd}	24,34 ^{de*}	13,34 ^{abc}	1,56 ^{abc*}	8,56 ^{def*}	25,57 ^{bc}	27,95 ^a
C30M	47,26 ^{bc}	16,57 ^a	1,59 ^a	10,41 ^a	27,74 ^{cd*}	13,75 ^{abc*}	1,62 ^{abc}	8,50 ^{ef*}	15,41 ^{de}	17,03 ^{cd}
CKG	79,92 ^a	15,89 ^a	1,49 ^{ab}	10,62 ^a	33,30 ^{bc*}	11,61 ^{cd*}	1,59 ^{ab}	7,31 ^{g*}	20,52 ^{bcd}	23,54 ^{bc}
VKG	32,53 ^{de}	13,28 ^{bcd}	1,36 ^{bcd}	9,79 ^d	25,73 ^{de}	12,58 ^{bcd}	1,51 ^{bc}	8,31 ^{fg*}	22,14 ^{bc}	13,04 ^{de}

3.3. Carbon mineralization and evolution of the microbial biomass

The amount of microbial biomass increased rapidly during incubation with amendments, reaching a maximum at day 15 for all treatments, except with the addition of regular compost (C_{control}) that induced a maximum at day 2 (**Fig.30**). Microbial biomass in non-amended soil remained unchanged during the incubation experiment. Regardless of additives, the content of microbial biomass was lower in soils amended with vermicomposts than in those amended with composts.

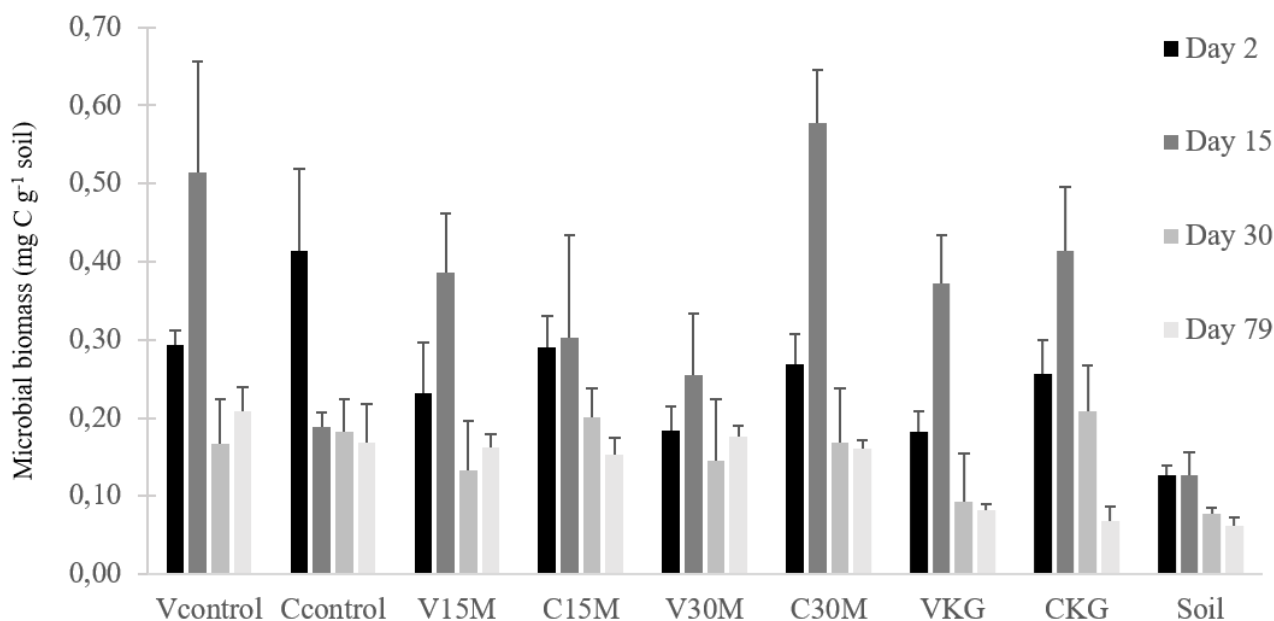


Figure 31 Evolution of the microbial carbon biomass in soils amended with various co-composts and co-vermicomposts.

Cumulative carbon amounts mineralised from non-amended and amended soils are presented on **Fig.31**. Soils amended with composts showed a higher carbon mineralization than non-amended soil, particularly soil amended with C30M (80.9 mg C-CO₂ g⁻¹ soil). Total carbon emitted from soil after compost application (**Fig 31.a**) increased in the order: control soil < C_{control} < C15M < CKG < C30M. By contrast, soils amended with vermicomposts showed lower or similar carbon emissions compared to non-amended control soil (**Fig 31.b**) and the carbon emissions followed this order: V_{control} < V15M < V30M < VKG < control soil. Therefore, on the short-term, the lowest carbon mineralization was observed with the application on soil of the regular vermicompost, with 43.9 mg C-CO₂ g⁻¹ soil. Increasing additive amount increased carbon mineralization with both compost and vermicompost amendments.

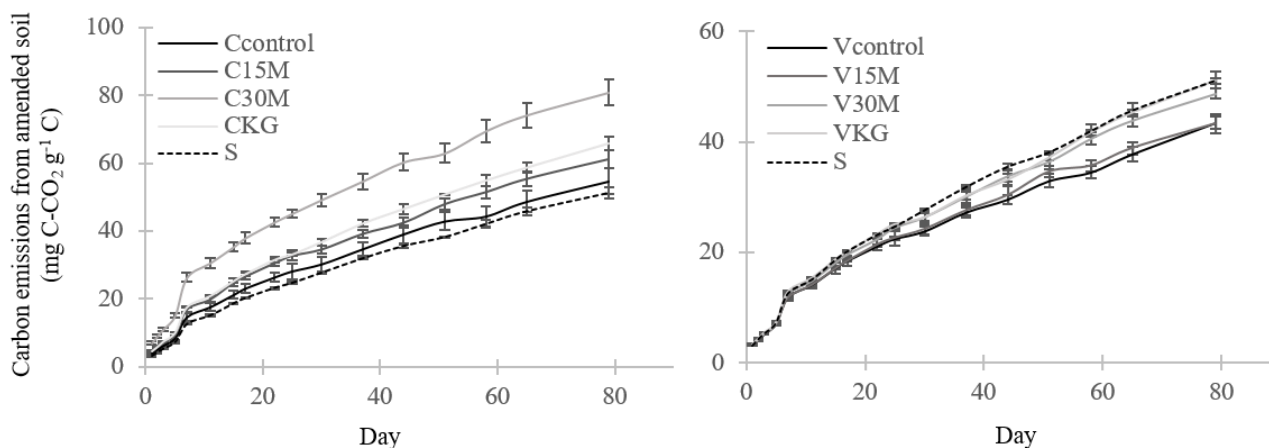


Figure 32 a) Cumulative carbon mineralisation from soil amended with co-composts : C_{control}= compost without additives; C15M= compost with 15% montmorillonite; C30M= compost with 30% montmorillonite; CKG= compost with 15% kaolinite+15% goethite: S= non-amended control soil. **b)** Cumulative carbon mineralization from soil amended with co-vermicompost: V_{control}= vermicompost without additives; V15M= vermicompost with 15% montmorillonite; V30M= vermicompost with 30% montmorillonite; VKG= vermicompost with 15% kaolinite+15% goethite: S= soil non-amended.

In order to describe the dynamics of carbon mineralization from amended soils, carbon mineralization data were fitted with a single-C pool and a two C pool model (**Table 18**). With the single pool model, the potentially mineralisable carbon pool was larger in soils amended with composts than with vermicomposts, and the size of this carbon pool increased with the amount of additives present in the amendment. Although V_{control} showed the lowest potentially mineralisable carbon pool, its degradation rate k was one of the highest ($k = 0.030 \text{ day}^{-1}$). According to a two-pool model, the labile carbon pool (C1) was higher in amended soils than in control soil. The application of composts led to higher proportions of labile carbon than when vermicomposts were applied. Inversely, the degradation rates associated with this pool (k_1) were lower in compost treatments than in vermicompost treatments. For instance, the application of C30M to soil led to the highest labile carbon pool ($32.7 \text{ mg g}^{-1} \text{ soil}$) while its degradation rate was the lowest (0.124 day^{-1}). The second pool of this model (C2) represents the slowly mineralisable carbon fraction. This fraction increased when soil was amended, except with V15M. The degradation rate k_2 related to this carbon pool was lower than k_1 and ranged from 0.002 to 0.007 day^{-1} . The R^2 values obtained with the two-pool model were higher for all treatments compared to those obtained with the single-pool model. The two-pool model was thus selected in the following.

Table 18 Carbon pools and mineralization rates, as estimated with two first-order exponential models.

C_{mineralized} = C x (1 - e^(-kx t))				
	C	k	t_{1/2}	R²
V_{control}	43,984	0,030	23,1	0,981
C_{control}	56,882	0,028	24,8	0,988
V15M	45,237	0,029	23,9	0,988
C15M	63,232	0,030	23,1	0,985
V30M	53,934	0,024	28,9	0,989
C30M	79,075	0,037	18,7	0,981
VKG	56,711	0,023	30,1	0,984
CKG	70,086	0,028	24,8	0,988
soil	57,993	0,024	28,9	0,990

C_{mineralized} = C₁ x (1 - e^(-k1x t)) + C₂x (1 - e^(-k2x t))							
	C₁	k1	t_{1/2 (1)}	C₂	k2	t_{1/2 (2)}	R²
V_{control}	12,09	0,149	4,6	192,5	0,002	347	0,997
C_{control}	13,38	0,152	4,6	116,27	0,005	139	0,997
V15M	10,63	0,157	4,4	87,76	0,006	115	0,997
C15M	17,48	0,139	5	183,33	0,003	231	0,997
V30M	10,7	0,157	4,4	129,58	0,004	173	0,998
C30M	32,69	0,124	5,6	197,96	0,003	231	0,991
VKG	11,71	0,162	4,3	192,85	0,003	231	0,997
CKG	14,67	0,163	4,2	125,5	0,007	99	0,998
soil	10,57	0,153	4,5	124,48	0,005	139	0,998

The two-model was used to predict the carbon mineralization in amended soil on a period of 2 years after organic amendment application (**Fig. 32**). Although on the short-term the soil amended with vermicompost ($V_{control}$) released less CO₂ than the non-amended control soil, this trend was not predicted to last on the long-term. Moreover, the application of composts produced with montmorillonite seemed to increase carbon mineralization more than the application of a regular compost. On the contrary, vermicomposts with montmorillonite appeared to decrease soil carbon emissions compared to the regular vermicompost. With kaolinite and goethite as additives, the opposite tendencies were observed. From all organic amendments, only V15M might decrease carbon mineralization from soil on the long term.

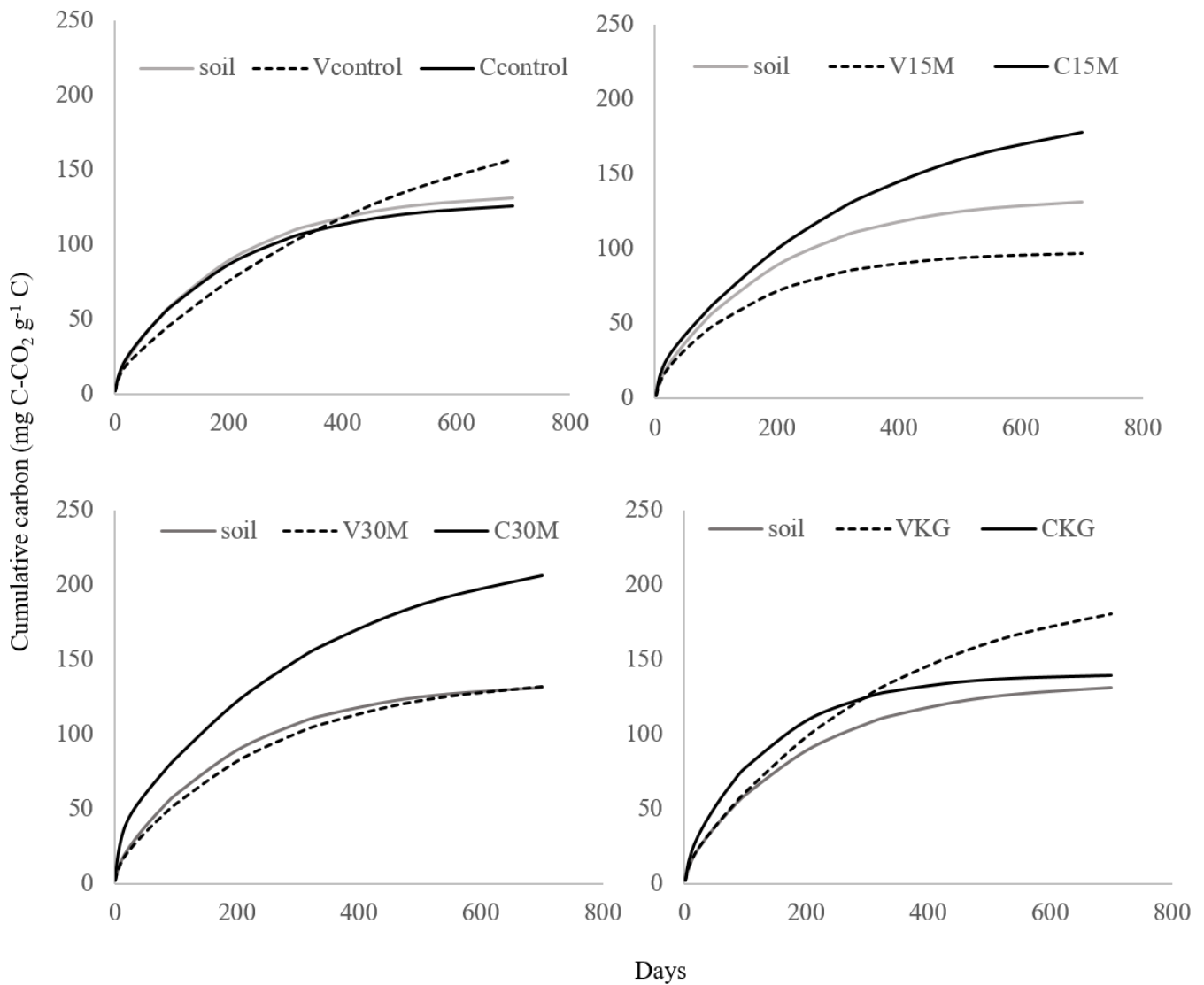


Figure 33 Predicted data of cumulative carbon mineralisation from soil amended with co-compost. C_{control} : compost without additives; C_{15M} : compost with 15% montmorillonite; C_{30M} : compost with 30% montmorillonite; CKG : compost with 15% kaolinite+15% goethite; V_{control} : vermicompost without additives; V_{15M} : vermicompost with 15% montmorillonite; V_{30M} : vermicompost with 30% montmorillonite; VKG : vermicompost with 15% kaolinite+15% goethite

3.4. Carbon repartition in fractions of amended soils

Soil organic carbon distribution among separated carbon fractions is shown on **Fig. 33**. The largest part of carbon was found in the silt and clay fraction (S+C), representing more than 45% of the total carbon. The POM were the second largest fraction (20-33%), except for V_{control} and C_{control} amended soil. In both treatments, relative proportions of carbon were higher in POM than in (S+C) fractions. Therefore, soil amended with regular compost and vermicompost recorded the highest proportion of carbon in the POM fraction among all organic materials. In

addition, carbon distribution among POM and (S+C) fractions were not different between a compost and a vermicompost obtained with the same amounts and type of mineral additives. Increasing amounts of additives (15% to 30%) increased the relative contribution of the S+C fraction and reduced that of the POM fraction, regardless of the composting process, up to a proportion similar to that observed for non-amended soil. The proportion of the recalcitrant fraction (rSOC) significantly decreased by approximately 2% compared to the non-amended control soil when vermicompost, compost and C30M were applied. Stable aggregates (SA) significantly increased by 5-10%, compared to non-amended soil, with the application of V15M, VKG and CKG amendments. Finally, the DOC fractions recorded the lowest contribution of carbon for all treatments, less than 2%, except for C15M with 4%. The addition to soil of vermicomposts significantly decreased the contribution of DOC to the total organic carbon while with composts, this fraction remained unchanged or increased compared to the non-amended soil.

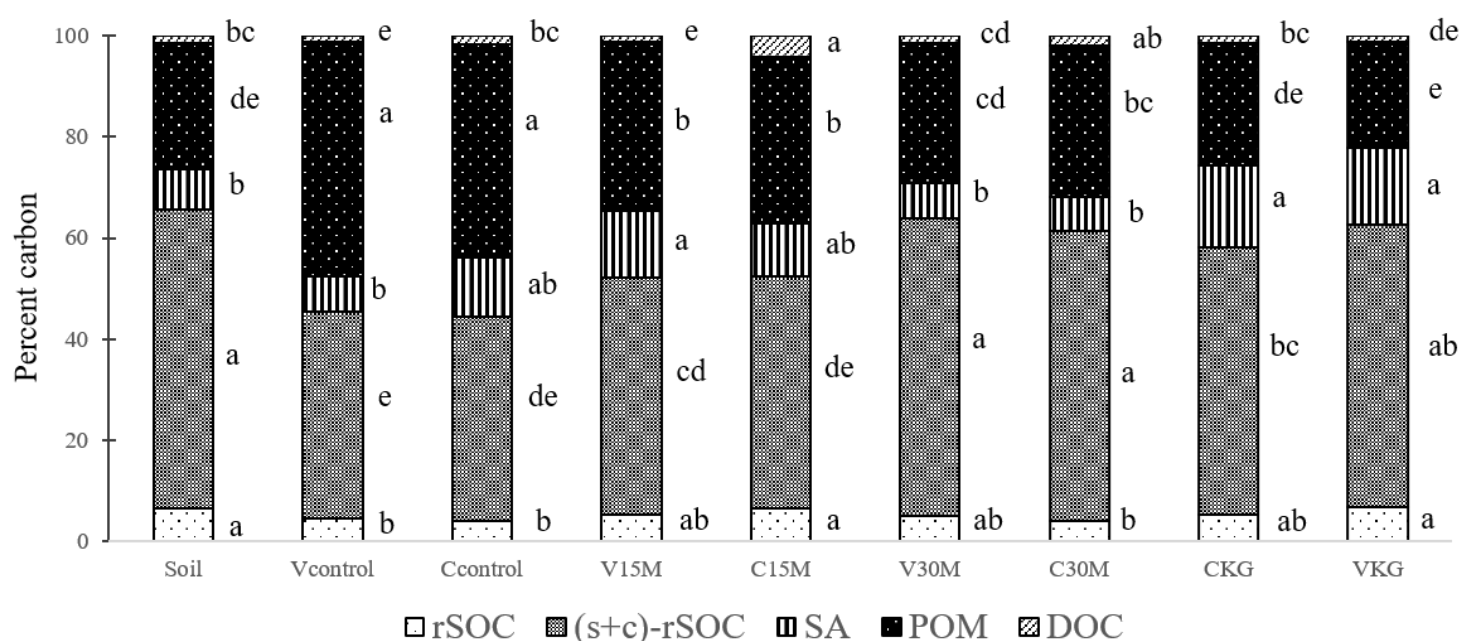


Figure 34 Relative contribution (% of total soil C) of soil organic carbon fractions for non-amended soils and soils amended with co-composts and co-vermicomposts. Dissimilar small letters indicate significant differences among treatments for a same fraction at $p < 0.05$ (Kruskal-Wallis test). POM: particulate organic matter; SA: sand fraction and stable aggregate; (s+c)-rSOC: silt and clay fraction; rSOC: chemically resistant fraction; DOC: dissolved organic carbon fraction.

3.5. Principal component analyses

Principal component analysis was carried out with all compost and vermicompost parameters mentioned in **Table 17** and also with carbon mineralization from amended soils (**Fig. 34**). The first two components explained 75.54% of the total variability. Significant separation between compost and vermicompost treatments are observed. Organic amendments obtained with clay also separate on the PCA plan from regular composts and vermicomposts (**Fig. 34, a**). NO_3^- content was positively correlated with the amount of carbon mineralized during composting process and the electrical conductivity (**Fig. 34, b**). Inversely, these variables were negatively correlated with the amount of carbon mineralized from amended soil. Finally, DOC content in the end-products was neither correlated to carbon mineralized in amended soil nor to carbon mineralized during composting processes.

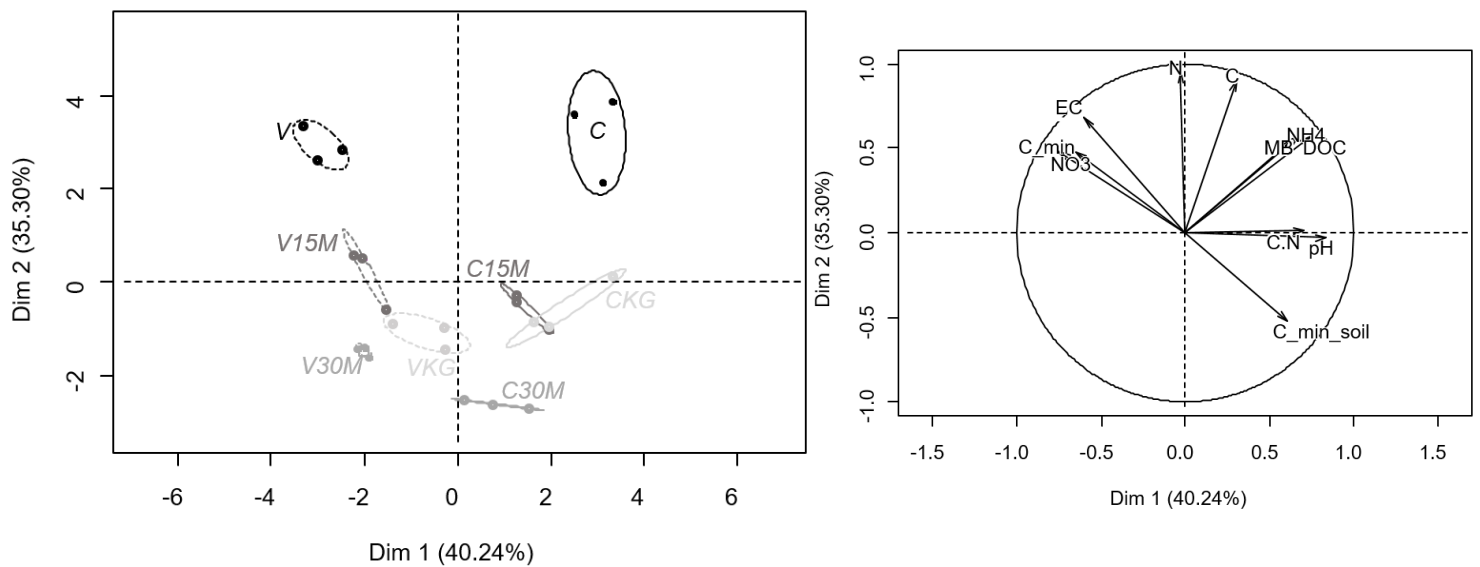


Figure 35 a) Principal component analysis of characteristics of co-composts and co-vermicomposts and their mineralization in soil. b) Correlation circle. EC: electrical conductivity; C_min: carbon mineralized during composting process; C_min_soil: carbon mineralized in amended soil; MB: microbial biomass; DOC: dissolved organic matter of (vermi)compost; N: total nitrogen; C: total organic carbon.

4. Discussion

4.1. Effect of co-compost application to soil on organic matter mineralization and microbial biomass carbon

The diverse composition of the composts used in this study resulted in significantly different effects on soil carbon mineralization (**Fig. 31 a**). The addition of compost, in agreement with Ribeiro *et al* (2010), to an arenic cambisol soil induced an increase of soil carbon mineralization. We also observed this increase for addition of co-composts in our study.

Labile carbon fraction is a main source of mineralized carbon on the short-term (Christensen, 2001) and, exogenous labile carbon has been shown to be quickly mineralized (Peltre *et al*, 2012, Bernal *et al*, 1998). The drastic decrease of the amended soil DOC observed from day 2 to day 79 suggests that the labile carbon added with the amendments was rapidly decomposed and mineralized. According to Mondini *et al* (2007), the amount of CO₂ evolved from the soil is related to the degree of stabilisation of the added organic matter, and thus the amount of DOC. Therefore, composts presenting a high DOC have been showed to have a high mineralization rate in soil (Trinsoutrot *et al*, 2000; Bustamante *et al*, 2007; Flavel and Murphy, 2006). However, this is not the case in our study and we did not observe any correlation between compost DOC and mineralized carbon from soil amended. In our study, the presence of clay within composts, which is supposed to protect and stabilize organic carbon during composting, appears to increase carbon mineralization after amendment to soil, relative to a regular compost (**Fig. 31**). Moreover, this effect appears to be inversely proportional to the amount of carbon mineralized during composting (**Fig. 35**).

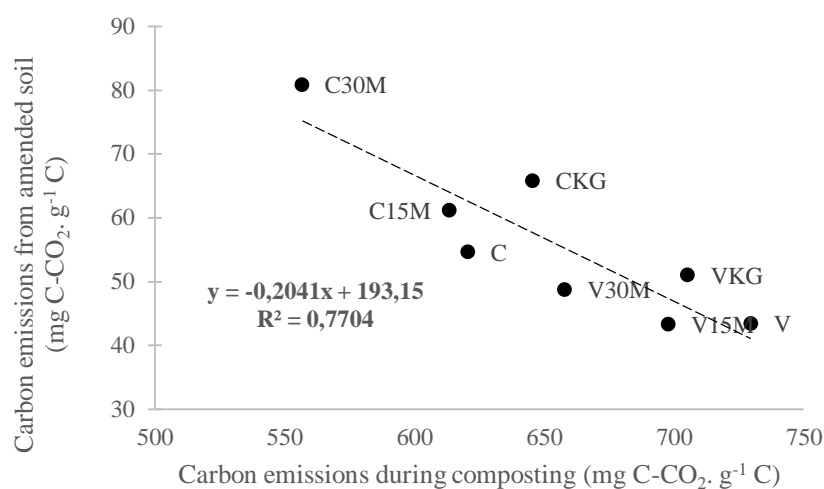


Figure 36 Relationship between carbon mineralized during the composting process and carbon mineralized from amended soil.

In previous studies, carbon stabilization during composting was generally enhanced by increasing the composting duration (Cambardella *et al*, 2003; Bernal *et al*, 1998). Here, we used the same composting duration (6 months) and added minerals to enhance carbon stabilization. Our composting process may not last long enough for enhancing carbon stabilization through mechanisms of physical and chemical protection of carbon comparable to those existing in natural soil (Sollins *et al*, 1996). However, Bolan *et al* (2012) showed that carbon mineralization of a soil amended with a 3-month co-compost of manure and minerals (allophane, gibbsite and goethite) was lower than with a regular compost. The initial feedstock of the co-compost might have more influence on carbon stabilization with minerals than the composting duration. In addition, the soil type greatly influences the decomposition of exogenous organic matter (Busby *et al*, 2007; Bolan *et al*, 2012). Therefore, future studies should assess carbon mineralisation of these co-composts in other soils and compare composts obtained from various feedstocks.

The higher CO₂ production from soil amended with co-composts compared to compost might also be related to an increase of microbial biomass due to mineral addition (Wei *et al*, 2014). According to **Fig. 30**, microbial biomass carbon was the highest 15 days after co-compost application while application of a regular compost only increased soil microbial biomass the first days. This addition of exogenous clay may stimulate soil organic matter decomposition, through enhancement of microorganism protection from predators (von Lützow *et al*, 2006; Zhang *et al*, 2013) and increase of the surface area available for microbial growth (Giardina and Ryan, 2000). However, the proportion of added minerals is small and their effect on

microbial biomass is mainly observed 15 days after co-compost application. The effect of minerals on carbon mineralization requires further investigation. Soil microbial communities might also change after mineral addition, decreasing the physical protection of exogenous organic matter by minerals (Schimel and Schaeffer, 2012). The observed increase of soil microbial biomass carbon underlines that the addition of minerals within compost is likely to positively affect the microbial growth in soil, and thus indirectly soil fertility, as mentioned by Bolan *et al* (2012).

4.2. The application of co-compost changed soil carbon distribution and soil nitrogen forms

Relative to the non-amended soil, soil organic carbon was increased on the short term by organic amendment application, as already observed by Ngo *et al* (2012). However, the carbon distribution in soil fractions, and particularly in the labile and recalcitrant pools, depends on the applied organic materials.

The application of compost and co-composts increased DOC amount in soil (**Table 17 and Fig. 33**), which might be positive for the improvement of soil fertility, since DOC contain easy sources of energy (Christensen, 2001). Application of co-composts, especially those with montmorillonite, might change the amount and composition of dissolve organic matter from soil, leading to larger mineralisable carbon fractions (Chantigny, 2003). Bol *et al* (1999) suggested that some organic amendments may desorb indigenous dissolved organic matter and thus change the quantity of DOC in amended soil (Wright *et al*, 2008). The recalcitrant fractions of carbon were also affected by compost and co-compost addition. The proportion of carbon present in stable aggregates or in the chemically recalcitrant fractions was decreased by the application of regular compost and also, in a lesser extent, of co-composts. Both results imply that additional carbon might be emitted on a longer term, which is also suggested by the predictions of the first order two pool (labile and recalcitrant) model of carbon mineralization (**Fig.32**). However, Chowdhury *et al* (2014) concluded on the contrary that the carbon remaining in soil amended with co-compost produced with alkaline materials, was more associated to the non-labile and residual C fractions than to the exchangeable and labile fractions. Differences in mineral and soil characteristics may explain the opposite trends obtained in our study on soil carbon repartition.

Beside C storage, adding organic amendments to soil has also the objective of improving the soil fertility. According to our results (**Table 17**), total soil nitrogen after incubation is largely increased by the addition of compost and co-composts. Similar results were previously observed by Bustamante *et al* (2010). The effects on total soil nitrogen amounts were not significantly different between the compost and the different co-composts, which, however, contain nitrogen with different availability. Compared to the regular compost, the presence of minerals within compost increased the amount of NO_3^- , a nitrogen form readily taken up by plants (Andrews, 1986).

4.3. Co-vermicompost with minerals: an efficient strategy to increase soil carbon storage?

Regular vermicompost application to soil leads to a lower carbon mineralization than regular compost application (**Fig. 31**). Chaoui *et al* (2003) already observed such trends, underlining that the compost contained much more labile carbon than the vermicompost. In addition, soil microbial biomass carbon was lower with vermicompost addition compared to that of the compost amended soil (**Fig. 30**), regardless of the presence of mineral additives. These observations suggest that energy sources of vermicompost are less available to the soil microbial biomass than those of compost. However, previous studies (Tejada and Benitez, 2011; Ngo *et al*, 2011) showed on the contrary that vermicompost application tends to increase the biological activity of soils compared to compost, due to a lower contribution of recalcitrant compounds. However, the access of soil microbial biomass to vermicompost energy sources might be limited since organic matter experience further aggregation and maturation within worm casts (Bossuyt *et al*, 2005, Atiyeh *et al*, 2000). Therefore, this organic amendment has a potential to increase soil organic matter levels, at least on the short-term.

Furthermore, similarly to compost, vermicompost application changed the carbon repartition in soil fractions by reducing the proportion of carbon in stable aggregates (**Fig. 33**) and increasing the potentially mineralisable carbon (**Table 18**). As a consequence, on the long term, vermicompost might lead to higher CO_2 emissions than a regular compost (**Fig. 32**).

The effect of vermicomposts obtained with minerals on the soil carbon repartition is different from that of the regular vermicompost (**Fig. 33**): the potentially mineralisable carbon fractions (**Table 18**) are modified, particularly with a vermicompost obtained with a low amount of montmorillonite. The carbon mineralization rate of both labile and recalcitrant carbon pools

decreased when montmorillonite is used while it increased with kaolinite and goethite (**Table 18**). These results suggest that carbon from the co-vermicompost with montmorillonite was more stabilized during vermicomposting than composting, enhancing soil carbon stocks. While previous studies showed the importance of worms to stabilize organic matter within casts in soil (Shipitalo and Protz, 1989), our study demonstrates that this stabilization may also occur during co-vermicomposting and seems to persist after application on soil. However, low carbon mineralization might also be related to a potential shift of soil microbial communities and activities induced by the application of co-vermicomposts, as already observed by Lazcano *et al* (2013) with vermicomposts.

In addition to increasing soil carbon stocks, the addition of co-vermicomposts largely improved the soil fertility in terms of nutrient availability as compared to the equivalent co-composts. However, nitrogen forms readily taken up by plants slightly decreased in the presence of minerals within casts. Finally, the PCA further illustrates that amendments separate from each other according to their composting process and also their mineral matrix (**Fig. 34**). With the vermicomposting process, final products have higher NO_3^- contents than with composting and important amount of CO_2 was emitted during the process. These characteristics appear to be well correlated to the carbon mineralized in amended soil (**Fig. 34, b**). Similar to Campitelli and Ceppi (2008), the total OC and the microbial biomass from exogenous organic matter were not correlated with carbon emitted from soil amended, neither with composts nor with vermicomposts. The carbon emitted during co-composting or co-vermicomposting processes with minerals might be used as an indicator to predict soil carbon mineralisation on the short-term since it was negatively correlated to the amount of carbon mineralised in soil from the corresponding co-composts and co-vermicomposts.

5. Conclusion

The stabilisation of exogenous organic carbon increases its potential benefits as soil amendment, such as carbon storage in soil and improvement of soil fertility. Although mineral addition was effective to reduce carbon mineralization and to stabilize carbon during the co-composting process (**Chapter 2**), the application of these co-composts on an arenic cambisol soil did not increase soil carbon storage on the short term. We suggest that the presence of minerals might stimulate soil microbial growth and modify the carbon repartition within soil fractions, increasing the amount of potentially mineralisable carbon. Moreover, the carbon

mineralized from amended soil is predicted to remain at a high level two years after co-compost application, especially with montmorillonite.

The use of worms during composting led to an organic amendment richer in nutrients (**Chapter 3**) and able to increase soil bio-available nitrogen content. In addition, vermicomposts appeared more stable in soil than composts. Nevertheless, the positive effect of vermicompost was only observed on the short term, while after one year, the mineralized soil carbon is predicted to increase compared to soil amended with composts. Similarly to co-composts, the application of co-vermicompost with minerals tends to increase soil carbon mineralization compared to the regular vermicompost on the short term. However, on a longer term, their application might result in an increase of soil carbon stocks, in particular when montmorillonite is used as additive. Therefore, our results suggest that the protective effect of clay minerals on exogenous organic matter applied to soil is persistent only if the fresh organic matter is composted with worms. However, further works are needed to assess these effects in field experiments and with soil with different textures in order to generalize the results obtained in this study.

Chapter 5

Growth of *Arabidopsis Thaliana* in potting media made of co-vermicompost and co-compost with clay minerals

Article in preparation

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1. Introduction

Peat moss is widely used in horticultural and market gardener industries as a potting media due to its physical characteristics (Raviv *et al*, 1986). However, in addition to its high cost and decreasing availability, the impact on the environment is not negligible since it is exported from wetlands, a large belowground carbon stock in the biosphere (Kern *et al*, 2017; Yu *et al*, 2012). Moreover, Bachman and Metzger (2008) underlined that peat moss does not efficiently avoid nutrient leaching due to its low ion exchange capacity, leading also to environmental issues when chemical fertilizers are applied. Therefore, there is a need to find a sustainable alternative substrate to peat moss.

Composts and vermicomposts, obtained from the transformation of organic wastes into valuable and marketable organic amendments and fertilizers have been shown to efficiently improve plant growth (Arancon *et al*, 2004; Atiyeh *et al*, 2001, 2002). For instance, Arancon *et al* (2008) and Zaller (2007) showed that substituting a commercial potting media by a vermicompost increased germination, flowering of petunias and tomato yields. However, plant growth appeared to be dose dependent and the best results were obtained with 20% substitution (Subler *et al*, 1998; Atiyeh *et al*, 2000). Moreover, plant varieties responded differently to the vermicompost application (Zaller, 2007). Beside plant growth improvement, the application of organic materials avoided some plant diseases such as *Pythium*, in particular when vermicompost was used (Chaoui *et al*, 2002; Singh *et al*, 2008). The process and duration of composting and vermicomposting must be controlled to avoid negative impact of the end-product on plant growth (Hartz and Giannini, 1998). For instance, a negative impact on seed germination was observed when compost duration was too short.

Although composting and vermicomposting appear as sustainable methods to produce organic amendments and fertilizers, they might have negative environmental impacts, such as those related to the large amounts of greenhouse gases such as CO₂, N₂O or CH₄ released by microbial activities during their production process (Nakasaki *et al*, 1985), or the leaching of nutrients and heavy metals in the environment after their application to soils (Fang *et al*, 2017; Page *et al*, 2014). To avoid nutrient leaching and limit greenhouse gases (GHG), several studies suggested to add exogenous material as biochar or minerals to compost or vermicompost, able to adsorb heavy metals (He *et al*, 2016; Awasthi *et al*, 2016), to trap the excess of nutrients (Agyarko-Mintah *et al*, 2017; Iqbal *et al*, 2015) or to limit GHG emissions (Agyarko-Mintah *et al*, 2016; Bolan *et al*, 2012). However, few studies investigated the impact of these compost additives on the plant development. For instance, Chen *et al* (2010) observed that addition of

bamboo charcoal and bamboo vinegar during pig manure composting increased germination index up to 95%. On the contrary, Li *et al* (2012) observed that bentonite addition inhibited the growth of mustard root, all the more that the amount of bentonite increased. Similar results were reported with sludge co-composted with lime and fly ash (Samaras *et al*, 2008; Fang and Wong, 1999). Therefore, the additive used during composting may appear as a plant growth simulator or inhibitor, mainly depending on its amount and characteristics.

There is a lack of knowledge in the literature on the influence of co-vermicompost on plant growth. In order to close this knowledge gap, we have produced composts and vermicomposts from lettuce, apple, cardboard, residual maize and spent coffee grounds with and without a clay mineral (30% montmorillonite). The mineral type and amount were selected according to their effect on carbon mineralization reduction during composting (**Chapter 2**). A commercial potting media was substituted by 7% (v/v) of these different organic materials and a plant model, *Arabidopsis Thaliana*, was sown to follow its germination, growth and seedling yields. The main objectives of the current study were to (1) assess the effect of clay co-composts on plant growth and biomass and (2) compare the effects of clay co-composts and co-vermicomposts on these plant parameters. We hypothesized that the presence of clay minerals retains nutrients within the organic materials and thus, limit plant growth and nutrient uptakes. However, the presence of worms during co-composting may counterbalance these effects: worm casts may improve and promote growth and yields of plants through an increase of nutrient availability within casts. Results should help to decide whether regular composts and vermicomposts might be replaced by co-composts and co-vermicomposts with minerals without altering plant development.

2. Materials and methods

2.1. Potting media and organic amendments

The commercial potting media (CMP) base used was a mixture of manure, peat moss and composted barks (Florentaise, France) with 60% of organic matter, a capacity of water retention of 700 mL L⁻¹ and an electrical conductivity of 80 mS m⁻¹. It was either used as is or 7% (v/v) substituted with organic amendments: compost (C_{control}), vermicompost (V_{control}), compost with 30% montmorillonite (C30M) or vermicompost with 30% montmorillonite (V30M). All organic amendments were produced from lettuces, apples, residual maize, spent coffee ground and pieces of cardboard. *Eisenia foetida* and *andrei* (La ferme du Moutta, France) were used during vermicomposting. Montmorillonite was purchased from Sigma-Aldrich and was added

at the beginning of the composting process for both C30M and V30M treatments (addition of 30% dry weight of initial organic wastes). Main characteristics of the commercial potting media base and of the organic amendments are mentioned in **Table 19** and **Table 20**.

Table 19 Nitrogen and carbon contents of the organic material used as potting media substitutes.

	Compost		Vermicompost	
	control	30M	control	30M
Total N (mg g⁻¹)	34,8	15,4	35,2	16,5
Total C (mg g⁻¹)	381,2	179,9	331,2	152,5
C/N	11,0	11,7	9,4	9,2
NO₃⁻ (mg g⁻¹ N)	0,08	0,01	16,46	10,4
NH₄⁺ (mg g⁻¹ N)	1,51	1,12	0,92	1,08
NH₄⁺/NO₃⁻	19,44	104,7	0,06	0,1

Table 20 Main characteristics of the commercial potting media, the composts and vermicomposts, with and without montmorillonite. Different small letters indicate significant differences between substrates (n=3).

	pH	EC (dS m ⁻¹)	Ca (g kg ⁻¹)	K (g kg ⁻¹)	Mg (g kg ⁻¹)	P (g kg ⁻¹)	Cr (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Pb (mg kg ⁻¹)
Commercial potting media	6,5 ^e	0,8 ^c	7,0 ^e	1,8 ^c	0,9 ^c	0,7 ^c	41,5 ^a	7,7 ^d	5,7 ^c
C_{control}	8,5 ^a	1,8 ^{ab}	26,5 ^b	17,2 ^a	3,5 ^{bc}	2,7 ^a	19,1 ^b	54,7 ^b	20,8 ^a
V_{control}	7,7 ^c	3,3 ^a	39,7 ^a	17,5 ^a	3,8 ^{ab}	3,0 ^a	13,6 ^b	65,2 ^a	17,4 ^a
C30M	8,2 ^b	1,2 ^{bc}	10,2 ^d	11,1 ^b	3,8 ^{ab}	1,1 ^b	22,0 ^{ab}	28,2 ^c	4,3 ^c
V30M	7,4 ^d	1,4 ^b	16,1 ^c	10,1 ^b	4,0 ^a	1,2 ^b	21,6 ^b	31,9 ^c	8,4 ^b

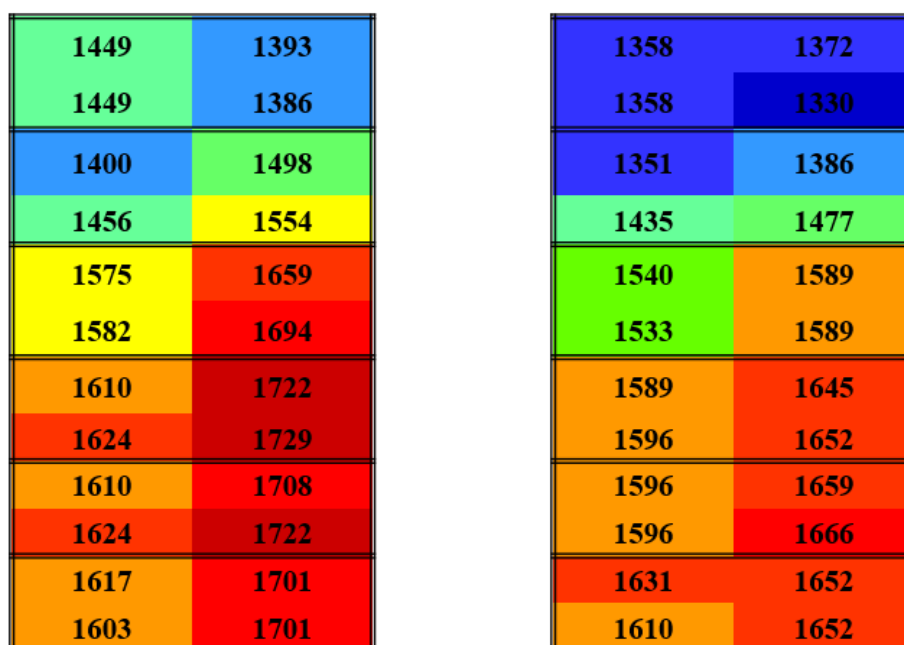
2.2. Experimental set up

Six treatments were carried out as follows: (1) CMP + 7% compost (v/v); (2) CMP +7% vermicompost (v/v); (3) CMP + 7% C30M (v/v) and (4) CMP + 7% V30M (v/v). In addition, two controls were used: (5) unsubstituted CMP and (6) a structural control containing CMP plus 7% montmorillonite. In order to assess if any differences in growth responses were nutrient-mediated, in a series of these 6 treatments plants only received water and in another similar series plants received once a week a nutrient solution (NPK 6-6-6 at 5mL L⁻¹).

A. Thaliana Columbia (purchased from European bank NASC) seeds were placed in an agar-agar solution and kept in the refrigerator for 3 days before planting. Several seeds were

dispensed with a micropipette at the surface of the substrate. Germinated plants were thinned twice (7 days and 14 days after seedling) to obtain 5 homogeneous plants per pot. Sixteen replicates were prepared for each treatment: 4 replicates were destroyed at day 26, 4 at day 29, 4 at day 33 and the last 4 replicates were conducted until seed harvest. Shoot and root biomasses were measured at days 26, 29 and 33.

All treatments were placed in a controlled climatic chamber. The light was applied during 8h to simulate short days and the intensity was approximately of 1500 lumens per week (**Fig.36**). A week rotation of pots has been established to reach this value.



Front door of the chamber

Figure 37 Repartition of the light intensity in lumen per week in the controlled chamber.

2.3. Plant and substrate analysis

2.3.1. Seed, root and shoot biomass

Root and shoot dried biomasses were separated and quantified on each destroyed replicate. Briefly, shoot biomass was separated from roots at collar level. Pot was cut to delicately extract soil/potting media. The substrate was placed on a 1 mm sieve and immersed in water in order to remove soil aggregate. After that, roots were washed with a thin pair of pliers, a brush and sprinkled water. Seeds were harvested every week by shaking the mature plants.

2.3.2. Measurement of leaf surface area and projected surfaces

Photos of plants were regularly taken at day 26, 29 and 33 after seedling. Leaf surface areas were measured while plant was in the substrate. After removing the plant from the pot for biomass measurement, leaves were separated, disposed on a blue surface and a picture was taken for estimating total leaf area (**Fig.37**). The surface areas were estimated with the ImageJ software.



Figure 38 Examples of numerical photos serving for the calculation of leaf surface area before sampling (on the left) and for the total leaf area after sampling (on the right). The red square shows 1 replicate from day 26, containing 5 plants before destruction.

2.3.3. Heavy metal contents and nutrient contents

Total heavy metal contents and total nutrient contents of the substrates were measured at the laboratory of UPMC (Ivry-sur-Seine, France) with inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Briefly, organic materials, about 300 mg, were completely digested in vessel systems by using an acid mixture of HCl and HNO₃ in a microwave oven. Total elements were measured three times by spectrometry from an aliquot of the solution obtained after digestion. Three replicates per treatment have been digested.

Organic carbon and total nitrogen of roots and shoots were measured on three replicates from each treatment. Roots and shoots were ground into powder and analysed with a CHN analyser (varia Micro Cube, Elementar).

3. Results

3.1. Carbon and nitrogen composition of roots and shoots during plant growth

3.1.1. Non fertilized treatments

The elemental composition of roots and shoots of the plants grown in treatments without additional fertilizer is shown in **Table 21** and **Table 22**. At day 26, nitrogen content in *A. Thaliana* roots (**Table 21**) ranged from 1.72 to 2.92% and was significantly higher when 7% regular vermicompost was added to the potting media compared to other substrate mixtures. The lowest nitrogen contents were recorded for both control (non-substituted commercial potting media) with and without additional clay (1.84 % and 1.72% respectively). Potting media substitution with 30% montmorillonite compost and vermicompost (C30M and V30M) significantly reduced nitrogen content in roots compared to substitution with regular compost and vermicompost. The carbon content of plant organs at day 26 ranged from 42.21 to 46.35%. Contrary to what was observed for nitrogen, the root carbon content increased when clay was present in the substrates. However, the commercial potting media as control led to the highest carbon content and thus highest C:N ratio in roots at day 26.

The root nitrogen and carbon content tended to increase for all treatments between 26 and 29 days, except for regular vermicompost substituted media and for controls. Finally, at day 33, nitrogen content in roots decreased up to 60% compared to day 26, while carbon contents slightly increased. The highest decrease was observed for the structural control (from 1.84 % N to 1.11 % N). In potting media substituted with co-compost and co-vermicompost the decrease in root nitrogen content was the lowest (only 20% decrease between day 26 and day 33).

Elemental composition in shoots are given in **Table 22**. Compared to roots, nitrogen contents were higher in shoots while carbon content were lower. At day 26, shoot nitrogen content ranged from 4.76 to 5.81 % and carbon contents from 36.33 to 38.36 %. Substitution of potting media by organic materials increased carbon contents in shoots by 1-2% at day 26 but nitrogen contents were virtually unchanged compared to control substrates. Similar to roots, shoot nitrogen content decreased with time, except with C30M as substituent (4.76% at day 26 and 5.31% at day 33) while carbon content remained stable, reaching 37-38% for all treatments at day 33. The decrease of nitrogen content was more pronounced in control and structural control than in substrates with organic substitutes.

3.1.2. Fertilized treatments

Compared to the root nitrogen contents of plant cultivated without mineral fertilizer, the use of chemical fertilizer increased root nitrogen contents, in particular for plants grown in potting media substituted with regular compost and vermicompost, and in the control. When clay was present in the media (C30M, V30M and structural control), the nitrogen uptake by plants remained similar to the unfertilized treatment or only slightly increased (at day 26, 1.74% N in structural control with fertilizer and 1.84% without fertilizer). From day 26 to day 33, root nitrogen contents decreased for all fertilized treatments in the same range as for unfertilized ones (up to 60%). However, in the fertilized treatments, the larger decreases were observed in potting media substituted with organic amendments.

The root carbon contents were generally lower in treatments with mineral fertilizer compared to unfertilized treatments, especially at day 29. From day 26 to day 33, carbon contents ranged from 30.91% to 47.15%. The most important decrease of root carbon contents (26%) was observed with C30M substituted potting media while substitution with V30M increased by 28% carbon uptake by roots.

The nitrogen content in shoots was doubled with the addition of chemical fertilizer and the carbon contents decreased by approximately 10-15% for all fertilized treatments compared to unfertilized ones, resulting in lower C:N ratios in fertilized compared to unfertilized treatments. The shoot nitrogen content increased from day 26 to 33, and the highest content was recorded for both controls at day 33 (8% and 8.2% for control and structural control, respectively). Similarly, shoot carbon contents increased from day 26 to day 33 and the largest contents were significantly ranged in the order: $V_{30M} \geq C_{30M} > V_{control} \geq C_{control} > \text{Structural control} \geq \text{Control}$.

Table 21 Carbon and nitrogen contents in **roots** from plants cultivated in different substrates and fertilization rates. Different small letters indicate significant differences within a column for a similar fertilization rate (n=3).

		Day 26			Day 29			Day 33		
		N (%)	C(%)	C/N ratio	N (%)	C(%)	C/N ratio	N (%)	C(%)	C/N ratio
- fertilizer	C _{control}	2,57 ^b	44,67 ^b	17,44 ^e	2,81 ^a	44,43 ^{ab}	16,16 ^b	2,02 ^a	46,36 ^{ab}	23,29 ^{bc}
	V _{control}	2,92 ^a	42,21 ^c	14,53 ^f	2,71 ^a	43,60 ^{ab}	16,07 ^{ab}	2,19 ^a	44,15 ^c	20,32 ^c
	C30M	2,35 ^c	45,43 ^{ab}	19,34 ^d	2,71 ^a	43,81 ^{ab}	16,42 ^{ab}	1,87 ^{ab}	47,97 ^a	26,08 ^b
	V30M	2,12 ^d	45,13 ^{bc}	21,31 ^c	2,82 ^a	42,73 ^b	15,44 ^b	1,73 ^b	43,27 ^{bc}	24,98 ^{bc}
	Structure	1,84 ^e	45,24 ^b	24,65 ^b	1,94 ^b	49,33 ^a	25,77 ^a	1,11 ^c	47,59 ^a	43,75 ^a
	Control	1,72 ^e	46,35 ^a	26,93 ^a	1,64 ^b	43,46 ^b	19,56 ^{ab}	1,23 ^c	47,64 ^a	38,97 ^a
+ fertilizer	C _{control}	3,45 ^{ab}	43,98 ^{bc}	12,75 ^b	3,53 ^b	43,76 ^{bc}	12,40 ^b	2,56 ^{ab}	46,12 ^{ab}	18,50 ^b
	V _{control}	3,92 ^a	44,26 ^{bc}	11,40 ^b	2,94 ^{bc}	35,52 ^{cd}	12,50 ^b	3,24 ^a	40,83 ^{bc}	12,96 ^c
	C30M	3,53 ^{ab}	43,76 ^{bc}	12,40 ^b	1,74 ^c	47,15 ^a	27,50 ^a	2,13 ^{abc}	32,40 ^c	14,82 ^c
	V30M	2,94 ^{abc}	35,52 ^c	12,50 ^b	2,75 ^{bc}	45,59 ^{ab}	16,59 ^a	2,67 ^{ab}	45,64 ^{abc}	17,20 ^b
	Structure	1,74 ^c	47,15 ^a	27,50 ^a	6,33 ^a	30,91 ^d	4,88 ^c	1,20 ^c	45,15 ^a	38,01 ^a
	Control	2,75 ^{bc}	45,59 ^{ab}	16,59 ^a	6,00 ^a	31,25 ^d	5,28 ^c	1,85 ^{bc}	46,41 ^a	25,31 ^a

Table 22 Carbon and nitrogen content in **shoots** from plants cultivated in different substrates and fertilization rate Different small letters indicate significant differences within a column for a similar fertilization rate (n=3).

		Day 26			Day 29			Day 33		
		N (%)	C(%)	C/N ratio	N (%)	C(%)	C/N ratio	N (%)	C(%)	C/N ratio
- fertilizer	C _{control}	5,69 ^{ab}	37,49 ^{ab}	6,65 ^b	4,36 ^{ab}	39,74 ^{ab}	9,11 ^{ab}	5,82 ^{ab}	38,55 ^{ab}	6,63 ^{ab}
	V _{control}	4,76 ^a	38,36 ^{bc}	8,23 ^c	4,12 ^b	40,00 ^a	9,76 ^a	4,01 ^b	38,86 ^a	9,92 ^a
	C30M	4,76 ^{abc}	38,15 ^c	8,03 ^c	4,18 ^b	39,36 ^{ab}	9,54 ^a	5,31 ^b	37,34 ^{ab}	7,07 ^a
	V30M	5,21 ^{ab}	37,07 ^{abc}	7,12 ^b	3,87 ^b	40,14 ^a	10,43 ^a	5,29 ^b	37,47 ^a	7,10 ^a
	Structure	4,81 ^c	36,33 ^a	7,56 ^a	4,93 ^a	37,52 ^c	7,61 ^b	2,99 ^a	38,42 ^c	12,88 ^b
	Control	5,09 ^{bc}	36,39 ^a	7,15 ^a	4,89 ^a	38,08 ^{bc}	7,83 ^b	3,07 ^a	38,86 ^{bc}	12,83 ^b
+ fertilizer	C _{control}	6,33 ^b	30,91 ^a	4,88 ^{ab}	6,52 ^b	29,64 ^c	4,57 ^d	6,85 ^b	32,18 ^b	4,70 ^b
	V _{control}	6,00 ^{bc}	31,25 ^a	5,28 ^{ab}	5,71 ^c	33,50 ^b	5,89 ^c	6,75 ^b	32,09 ^b	4,77 ^{ab}
	C30M	6,52 ^b	29,64 ^{ab}	4,57 ^b	7,93 ^a	27,80 ^d	3,50 ^e	6,74 ^b	34,78 ^a	5,17 ^{ab}
	V30M	5,71 ^c	33,50 ^a	5,89 ^a	6,76 ^b	29,80 ^c	4,45 ^d	6,44 ^b	34,89 ^a	5,51 ^a
	Structure	7,93 ^a	27,80 ^b	3,50 ^c	2,57 ^c	44,67 ^a	17,44 ^a	8,21 ^a	30,65 ^{bc}	3,73 ^c
	Control	6,76 ^{ab}	29,80 ^{ab}	4,45 ^{bc}	2,92 ^d	42,21 ^a	14,53 ^b	8,01 ^a	28,85 ^c	3,62 ^c

3.2. Evolution of plant growth parameters

Several plant growth parameters have been measured at different times (26, 29 and 33 days after seedling), such as leaf area, projected leaf area (=total leaf area), number of leaves and shoot and root biomass in the different treatments, with and without additional fertilizer (**Fig. 39** and **Fig. 41**). All treatments had 5 plants per pot.

3.2.1. Non fertilized treatments

Without fertilizer, shoot and root biomass were significantly different between commercial potting media alone and substituted with composts and vermicomposts, in particular at day 33 after seedling (**Fig.39**). The presence of organic substituent significantly decreased plant shoot as well as root biomass. Moreover, addition of clay in the commercial potting media (structure control) also decreased the global plant biomass, especially shoot biomass. These effects were particularly pronounced at day 29 for the root biomass and at day 26 for the shoot biomass. However, the presence of clay in the substituted composts and vermicomposts significantly increased shoot and root biomass compared to regular compost and vermicompost.

The number of leaves was also larger in the commercial potting media alone, with almost 65 leaves per pot, followed by treatments with clay (C30M, V30M), then by structural control and regular compost and vermicompost with 45 leaves per pot. Furthermore, the mean weight of leaf (**Fig.38**) was largest with the commercial potting media (172, 226 and 409 μg at day 26, 29 and 33 respectively) and then with V30M, C30M substitution and structural control. Treatments with regular compost and vermicompost showed the lowest leaf weight, representing less than 100 μg .

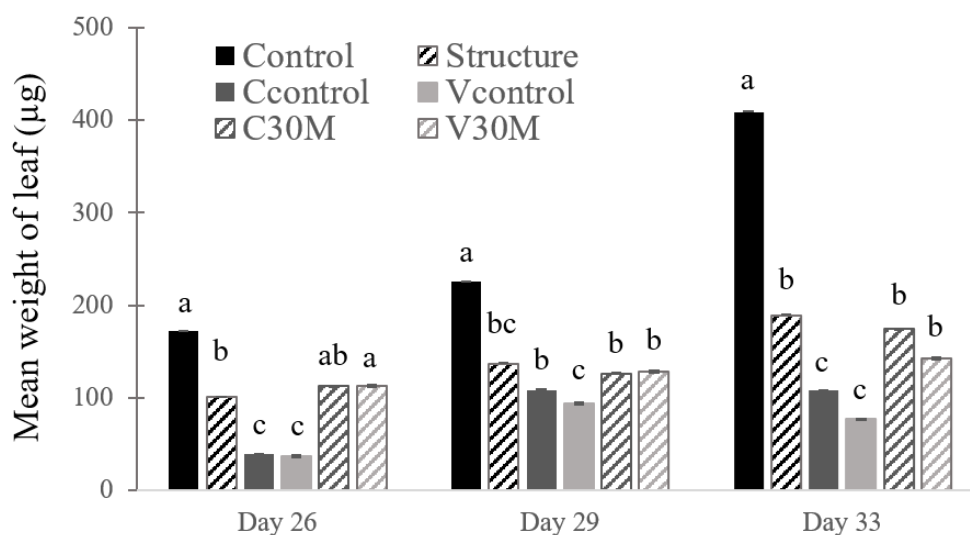


Figure 39 Mean dry weight of *A. Thaliana* leaf cultivated on different substrates without additional fertilizer. Different small letters indicate significant differences among treatments on the same day (n=4).

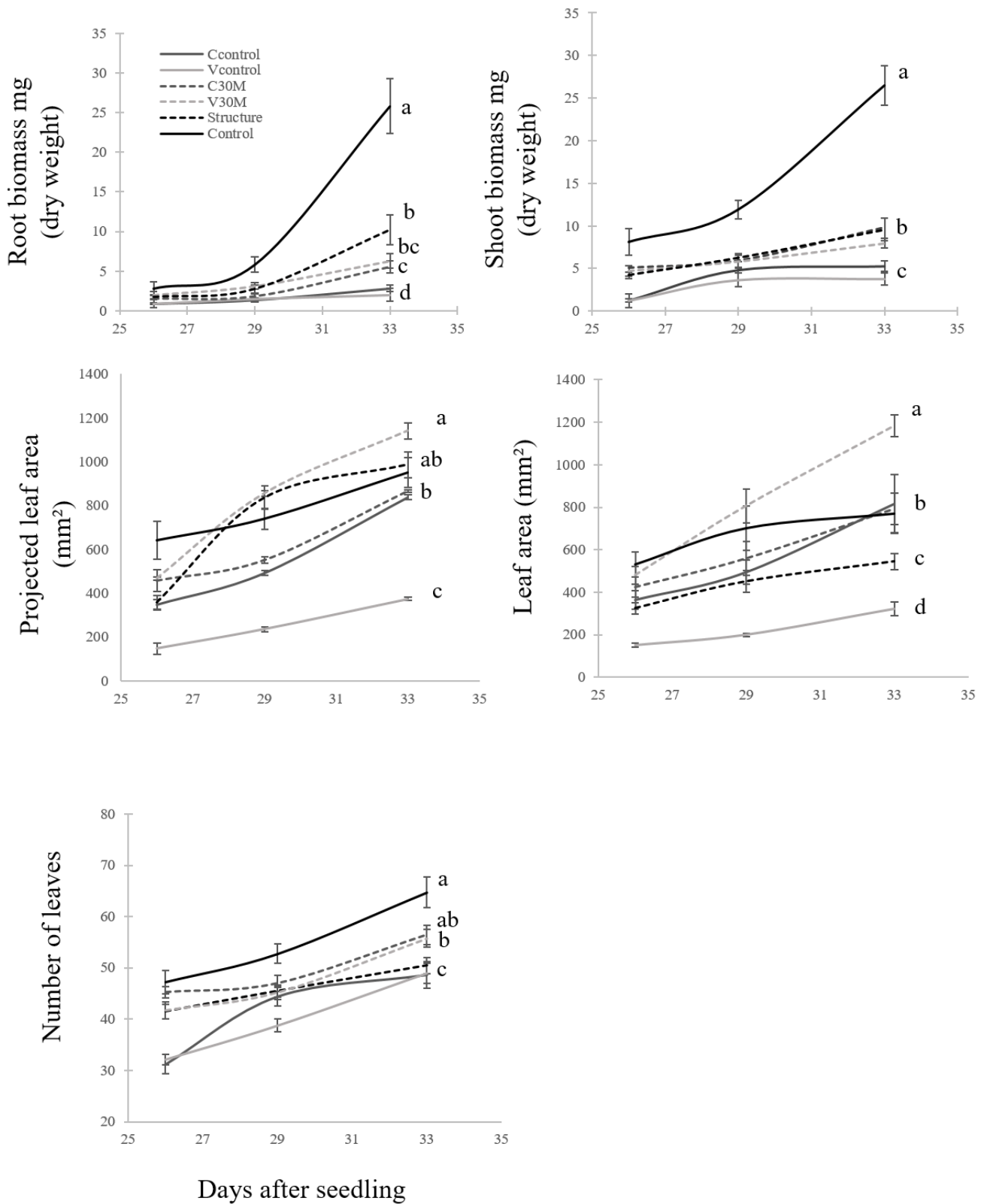


Figure 40 Evolution of different plant growth parameters when **no fertilizer** solution was added. Error bars indicate standard deviation. Different small letters indicate significant differences among treatments at day 33 (n=4).

The leaf area of plants in unfertilized treatments before their destruction increased with time in all treatments except with unsubstituted commercial potting media (control), in which it remained at 770 mm² after 29 days (**Fig.39**). The leaf area of *A.Thaliana* grown with vermicompost was the lowest while leaf area obtained with V30M was the highest, reaching 1185 mm² at day 33. Regular compost and C30M did not show any significant effect on leaf area compared to the control. The addition of clay in the commercial potting media (structural control) significantly decreased the leaf area by 30% compared to the control at day 33. The projected leaf area, which represents the total leaf area (leaves were separated from each other) was higher than the leaf area measured before plant destruction, mainly due to the superposition of leaves in the pots. However, for some treatments (V30M), values were quite similar suggesting that leaves were completely exposed to light. Inversely, for the structural control treatment, both leaf area estimates were very different, suggesting that leaves were hidden by each other.

3.2.2. Fertilized treatments

When fertilizer was applied weekly in the commercial potting media with clay (structural control), root and shoot biomasses were increased compared to the corresponding non-fertilized treatment (**Fig. 41**). Moreover, the fertilized structural control showed significantly higher root biomass than the fertilized control. At day 33, the root biomass of plants grown in regular compost and V30M substituted potting media were similar but 5 times lower than those of plants grown in the structural control. Finally, the lowest root biomass was recorded for treatments with regular vermicompost and C30M, reaching less than 10 mg per pot. Similar trends were observed with shoot biomass, except that controls were not significantly different. The number of leaves was also the largest in controls, leading to similar mean weight of leaf (**Fig.40**). Finally, regular the presence of vermicompost and C30M in the potting media decreased the mean weight of leaves compared to other mixtures and controls. Both leaf area estimates followed the same trend as other parameters, except that plants grown with regular vermicompost showed the lowest area, never exceeding 3000 mm² and staying unchanged between days 29 and 33.

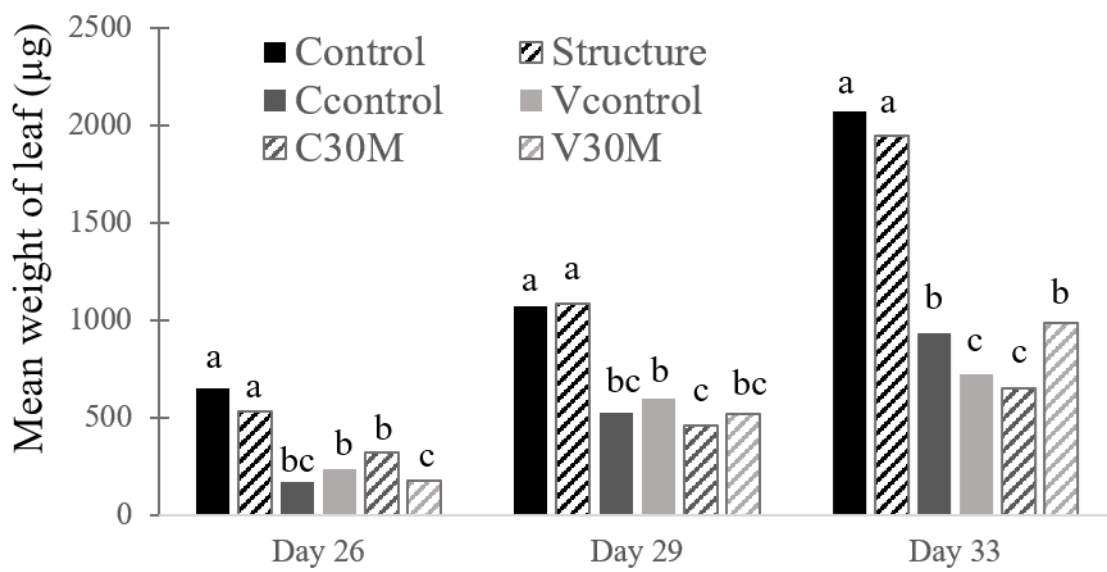


Figure 41 Mean dry weight of *A. Thaliana* leaf cultivated on different substrates with additional fertilizer. Different small letters indicate significant differences among treatments on the same day (n=4).

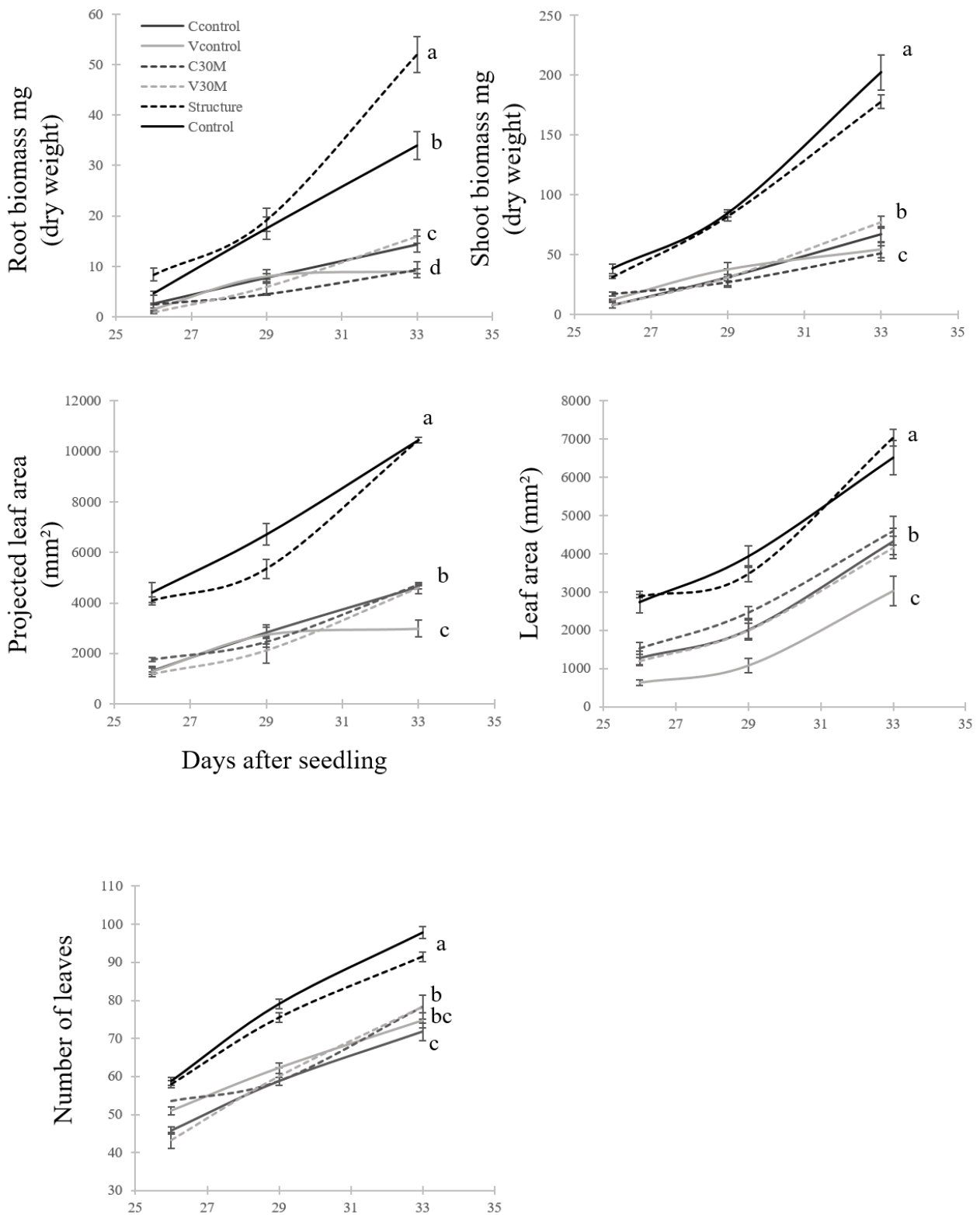


Figure 42 Evolution of different plant growth parameters when **additional fertilizer** solution was applied. Error bars indicate standard deviation. Different small letters indicate significant differences among treatments at day 33 (n=4).

3.3. Length of inflorescence and seed yields

The maximum length of inflorescence was measured (Fig. 42 and Fig. 43). Without fertilization, the length ranged from 10.2 to 31.6 cm. The lowest length was recorded when regular vermicompost was present in the potting media while the largest was observed when regular compost was applied. Substrates with clay showed length with intermediate values and quite similar among treatments (approximately 20 cm).

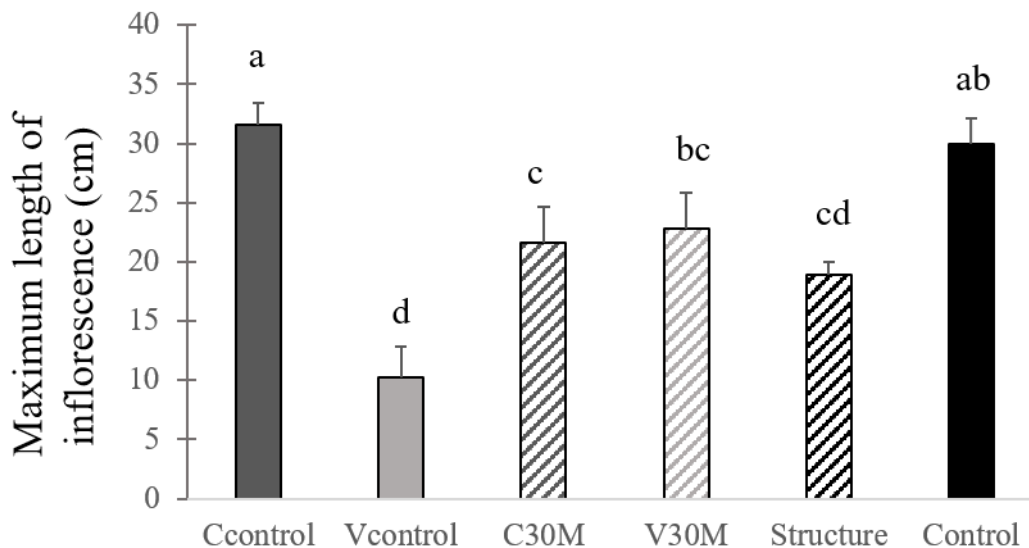


Figure 43 Maximum length of inflorescence of *A. Thaliana* without **additional fertilizer**. Error bars indicate standard deviation. Different small letters indicate significant differences among treatments (n=4)

Adding fertilizer once a week decreased the length of inflorescence of plants grown in regular compost substituted media compared to the corresponding treatment without fertilizer addition. By contrast, fertilizer application on vermicompost substituted media significantly increased the length, leading to the highest values among fertilizer treatments (more than 30 cm). In the structural control, no inflorescence was observed as all plants died before the end of the experiment.

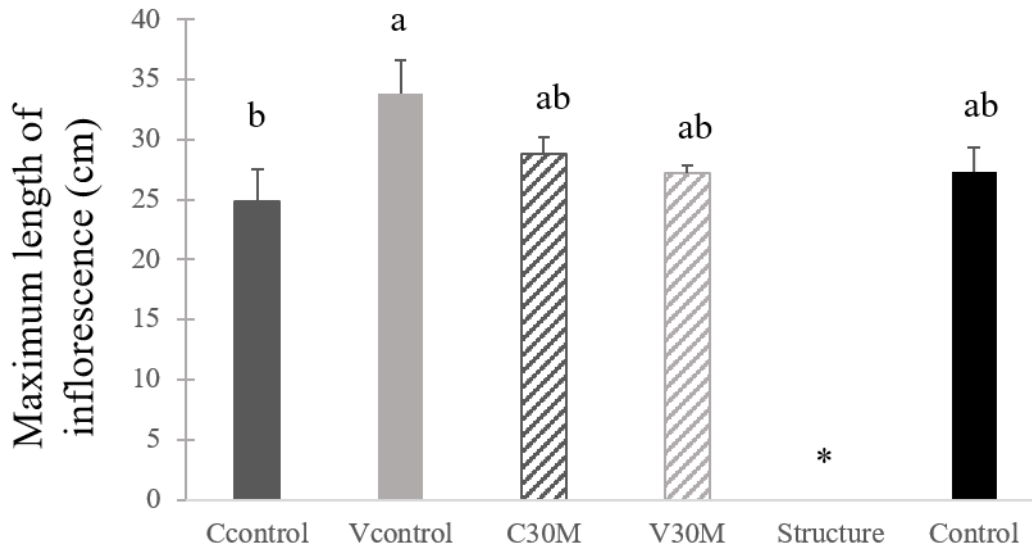


Figure 44 Maximum length of inflorescence of *A. Thaliana* with **additional fertilizer**. Error bars indicate standard deviation. Different small letters indicate significant differences among treatments (n=4). (*) indicate that no inflorescence was observed.

Seed yields are presented in **Table 23**. The total weight of seeds was not significantly different among treatments which received chemical fertilizer, except for the structural control due to an absence of inflorescence (**Fig. 43**). However, the presence of compost tended to decrease the total seeds harvested by a factor 2. For treatments without additional fertilizer, the lowest seed weight was recorded in the presence of vermicompost, followed by the structural control and the C30M treatment. The highest seed weight was obtained when regular compost is present in the potting media.

Table 23 Seed yields in μg per treatment. All seeds were harvested when plants died. Different small letters indicate significant differences among treatments (n=4).

	Total weigh of seeds (μg)	
	-fertilizer	+ fertilizer
C_{control}	62,2 ^a	52,9 ^{ab}
V_{control}	4,6 ^e	155,0 ^a
C30M	26,0 ^{cd}	163,7 ^a
V30M	42,3 ^{bc}	129,2 ^a
Structure	23,8 ^d	0,0 ^b
Control	58,7 ^{ab}	113,0 ^{ab}

4. Discussion

4.1. Growth parameters and seed yields with compost and vermicompost

Our results showed that a substitution of a commercial potting media with either compost or vermicompost is possible without affecting the seedling emergence of *A. Thaliana*. However, the growth parameters greatly differed according to the substrate used for substitution. Substitution of commercial potting media with 7% compost tended to decrease plant biomass and plant leaf area (**Fig. 39**). More pronounced differences were observed with application of vermicompost. By contrast, previous studies often observed that the application of either compost or vermicompost increased plant biomass and yields (Atiyeh *et al*, 2001; Hashemimajd *et al*, 2004).

Composts and vermicomposts used in this study do not present potential risks for plants as heavy metal contents are below the recommended values (legislation NF U44-051, Afnor 2006) and nutrient contents are not limited. Only high NH_4^+ contents in composts were noted (**Table 19**) compared to composts used in the literature (Sullivan and Miller, 2001). A high content of NH_4^+ may have negative effect on the plant growth, mainly due to a high phytotoxicity level (Bernal *et al* 2009). The low $\text{NH}_4^+ : \text{NO}_3^-$ ratio of our vermicompost (**Table 19**) suggests that nitrogen is mainly in forms readily available for plants. However, the substitution of commercial potting media by this vermicompost did not improve plant growth parameters (**Fig. 39**). By contrast, several studies observed a positive influence of vermicompost on the growth of either ornamental or edible plants, due to a large amount of bio-available nutrients (Aityeh *et al*, 2001). Since we observed the decrease of root and shoot biomass in the compost and vermicompost treatments also when the lack of nutrients was avoided with chemical fertilization, it cannot be attributed to nutrient availability in the potting media.

Electrical conductivity and pH of the compost and vermicompost may also explain a part of the limitation of plant growth (**Table 19**). As mentioned by Goh and Haynes (1987), plant growth is generally optimal for a pH comprised between 5.0 and 6.5. Our vermicompost and compost had pH of 7.7 and 8.5 respectively, which might have raised the pH of the growing medium, resulting in a plant growth limitation. Therefore, the pH of these types of compost and vermicompost from kitchen wastes would need to be corrected before use in potting media (Verdonck *et al*, 1987). Similarly, the relatively high electrical conductivity of our organic materials reflects a high salt concentration which may negatively influence plant growth (Atiyeh *et al*, 2001).

Although our vermicompost and compost have suitable physicochemical and nutrient properties for use as growing media according to Bernal *et al* (2009), except for the ammonium content for compost, their application did not improve *A.Thaliana* growth. We showed that 7% of compost or vermicompost in the potting media either delayed or inhibited plant growth. The low reproduction of *A.Thaliana* (seed yield) with vermicompost and no additional fertilizer suggests that the main mechanism occurring is an inhibition of growth, while with compost, a delay of the growth may explain the low biomass and leaf area observed at different steps, while final yields were similar to those in the control (**Table 23**). The plant growth limitation observed here is thus in contrast with other studies (Atiyeh *et al*, 2001; Hashemimajd *et al*, 2004), which might be explained by the different plants studied and the different origins of compost and vermicompost. The effects of composts or vermicomposts on plant growth have been shown to be closely related to the plant species and to the amount of compost in the media (Grigatti *et al*, 2007, Lazcano and Dominguez, 2011). In our study, the chosen 7% (v/v) substitution with compost or vermicompost is largely below the proportion recommended by several authors (Bachman and Metzger, 2008; Atiyeh *et al*, 2000), which is 20% (v/v). Above this dose, plant may show adverse effects such as an inhibition of seedling. However, Bachman and Metzger (2008) observed that for some varieties of tomatoes, a dose as low as 10% of vermicompost leads to a significant decrease of shoot and root biomass. In this way, a low application of vermicompost may be the most beneficial (Ali *et al*, 2007). Therefore, for the cultivation of *A. Thaliana*, a substitution dose lower than 7% (v/v) should be used.

In addition, the presence of vermicompost in potting media has already been shown to induce inhibitory effect on seed germination and early seedling development (Levinsh, 2011). As underlined by several authors, vermicompost may contain hormone-like as auxin-like, due to worm activities (Scaglia *et al*, 2016), promoting the plant growth development (Teale *et al*, 2006, Atiyeh *et al*, 2001). Nevertheless, when auxin-like compounds are present at high concentrations, it can reduce the growth rates and development of plants (Hopkins and Huner, 2004). Thus, the 7% dose of our vermicompost might contain auxin-like compounds in large amounts, which would inhibit plant growth.

4.2. Influence of clay application within compost and vermicompost

In soil, clay is a major physical component and it is conventionally omitted from potting media. However, previous studies (Ehret *et al*, 1998) observed that the addition of mica or

montmorillonite clay coupled with fertilizer application strongly improves plant biomass, flowering and harvest of geraniums, *Cotoneaster* and marketable cucumbers. These results are mainly correlated to an increase of water content and retention in the potting media. Our results are in line with these findings and may confirm that the co-application of a chemical fertilizer and clay in the potting media may increase plant biomass (**Fig. 41**). However, in view of a reduction of chemical fertilizer uses, different strategies of clay application must be found.

According to our results (**Fig. 39**), when clay is added directly to the potting media without fertilizer the plant growth is limited, particularly the leaf expansion, compared to the application of clay within co-compost and co-vermicompost. Moreover, compared to regular compost and vermicompost, the substitution of commercial potting media by these co-compost and co-vermicompost largely increased *A.Thaliana* growth parameters (leaf area and seed yields) (**Table 19**), although the concentration of nutrients such as Ca, K and P is almost divided per two in co-compost and co-vermicompost compared to compost and vermicompost (**Table 20**). This increase is more pronounced with vermicompost (V30M) than with compost (C30M), especially without additional fertilized. These findings invalidate our initial hypothesis that the presence of clay within organic fertilizers would negatively influence plant growth, due to the nutrient retention on minerals. The enhancement of plant growth with the application of co-compost and co-vermicompost may be mainly due to the improvement of the potting media structure, improving water retention, along with their lower pH and electrical conductivity compared to regular compost and vermicompost.

4.3. Nitrogen concentration in plant tissues depends on the substrate and the presence of clay

The ability of plants to capture nutrient resources in potting media is largely dependent on the physico-chemical parameters of the substrates (Kammann *et al*, 2015; Garcia-Gomez *et al*, 2002). According to our previous results (**Chapter 3**), the nutrient availability is higher in vermicompost than in compost for both nitrogen and phosphorus elements. In this chapter, we observed that nutrient content in tissues, especially nitrogen, differs according to the substrate and fertilization system (**Table 21 and Table 22**). When no additional fertilizer was applied, the addition of compost and vermicompost to a commercial potting media increased the nitrogen content in root and shoot tissues. The substitution of the commercial potting media by an organic fertilizer rich in nitrogen increased the leaf nutrient content, as already observed by

Pinamonti *et al* (1997). Nevertheless, no significant correlation was found between plant tissue nitrogen concentration and growth parameters. Atiyeh *et al* (2001) also observed an increase of the nitrogen concentration in tomato tissues due to high nitrogen content in the applied vermicompost. However these authors observed a large decrease of this concentration with time, while in our study the leaf nitrogen content drastically decreased with time only when no exogenous organic matter was applied, while with exogenous organic matter application, particularly with clay co-compost and co-vermicompost, the decrease with time was small. The slow decrease of nitrogen concentration in the presence of organic fertilizer may be explained by a slow but continuous release of nitrogen from co-compost or co-vermicompost (Chaoui *et al*, 2003).

5. Conclusion

The substitution of the potting media with composts and vermicomposts tended to inhibit plant growth development, even when all nutrients were shown to be available in the composts (**Chapter 3**). We suggest that this inhibition may be induced by a change of pH, electrical conductivity or physical structure of the potting media. The largest dry weights of plants, shoots and roots were observed with non-substituted potting media, while compost addition to potting media is generally suggested to slightly improve plant growth. However plant growth enhancements compared to a commercial potting media reported in the literature are often non-significant differences and generally dependent upon plant species and amount of compost or vermicompost applied. Moreover, the maturity and quality of the composts and vermicomposts assessed through estimates of their nutrient bio-availability and physicochemical parameters (**Chapter 3**) appeared optimum for plant growth. Estimating these parameters is thus not enough to predict the effects of composts and vermicomposts on plant growth. Further work is required to assess the optimum amount of compost/vermicompost to improve plant growth for different types of cultivated plants, and to understand the mechanisms behind plant growth enhancement.

In order to reduce the use of chemical fertilizer and peat moss in potting media, vermicomposts with clay additives may be a good compromise. Although the presence of clay during vermicomposting decreased the bio-availability of nitrogen and phosphorus (**Chapter 3**), the obtained vermicompost improved *A. Thaliana* growth, especially leaf expansion compared to a

regular compost and vermicompost. These improvements may be explained by an enhancement of the physical structure of the potting media by clay and a continuous release of nutrients from co-vermicompost. However, the mechanisms by which clay additives benefits plant development compared to regular organic fertilizers should be further investigated.

Chapter 6

Addition of worms during composting with red mud and fly ash reduces CO₂ emissions and increases plant available nutrient contents.

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1. Introduction

Composting and vermicomposting are used worldwide to reduce the degradability of waste organic matter in order to generate organic amendments for soil application (Tejada *et al*, 2010; Ngo *et al*, 2012). The end-products, rich in nutrients and organic matter, can also be used as plant growth media (Hashemimajd *et al*, 2004; Atiyeh *et al*, 2000). However, both transformation processes emit greenhouse gases (GHG) such as CO₂, N₂O and CH₄ (He *et al*, 2000; Velasco-Velasco *et al*, 2011) depending on the aeration of the composting pile (Hobson *et al*, 2005; Hao *et al*, 2001), the presence of worms (*i.e.* vermicomposting) (Chan *et al*, 2011), or the size of the composter (Martínez-Blanco *et al*, 2010). In the IPCC (2014) carbon budget, CO₂ emissions due to mineralization during composting are not considered as contributing to global GHG emissions due to their biogenic origin. However, they have similar impacts on the environment and on the climate change as non-biogenic emissions and are quantitatively important (Lim *et al*, 2016). Around half of the composted biomass is generally lost in the form of CO₂, depending on the composting process as discussed in **Chapter 2**. Reducing CO₂ emissions during composting may be a strategy to increase C sequestration and therefore contribute to climate change mitigation.

In recent years, new composting processes were developed, using bulking agents such as biochar (Chowdhury *et al*, 2014) or minerals (Awasthi *et al*, 2016) in order to enhance carbon stabilisation and compost quality. Bolan *et al* (2012) showed that the presence of minerals such as goethite or allophane during composting of poultry manure increased carbon stability. Other authors used alkaline minerals from industries such as coal fly ash, generated by coal combustion for electricity production (Wong *et al*, 1995; Fang *et al*, 1998; Chowdhury *et al*, 2015) or red mud, a product obtained during the process of alumina extraction from bauxite (Chowdhury *et al*, 2015, Wang *et al*, 2016, Zhou *et al*, 2017). The main advantages of these alkaline materials are their capacity of C and heavy metal stabilisation (Wong *et al*, 2009; Nadaroglu and Kalkan, 2012; Si *et al*, 2013), their high pH leading to pathogen decrease (Wong and Selvam, 2009), their high availability (Gomes *et al*, 2016) and their low cost as industrial wastes. These alkaline materials are thus valuable additives to improve composting procedures as they can decrease carbon mineralization and reduce dissolved organic carbon (DOC) of the end-product (Chowdhury *et al*, 2015).

One of the issues to address when using these bulking agents is their potential effect on organic matter (OM) decomposition and thus compost quality and maturity, that may affect the bio-availability of nutrients such as nitrogen (N) or phosphorus (P). According to Belyaeva and

Haynes (2012), the presence of red mud during composting immobilizes phosphates and thus limits the plant available P.

In the context of ecological engineering, addition of worms to compost may be used to enhance OM decomposition (Lazcano *et al*, 2008; Paradelo *et al*, 2009, 2010) and plant available nutrient contents of the end-products, in particularly P (Garg *et al*, 2006; Ghosh *et al*, 1999). In general, worms' presence during composting leads to greater CO₂ release than regular composting; in the presence of mineral bulking agents, worms enhanced carbon stability due to the simultaneous ingestion of OM and minerals (Barthod *et al*, 2016). Thus, during co-composting with alkaline materials, the addition of worms might limit the nutrient adsorption onto the additives and increase the carbon stability.

The main objective of this study was to test the benefits of worms during co-composting to incorporate industrial waste materials into high quality composts. In particular, we investigated (i) GHG emission potential during co-composting and (ii) end-product maturity and quality. We hypothesised that the worms would decrease GHG emissions during co-composting and lead to higher contents of available nutrients in the end-product.

2. Materials and methods

2.1 Fresh organic matter, additives and worms

Similar fresh organic matter and proportions used as in **Chapter 2** were employed in this study. Briefly, lettuces, apples, residual maize stubble, spent coffee ground and pieces of cardboard (2x3cm) were composted or vermicomposted. Lettuces and apples, products of organic agriculture, were obtained from a general store, the spent coffee ground from a public cafeteria, and the maize from a field in Grignon (France). The main characteristics of these materials are listed in **Table 4, Chapter 2**.

These organic materials were co-composted and co-vermicomposted (with *Eisenia andrei* and *Eisenia foetida*) with fly ash and red mud, alkaline by-products respectively from a coal fired power station (Alinta Energy, South Australia) and a bauxite mining site (Colmaco Alumina refinery, Queensland Australia). Red mud and fly ash have a pH of 10.4 and 10.6 and a specific surface area of 23 and 1 m² g⁻¹ respectively

2.2 Elemental and chemical analysis

Specific surface area of red mud and fly ash was measured by the Brunauer-Emmett-Teller (BET)/N₂-adsorption method. OC and N contents were measured using a CHN auto-analyser (CHN NA 1500, Carlo Erba). Dissolved organic carbon (DOC) contents were measured in 0.034 mol L⁻¹ K₂SO₄ extracts (1:5 w/v) using a total organic carbon analyzer (TOC 5050A, Shimadzu). A glass electrode (METTLER instruments) was used to measure pH and electrical conductivity (EC) in water extracts of (vermi)-composts (1:5). Elemental composition of (vermi)composts was measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) after digestion in a microwave system with HNO₃ and HCl. NO₃⁻ and NH₄⁺ contents were determined in 1M KCl extracts (1: 5 w/v). The extracts were filtered then analyzed by colorimetry on a continuous flow analyzer (Skalar, The Netherlands)

2.3 Incubation experiment and measurements of CO₂, CH₄ and N₂O emissions

Fresh organic materials were composted or vermicomposted in the presence of red mud or fly ash, at 22°C in the dark in the laboratory with 6 treatments (four replicates per treatment): (i) compost alone, (ii) compost with 15% (w/w) of red mud, (iii) compost with 15% (w/w) of fly ash, (iv) vermicompost alone, (v) vermicompost with 15% (w/w) of red mud, (vi) vermicompost with 15% (w/w) of fly ash. In the case of vermicompost treatments, worms were raised in a compost obtained from similar initial fresh organic matter as used in the experiment. Seven adult worms were chosen and cleaned to remove adhering soil/compost before estimating their body mass and added to the composted material.

The experiments were carried out in 2L jars, closed with a tissue to avoid contamination and arranged in a randomized block design. A mixture of fresh organic materials was established as described above (mixing lettuces, cardboard, spent ground coffee, maize and apples) in order to reach an initial C:N ratio of 40 and a moisture level of 70% (w/w). Water was sprinkled on jars at the beginning of the experiment to reach this moisture level that was maintained throughout the experimental period for all treatments. The incubation was stopped after 196 days.

The decomposition of organic materials was measured by monitoring the release of CO₂, using a MicroGC (Agilent, Santa Clara, USA) after the jars were closed during 3 hours. In the same way, CH₄ and N₂O emissions were measured at three different times (days 7, 56 and 196). After each measurement, all jars were opened until the next measurement to avoid high concentration of CO₂ inside.

2.4 Microbial biomass and phospholipid fatty acid analysis (PLFA)

Microbial biomass was measured in the end-products (after 196 days) using the chloroform-fumigation extraction method (Vance *et al*, 1987). Briefly, 5 g of fresh material was fumigated with CHCl_3 in a desiccator during 16h. The fumigated and non-fumigated samples were extracted using 0.034 M K_2SO_4 (1:4 w/v) and the extract was filtered and analyzed for DOC using a TOC analyzer. Microbial biomass C was calculated using the following equation:

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

Where MBC is the microbial biomass carbon, MB is the organic carbon extracted from fumigated and unfumigated compost. 2.22 is the coefficient used for calculation to take into account the efficiency of extraction.

Lipid profiles were established after extraction of 1g (freeze-dried) materials according to the method described by Frostegård *et al* (1991). To summarize, lipids were first extracted with a chloroform/methanol/citrate buffer solution, then separated by using a silicic acid column, and finally methanolysed. The final extract was analyzed with a GC-FID. The bacterial community is divided into two groups, Gram-positive and Gram-negative. The following n-alcanoic acids were considered as originating from Gram-positive bacteria: iso C15:0, anteiso C15:0, iso C16:0 and iso C17:0 (ref). The Gram negative bacteria specific compounds were: C17:0, cis C18:1 ω 9, trans C18:1 ω 9 and C19:0. We considered C18:2 ω 6,9 acid as fungal marker.

2.5 Phosphorus fractionation

Final compost and vermicompost without additives, and treatments with red mud were subjected to the Hedley P fractionation (Hedley *et al*, 1982). Triplicate samples (0.5g, air-dried and grinded) were put in polyethylene tubes and sequentially extracted with 30 mL distilled water, 0.5 M NaHCO_3 at pH 8.5, 0.1 M NaOH and finally with 1M HCl by shaking the samples for 16h. For each extraction, the solution was filtered (0.45 μm) and stored at 4°C until analysis for P quantification. The residual P was extracted with a NaBrO solution from the HCl fraction. The inorganic P concentrations for each extracted solution, including the residual fraction were quantified by colorimetry and the total P was estimated after an oxidation with a NaBrO solution. The concentration of organic P (Po) was calculated as the difference between total P (Pt) and inorganic P (Pi).

The H_2O and NaHCO_3 fractions represent the most readily soluble P pool, usually available to microbes and plants on the short term (i.e., bioavailable form). The NaOH fraction is considered

as the moderately labile pool and the HCl is the non-labile inorganic P (Cross and Schlesinger, 1995; Ivanoff *et al*, 1998).

2.6 Calculation and statistical analysis

The emitted CO₂ amounts were fitted to a first-order model (Marquardt-Levenberg algorithm), using the following equation:

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

where C is the mineralised carbon in CO₂ (mg g⁻¹ compost) at the time t (day), C_{min} is the potentially mineralisable carbon (mg g⁻¹ compost), and k is the rate constant of carbon mineralization (day⁻¹).

All reported data are the arithmetic means of three or four replicates. A Kruskal-Wallis test was performed to assess the difference significance of end-product characteristics and of gas emissions. 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 End-product characteristics

Table 24 Characteristics of the end-products obtained after co-composting and co-vermicomposting with alkaline materials Data are presented as means (n=4). Different small letters indicate significant differences between treatments (Kruskal-Wallis test, $p < 0.05$). C_{control} : compost; V_{control} : vermicompost; CRM: compost +red mud; VRM: vermicompost+ red mud; CFA: compost + fly ash; VFA: vermicompost+ fly ash.

End-products	pH	EC (dS m ⁻¹)	Total N (mg g ⁻¹)	Total C (mg g ⁻¹)	DOC (mg C g ⁻¹)	C:N	NO ₃ ⁻ - N (mg kg ⁻¹)	NH ₄ ⁺ - N (mg kg ⁻¹)	NH ₄ ⁺ NO ₃ ⁻
C_{control}	8.5 ^d	1.98 ^{bc}	34.81 ^a	381.19 ^a	37.93 ^{bc}	11.08 ^{ab}	2.6 ^b	52.1 ^a	20.12 ^b
V_{control}	7.5 ^e	3.11 ^a	35.22 ^a	331.17 ^a	13.84 ^{de}	9.42 ^{bc}	576.0 ^a	31.9 ^b	0.06 ^c
CRM	9.7 ^a	2.47 ^{cd}	20.32 ^{bc}	250.83 ^b	52.88 ^a	12.49 ^a	1.8 ^c	74.5 ^a	41.40 ^a
VRM	9.2 ^b	2.56 ^{ab}	19.89 ^c	211.58 ^c	47.35 ^{ab}	10.75 ^{ab}	283.5 ^a	20.2 ^c	0.07 ^c
CFA	8.6 ^c	1.41 ^d	25.40 ^b	253.28 ^b	25.91 ^{cd}	10.10 ^b	2.6 ^{bc}	59.1 ^a	22.63 ^b
VFA	7.8 ^{de}	1.55 ^{ab}	23.40 ^{bc}	202.35 ^c	6.61 ^e	8.64 ^c	465.8 ^a	19.4 ^c	0.04 ^c

All the characteristics of the end-products are presented in **Table 24**. Alkaline materials (fly ash and red mud) tended to raise the end-product pH above that of the non-amended compost (C_{control}), which showed a pH of 8.5. The use of bulking agents during vermicomposting also increased the pH compared to vermicompost control (V_{control}), which showed a near neutral pH. All EC values of vermicomposts were significantly above those of corresponding composts. Addition of red mud during composting or vermicomposting did not significantly influence EC and addition of fly ash significantly decreased the EC for composting without worms only (CFA compared to C_{control}). The presence of alkaline amendments led to lower N contents of final composts and vermicomposts. Similarly, the total carbon content was reduced with the addition of fly ash. Lower C contents were noticed for products resulting from the vermicomposting process compared to the composting process. Dissolved organic carbon (DOC) was the highest in treatments with red mud (CRM and VRM). Fly ash did not significantly influence the DOC of the final product although DOC tended to be lower for fly ash treatments (CFA and VFA) compared to regular compost and vermicompost (C_{control} and V_{control}). Composting with worms generally led to lower DOC of the end-product, with a minimum of 6.61 mg C g⁻¹ when worms and fly ash were combined. Finally, the nitrate (NO₃-N) content of the end-products was more affected by worms than by alkaline materials. Indeed, vermicomposting led to NO₃-N contents up to 576 mg kg⁻¹, while NO₃-N contents of compost without worms ranged from 1.8 to 2.6

mg kg⁻¹. Inversely, higher NH₄⁺-N contents were recorded for compost than vermicompost end-products. Red mud and fly ash addition did not change this content compared to the compost without additives (C_{control}). By contrast, adding alkaline materials during vermicomposting significantly decreased the NH₄⁺-N contents.

3.2 Gas emissions and decomposition rate

N₂O, CH₄ and CO₂ were monitored during composting and vermicomposting. The N₂O emissions were largest at the first analysed date, day 7 (**Table 25**). These emissions ranged from 0.022 to 0.771 µg N₂O g⁻¹ h⁻¹ and the highest emissions were recorded for non-amended and alkaline material amended vermicomposts (V_{control}, VFA and VRM). Addition of red mud and fly ash tended to increase N₂O emissions at day 7, in vermicomposting as well as in composting treatments. The presence of alkaline materials, especially red mud, decreased the emissions at day 56, but did not influence them at day 196. Bulking agent addition did not significantly influence methane emissions, except for fly ash in composting treatments, which significantly increased CH₄ emissions at days 56 and 196.

Table 25 Emissions of N₂O and CH₄ from composts and vermicomposts non amended and amended with alkaline materials at days 7, 56 and 196. Data are presented as means (n=4). Different letters indicate significant differences between treatments (Kruskal-Wallis test, p< 0.05). C_{control}: compost; V_{control}: vermicompost; CRM: compost +red mud; VRM: vermicompost+ red mud; CFA: compost + fly ash; VFA: vermicompost+ fly ash.

	<i>- Day 7 -</i>		<i>- Day 56 -</i>		<i>- Day 196 -</i>	
	µg N ₂ O g ⁻¹ h ⁻¹	µg CH ₄ g ⁻¹ h ⁻¹	µg N ₂ O g ⁻¹ h ⁻¹	µg CH ₄ g ⁻¹ h ⁻¹	µg N ₂ O g ⁻¹ h ⁻¹	µg CH ₄ g ⁻¹ h ⁻¹
C_{control}	0.022 ^c	0.024 ^a	0.010 ^{bc}	0.026 ^{bc}	0.010 ^{bc}	0.025 ^b
CFA	0.085 ^{bc}	0.021 ^a	0.009 ^{cd}	0.074 ^a	0.010 ^{bc}	0.080 ^a
CRM	0.171 ^b	0.019 ^{ab}	0.007 ^d	0.024 ^c	0.007 ^c	0.025 ^b
V_{control}	0.526 ^a	0.018 ^b	0.018 ^a	0.040 ^a	0.020 ^a	0.024 ^b
VFA	0.542 ^a	0.019 ^{ab}	0.011 ^{ab}	0.063 ^a	0.024 ^a	0.027 ^{ab}
VRM	0.771 ^a	0.018 ^b	0.012 ^{bc}	0.033 ^a	0.013 ^{ab}	0.024 ^b

The cumulative CO₂ emissions during composting ranged from 519 to 730 mg CO₂-C g⁻¹ compost (**Figure 45**). The addition of alkaline materials to compost generally decreased the carbon emissions compared to non-amended compost (C_{control}). With fly ash (CFA), the 5% emission decrease was not significant and with red mud (CRM), the 16.5% decrease was

significant. Worm presence during vermicomposting increased carbon mineralization compared to composting. Compared to control vermicompost (V_{control}), carbon emissions were decreased by 5% during vermicomposting with fly ash (VFA) and by 13% with red mud (VRM).

Estimates of the amount of carbon potentially degradable into CO_2 and the mineralisation rate constant k (day^{-1}) given by the equation 1 are summed up in **Table 26**. During composting, the concentration of degradable carbon was significantly decreased with red mud, while with fly ash, the decreasing trend was not significant. However, red mud addition significantly increased the degradation rate constant k (CRM rate larger than that of C_{control}). Non-amended vermicompost (V_{control}) had the highest potential degradable carbon with $764 \text{ mg CO}_2\text{-C g}^{-1}$ compost, followed by VFA and VRM. Similarly to composting, only the red mud addition significantly decreased this carbon pool during vermicomposting. The rate constant k was similar for all the vermicomposting treatments.

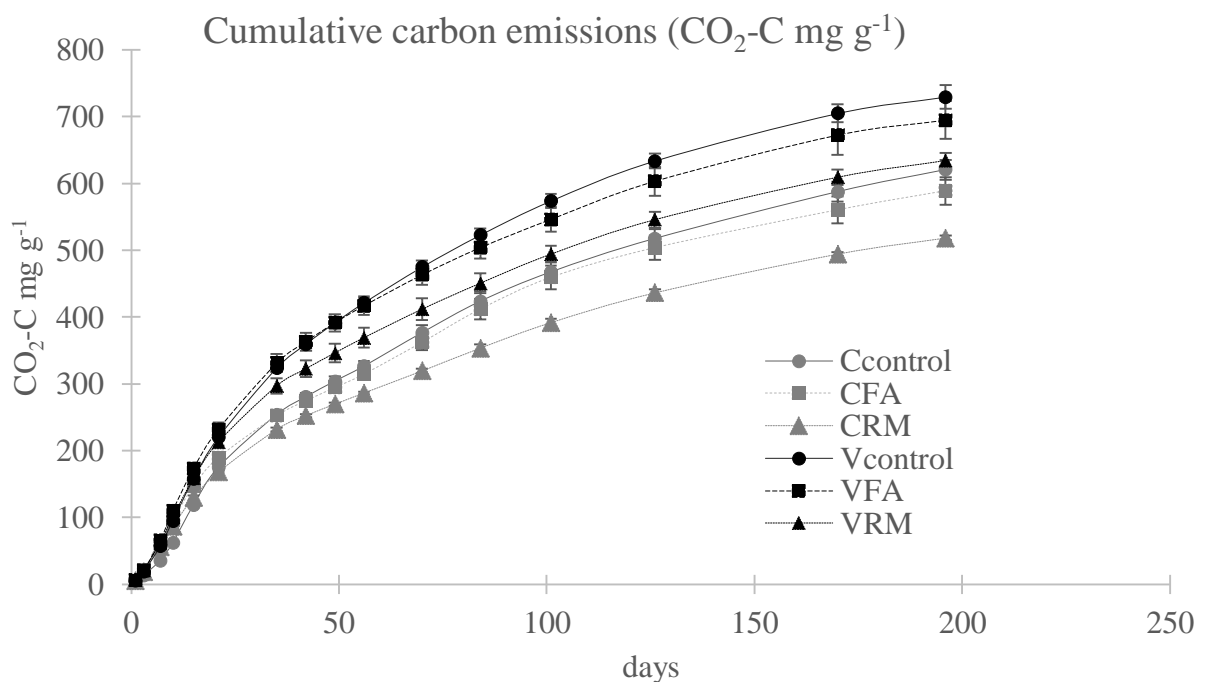


Figure 45 Cumulative CO_2 emissions during composting and vermicomposting with and without 15% alkaline materials. C_{control} : compost; V_{control} : vermicompost; CRM: compost + red mud; VRM: vermicompost + red mud; CFA: compost + fly ash; VFA: vermicompost + fly ash. Bars represent the standard deviation of the mean ($n=4$).

Table 26 Effect of the addition of alkaline materials on the mineralisation rate constant k (day^{-1}) and the potential degradable carbon pool (Cmin) during composting and vermicomposting. C_{control} : compost; V_{control} : vermicompost; CRM: compost +red mud; VRM: vermicompost+ red mud; CFA: compost + fly ash; VFA: vermicompost+ fly ash. Data are presented as means ($n=4$). Different letters indicate significant differences between treatments (Kruskal-Wallis test, $p < 0.05$).

	C min potential (mg g^{-1})	k (day^{-1})
C_{control}	669.99 ^{bc}	0.0123 ^c
V_{control}	764.01 ^a	0.0145 ^{ab}
CRM	528.51 ^d	0.0147 ^{ab}
CFA	614.66 ^c	0.0140 ^{bc}
VRM	642.60 ^{bc}	0.0159 ^{ab}
VFA	706.26 ^{ab}	0.0167 ^a

3.3 Microbial biomass amounts and communities

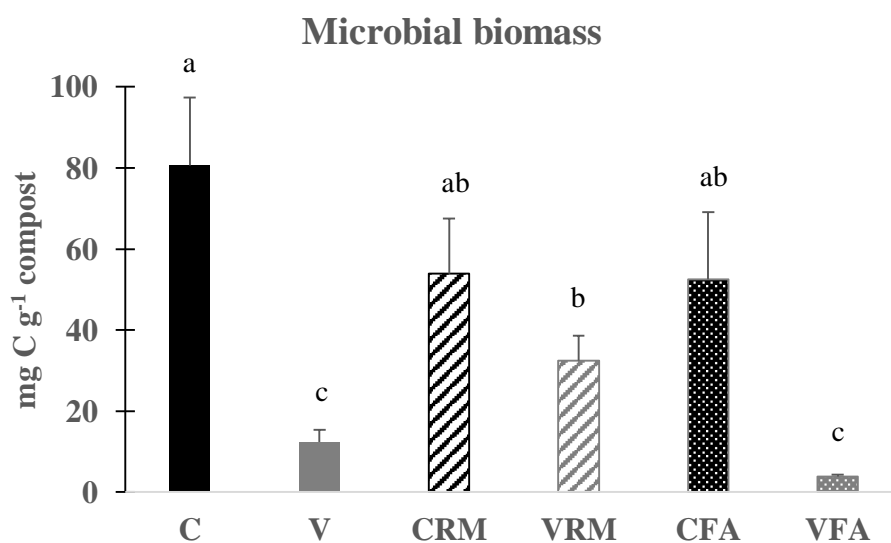


Figure 46 Microbial biomass amount after 196 days of composting and vermicomposting process with and without alkaline materials. C_{control} : compost; V_{control} : vermicompost; CRM: compost +red mud; VRM: vermicompost+ red mud; CFA: compost + fly ash; VFA: vermicompost+ fly ash. Bars represent the standard deviation of the mean ($n=4$). Different small letters indicate significant differences between treatments (Kruskal-Wallis test, $p < 0.05$).

Amounts of microbial biomass carbon of composts produced without worms (**Fig. 45**) at the end of the composting experiment (196 days) were not significantly different in treatments with alkaline materials compared to control compost. However, the presence of red mud and fly ash both tended to decrease microbial biomass (80.6 mg C g⁻¹ for C_{control} and, 53.9 and 52.4 mg C g⁻¹ for CRM and CFA respectively). Worm presence decreased microbial biomass compared to the corresponding composting treatments. Compared to vermicompost (V_{control}), microbial biomass was significantly increased in vermicomposts with red mud (32.5 mg C g⁻¹) and tended to decrease in those with fly ash (3.86 mg C g⁻¹).

Based on the mineralization and biomass amount data, only the treatments with red mud (composting and vermicomposting) were selected, along with the controls, for the following analyses (PLFA, nutrient and heavy metal quantification). This alkaline material indeed induced large and significant changes of mineralization and biomass parameters, while changes observed with fly ash were not significant.

The relative proportions of microbial markers in the PLFA extracted from the end-products are shown in **Fig. 46**. The lowest relative abundance of fungus biomarkers was found in the red mud vermicompost, accounting for only 10% of the total extracted PLFA (**Fig. 46, c**). The Gram-positive bacteria markers represented approximately 35% of the total PLFA for all treatments. Finally, Gram-negative bacteria markers had a lower abundance in the composts (C_{control} and CRM) than in the vermicomposts. Addition of red mud only influenced the relative abundance of fungus biomarkers in vermicompost.

In all treatments, the ratio of Gram+ to Gram- biomarkers (**Fig. 46, a**) was similar, ranging from 0.65 to 0.82. The ratio of fungal to bacterial biomarkers (**Fig. 46, b**) was larger for vermicomposts (V and VRM) as compared to composts. The presence of red mud did not influence these ratios neither for composts nor for vermicomposts.

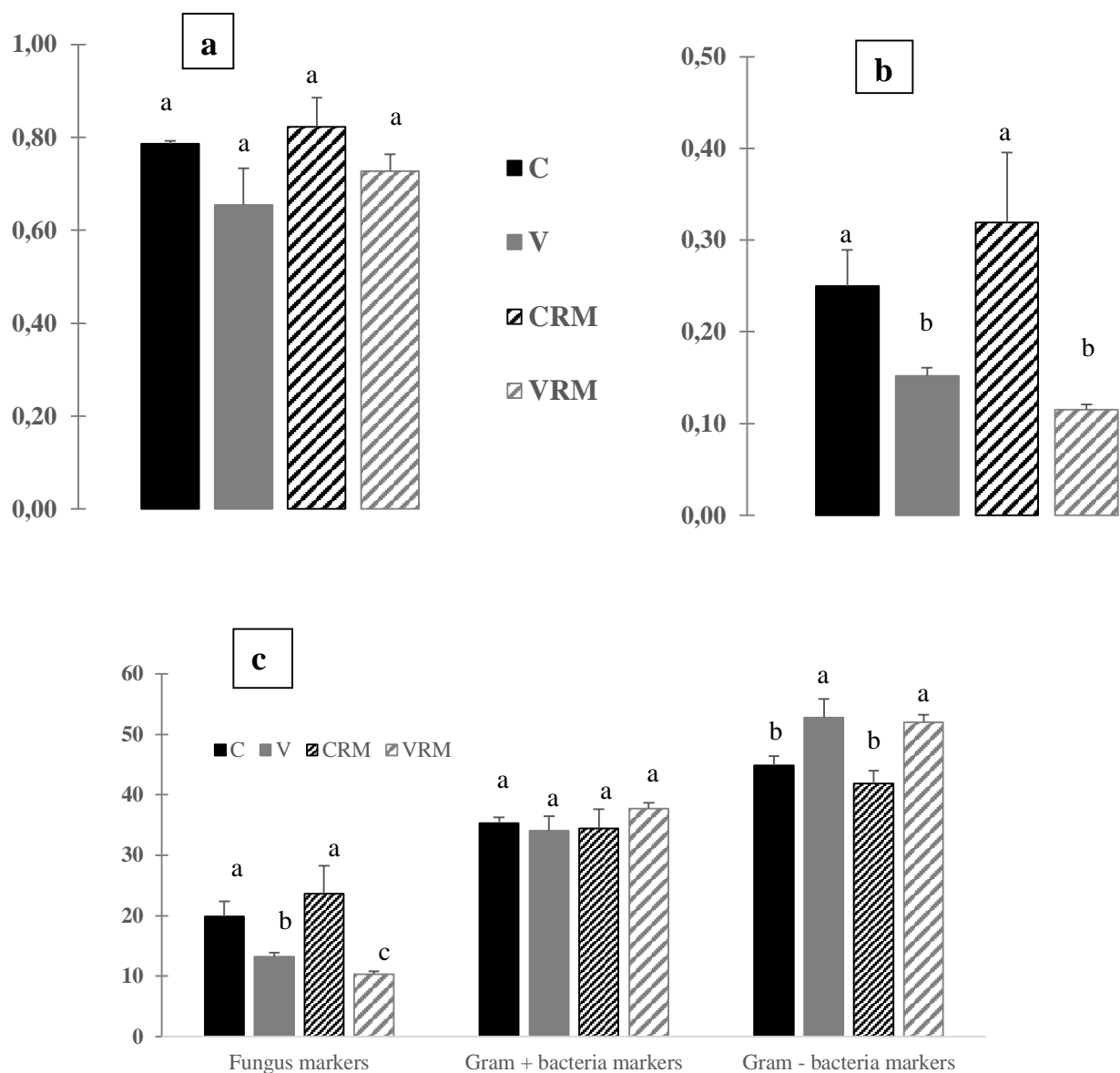


Figure 47 Microbial and fungal PLFA markers in the composts and vermicomposts obtained with and without red mud. C_{control}: compost; V_{control}: vermicompost; CRM: compost+red mud; VRM: vermicompost+red mud. Bars represent the standard deviation of the mean (n=3). Different small letters indicate significant differences between treatments (Kruskal-Wallis test, p< 0.05). a) Ratios of specific markers of Gram+ and Gram-; b) Ratios of specific markers of fungi and bacteria; c) Relative abundance of fungus, Gram+ and Gram- markers.

3.4 Phosphorus fractions and nutrient contents

Total nutrient and heavy metal (Ca, K, Mg, P, Zn and Pb) contents and phosphorus fractionation measured in compost and vermicompost controls (C_{control} and V_{control}) and in red mud treatments (CRM and VRM) in **Table 27** and **Figure 47**, respectively. The total nutrient and heavy metal concentrations (**Table 27**) were also measured in the initial red mud. Nutrient contents were higher in vermicompost than in compost, but only Ca and Zn contents were significantly different. Red mud presence resulted in a decrease of total Ca, K, Mg, Zn and P for end-products

of both processes, and an increase of Pb concentrations. Vermicompost with red mud (VRM) tended to show higher nutrient and heavy metal contents than red mud-compost (CRM), but only total P contents were significantly different (1480 mg kg⁻¹ and 1167 mg kg⁻¹ respectively).

Table 27 Nutrient and heavy metal contents in the composts and vermicomposts, with and without red mud and in the initial red mud. C_{control}: compost; V_{control}: vermicompost; CRM: compost +red mud; VRM: vermicompost+ red mud; RM: initial red mud. Data are the means of 3 replicates. Different letters indicate significant differences between treatments (Kruskal-Wallis test, p< 0.05).

End-products	Ca (g kg ⁻¹)	K (g kg ⁻¹)	Mg (g kg ⁻¹)	P (g kg ⁻¹)	Zn (mg kg ⁻¹)	Pb (mg kg ⁻¹)
C _{control}	26.476 ^b	17.165 ^a	3.460 ^a	2.684 ^a	54.75 ^b	20.81 ^{ab}
V _{control}	39.689 ^a	17.550 ^a	3.806 ^a	3.004 ^a	65.21 ^a	17.38 ^b
CRM	22.266 ^b	8.673 ^b	2.136 ^b	1.167 ^c	31.70 ^c	24.49 ^{ab}
VRM	27.326 ^b	11.948 ^b	2.675 ^b	1.480 ^b	42.87 ^c	27.50 ^a
Initial RM	10.820	1.844	0.931	0.660	7.69	5.73

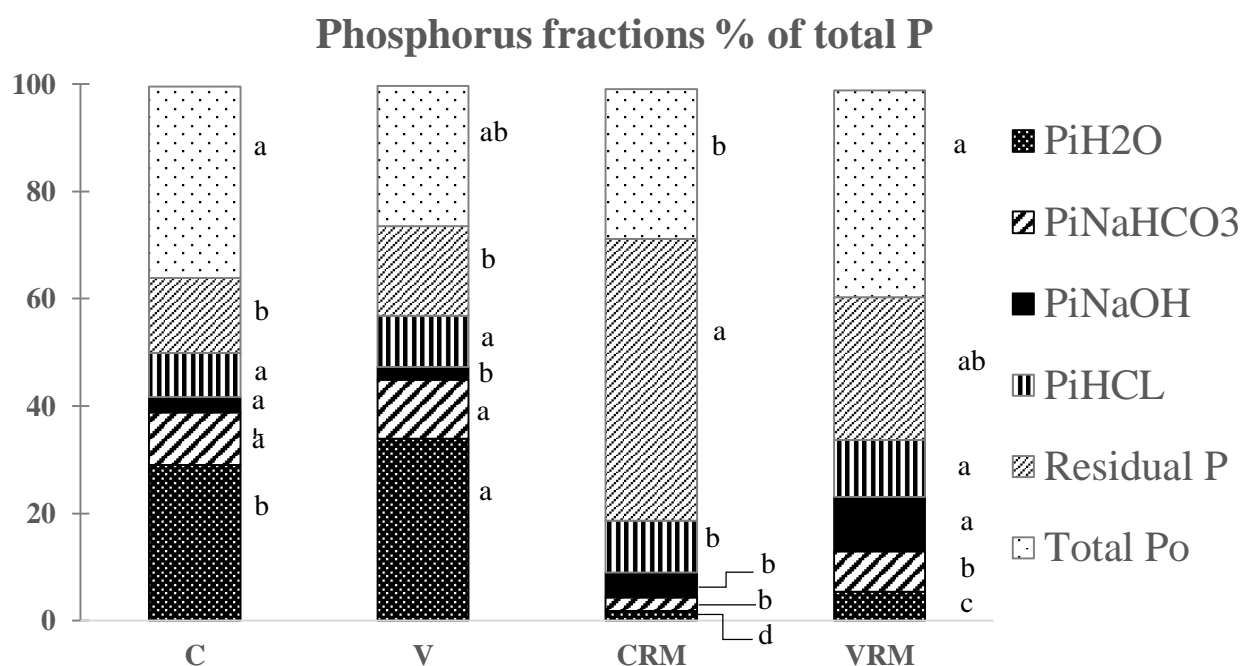


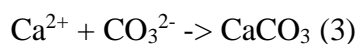
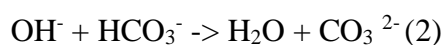
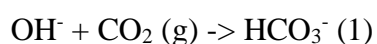
Figure 48 Phosphorus fractions in 196 day composts and vermicomposts with or without red mud. C_{control}: compost; V_{control}: vermicompost; CRM: compost +red mud; VRM: vermicompost+ red mud. Different small letters indicate significant differences between equivalent fractions (Kruskal-Wallis test, p< 0.05).

Results from phosphorus Hedley fractionation are given in **Fig. 47**. Phosphorus was mostly inorganic, the organic P proportions ranging from 26.2% to 38.5%. P fractions were similar between C and V treatments, except the P_i NaOH fractions, which were slightly higher in compost (2.9%) than in vermicompost (2.3%) and water-soluble P (P_i H₂O), which was higher in vermicompost as compared to compost. The latter fraction was the largest one in regular treatments. Red mud addition (VRM and CRM) strongly decreased the water-soluble and Na HCO₃ P fractions (4.3% and 12.9% in CRM and VRM respectively) compared to the corresponding controls. By contrast, the more recalcitrant P fractions, P_i HCL and P_i NaOH, were larger in red mud treatments, particularly without worms (CRM). The residual P fractions also significantly increased when red mud was added during composting (CRM), but the increase was not significant for vermicomposting (VRM). The total organic P was decreased by red mud addition only with composting (CRM).

4. Discussion

4.1 Alkaline materials influenced GHG emissions, in particular CO₂

Influence of red mud and fly ash on CO₂ emissions were contrasted. While red mud reduced significantly CO₂ emissions during composting, fly ash addition had no effect. This may be related to the different origins of emitted CO₂. Red mud is highly alkaline due to the presence of residual NaOH, used during the industrial process of alumina production (Wang *et al*, 2008). The contribution of Ca and NaOH to red mud may reduce CO₂ emissions due to carbonate formation:



The high Ca contents in red mud (**Table 27**) might shift the chemical equilibrium (2) towards carbonate formation (Si *et al*, 2013).

Fly ash, as a waste product of clean-coal combustion used to mitigate gaseous emissions (*eg.* used in power plants) has a higher pH compared to red mud. Since no sodium hydroxide is used during this process, the slight decrease of CO₂ emissions during composting may not be explained by carbonate formation.

Therefore, the origin of the waste products is important to consider to understand its impact on CO₂ emissions during composting. When industrial wastes are used for co-composting, specific chemical processes may interfere with biological CO₂ emissions.

During composting without alkaline amendment, N₂O emission rates, ranging from 0.010 to 0.022 mg N₂O kg⁻¹ h⁻¹ were similar to those recorded by Andersen *et al* (2010) and methane emissions ranged from 0.024 to 0.026 mg CH₄ kg⁻¹ h⁻¹. N₂O emissions drastically decreased with composting time for all treatments, due to the removal of NH₄⁺ through nitrification. Alkaline amendments had no effect on N₂O emissions, while CH₄ emissions increased when composts were produced with fly ash. During composting, methane is mainly formed in anaerobic microsites (Beck-Friis *et al*, 2000), which may develop more in the presence of fly ash, showing the highest initial pH. Fly ash may increase methanogen bacterial activity, known to be inhibited by acidic pH (Taconi *et al*, 2008). As methane has much stronger greenhouse effects than CO₂ (IPCC, 2014), our results suggest that changes in methane emissions may be of concern with fly ash use during composting.

4.2 Alkaline materials influenced the qualities of the final products

While both alkaline materials decreased the amount of microbial biomass, the presence of red mud also significantly increased OM degradation rate. Wong *et al* (1995) also observed that alkaline materials enhance the decomposition efficiency of organic materials. They suggested that alkaline materials create a suitable environment for the development of microbial communities and their activities, in particular by increasing the pH. Such microbial enhancement is not supported by our microbial data (**Fig. 46**), showing reduced microbial respiration and biomass amount for compost with red mud and fly ash. Similar ratios (from 0.79 to 0.82) of Gram⁺ on Gram⁻ were measured in treatments with and without red mud, which may indicate similar compost maturity (Klamer and Bååth, 1998).

Contents of nutrients, essential for plant nutrition are also important to assess the compost quality. Bernal *et al* (2009) proposed to use the ratio NH₄⁺:NO₃⁻ to assess compost maturity, and a value of 0.16 as the upper limit for a mature compost. Our NH₄⁺:NO₃⁻ ratios ranging from 20.12 to 41.88 indicate that composts have not yet reached maturity (**Table 24**).

While red mud addition did not affect Pb and Ca contents in compost, other nutrient contents decreased with its presence. According to our data (**Fig. 47**), addition of red mud during composting led to a drastic decrease of the easily extractable P fractions. This is in line with previous studies suggesting red mud efficiency to retain water-soluble P (Wang *et al*, 2008;

Belyaeva and Haynes, 2012). This retention is probably due to the specific adsorption, through ligand exchange, of soluble P on red mud iron oxides, and the precipitation of calcium phosphates with the high red mud Ca contents, favoured by their high pH (Belyaeva and Haynes, 2012). Finally, in a perspective of applying these composts to soil, their heavy metal contents must be below the legal threshold, defined in Europe by each country, generally below 200 mg kg⁻¹ OM. In our case, heavy metal contents of all treatments are approximately ten times lower than this value (**Table 27**).

The end-product DOC concentrations were increased with red mud and decreased with fly ash addition compared to the C_{control}. Chowdhury *et al* (2015) reported similar results for composts produced with fly ash but observed also lower DOC concentrations with red mud, which can be explained by their different initial feedstock (poultry manure and biosolids). Decreased DOC in the end-product of fly ash treatment suggest that the remaining carbon contains less readily decomposable compounds as confirmed by the work of Chowdhury *et al* (2015), who showed reduced mineralisation after addition to soil. According to our results, co-composting with red mud might thus (1) shorten the composting period and (2) lead to C stabilisation through carbonate formation, as discussed above. Both substrates had negative effects on microbial biomass development.

4.3 Worms increased co-compost qualities but also GHG emissions

CO₂ emissions from vermicomposting were higher than those of composting treatments (**Fig. 44**). Nigussie *et al* (2017) suggested that the higher cumulative CO₂ emissions during vermicomposting might result in a more advanced stage of stabilisation of the final product. In vermicomposts, higher relative abundance of the markers of Gram – bacteria (**Fig. 46**), which appear in the last phase of composting (Klamer and Bååth, 1998), supported this hypothesis. However, total microbial biomass was greatly reduced in vermicomposts (**Fig. 46**). Higher degradation rates for vermicomposts may be explained by worm effects on waste material (fractionation, burying and ingestion) leading to a high activity of microbial communities (Domínguez *et al*, 2010, Aira *et al*, 2006). After removal of easily degradable material, recalcitrant compounds and microbial products accumulate and may further be ingested by worms (Gómez-Brandon *et al*, 2011; Huang *et al*, 2013), which may explain the decrease in total biomass. The decrease of the fungal/bacterial ratio with worms (**Fig. 46**) seems to indicate a preferential worm feeding on fungi. The vermicompost end-product, rich in nitrogen

compounds (Ngo *et al*, 2011) and low in DOC (**Table 24**) is thus low in energy available for microbial biomass.

Vermicomposting with alkaline materials led to a decrease of CO₂ emissions, in proportions similar to those observed for composting with fly ash and red mud (approximately 5% with fly ash and 16% with red mud). These results may indicate that worms did not enhance the stability of the material, contrary to our initial hypothesis. In contrast to compost treatments, with vermicomposting, microbial biomass was increased in red mud treatment and decreased in fly ash treatment. It seems that red mud addition impedes worm feeding on microbes.

While CH₄ emissions were not different between compost and vermicompost treatments with and without alkaline materials, N₂O emissions were higher from vermicompost treatments as compared to compost treatments, in particular in the first days. Some previous studies obtained similar results (Hobson *et al* 2005) but others observed the opposite trends (Wang *et al* 2014; Nigussie *et al* 2017), probably due to the different composted materials. The high N₂O emissions that we observed are likely due to the denitrification mechanisms occurring in the worm gut (Lubbers *et al*, 2013).

Composting with worms increased the NO₃⁻ content and decreased the NH₄⁺ content of the final product compared to composting without worms. Similar observations were made by several authors (Nigussie *et al*, 2017; Lazcano *et al*, 2008) and tentatively explained by the organic N mineralisation and/or the limitation of N₂ losses through denitrification. Concerning the NH₄⁺:NO₃⁻ ratio, all vermicompost end-products were under the preconized threshold (**Table 24**), suggesting that worms improve compost maturity.

Nutrient contents, especially Ca, P and Zn (**Table 27**) were higher in vermicompost (V_{control}) than in compost (C_{control}), and decreased with red mud addition. Vermicomposting particularly increased nitrate content and P soluble fractions compared to composting. This is in agreement with the results of Ghosh *et al* (1999) and may be explained by a rapid decomposition of OM and thus the conversion of insoluble P to more soluble forms. Vermicompost with red mud showed higher nitrate content and higher soluble P proportion than red mud compost, suggesting that worms' presence could also counterbalance N immobilisation and P adsorption to the inorganic matrix, which has a great influence on P availability in co-compost produced with red mud (Belyaeva and Haynes, 2012).

5. Conclusion

Vermicomposting and composting are processes leading to the stabilisation of organic matter due to removal of easily decomposable compounds by microbial degradation. They thus emit CO₂ and other GHG. In order to reduce these emissions and increase the carbon stability of the end-products, alkaline materials (red mud and fly ash) were added at the initial phase of composting. Both materials decreased CO₂ emissions but only red mud decreased them drastically without increasing other GHG emissions. Formation of carbonates in addition to negative effects on microbial activity could be the main reasons for this CO₂ emission decrease. The nutrient and heavy metal contents, parameters essential to consider for compost use as plant growth media, appeared to be decreased by red mud addition during composting, suggesting nutrient retention on red mud, especially for soluble P. Decrease of available nutrients in compost due to red mud addition could be counterbalanced by the addition of worms. Worms' presence increased CO₂ emissions but combined to red mud, it leads to similar GHG emissions, better carbon stabilization, compost maturity and nutrient availability compared to a classic compost.

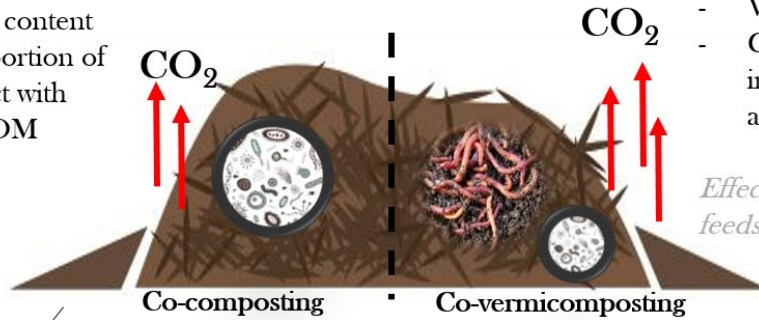
Conclusions and future perspectives

Treating organic wastes has become a critical social and environmental problem. Traditionally, two processes are well-known to transform organic wastes into a valuable organic fertilizer and amendment, composting and vermicomposting. Even if they are based on biological degradation, both require specific controls in order to reach an optimum of parameters such as moisture content, aeration and temperature. Although composting and vermicomposting are considered as suitable and environment-friendly strategies to recycle organic wastes, their carbon footprint is not negligible due to a large amount of CO₂ released in the atmosphere.

To limit this negative aspect, additives might be used in order to optimize the composting process and also to limit its environmental impact. Recently, clay minerals and iron oxides raised interest as additives to compost due to their capacity to protect carbon from further mineralization (Bolan *et al*, 2012). However, little is known on the effects of these minerals on the maturity and quality of the end-products and on the mechanisms potentially induced by their presence on carbon protection, nutrient availability, etc. Moreover, co-composting was previously only performed without worms, even if worms may play a key role in these systems. Therefore, the present study investigated this knowledge gap of co-composting and co-vermicomposting with minerals and focused on three essential aspects: (1) the carbon stability during processes, (2) the physico-chemical characteristics of the end-products and their ability to be used as potting media and, (3) the changes induced by their application on soil properties. For this aim, we produced different composts with minerals, with and without worms, from fresh organic matter, in order to quantify carbon emissions, characterize the end-products and chemical changes induced by different amounts and mineralogical properties of the additives used. End-products were used as organic amendment on an arenic cambisol and as potting media in a plant growth experiment. The **figure 49** summarizes the main conclusions of this study and perspectives, further works required to fully understand these new composting systems.

Chapter 2 and 6: Carbon protection occurs during composting but depends on the mineralogy of the additives; worms enhance organo-mineral associations

- Low DOC content
- Small proportion of clay interact with degraded OM



- Very low DOC content
- Great proportion of clay interact with degraded and intact OM

Effects with different initial feedstocks? Worms?

Chapter 3 and 6: nutrient availability decreases due to mineral adsorption; worms counterbalance this negative effect of minerals



- High $\text{NH}_4^+ : \text{NO}_3^-$ ratio
- Low amount of soluble phosphorus;
- Low EC

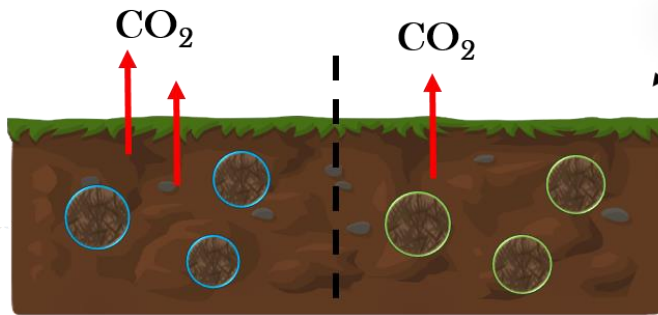
Shift of microbial communities/ activities?

- $\text{NH}_4^+ : \text{NO}_3^-$ ratio < 0.5
- soluble phosphorus higher than in co-compost
- Relative high EC



Chapter 4: carbon mineralization from amended soil is negatively correlated to carbon mineralization during co-composting; co-vermicompost allows to increase soil carbon storage

Influence of soil texture? Climate?



Arenic cambisol

- No negative effect on soil microbial biomass
- Protective effect of mineral persistent only within co-vermicompost



- Physico-chemical parameters do not allow plant growth prediction
- Potential growth inhibitor within co-vermicompost and co-compost

Chapter 5: plant growth is highly dependent on the substrate; low concentration of vermicompost should be recommended; co-vermicompost enhances some growth parameters

Optimum amount? Mechanisms occurring in the rhizosphere? Other plant species?

Figure 49 Summary of the main conclusions and perspectives induced by this study

1. The carbon protection of organic matter during composting and persistent effect of mineral protection in amended soil: reduction of GHG and potential increase of soil carbon stocks?

As discussed in the state of the art (**Chapter 1**), carbon protection in soil is favoured by the presence of minerals, through organo-mineral associations. Organo-mineral associations formed in soil greatly depend on several factors as the clay mineralogy (2:1 or 1:1 clay) and thus the specific surface area which potentially interacts with organic matter (Kleber *et al*, 2015), the presence of oxides, and the composition of the organic matter. In addition, minerals may reduce the accessibility of organic matter for microdecomposers (Sollins *et al*, 1996) through the formation of aggregates of different sizes. In this study (**Chapter 2**), fresh organic matter was decomposed based on the composting process with and without minerals in different proportions, allowing to assess the potential formation of organo-mineral associations, according to the added mineral matrix. Beside the influence of mineral matrix on carbon protection during composting, worms were also added in order to investigate their potential effect on carbon and organo-mineral associations. Indeed, this biotic compartment is considered as a major factor in soil to form aggregates and increase carbon protection (Bossuyt *et al*, 2005). However, their influence on carbon protection is still poorly understood as soil ingestion and destruction through worm gut might lead to destabilization of the soil structure, and increase GHG emissions (Lubbers *et al*, 2013).

We designed an experimental approach in this study to build a simple model of organic matter decomposition with minerals and worms, since pure minerals were tested and the initial organic matter was known. After 196 days of co-composting and co-vermicomposting, a high amount of 2:1 clay minerals significantly decreased carbon emissions. Thus, the carbon mineralization of fresh organic matter seemed to be driven by the presence and characteristics of minerals. However, different mechanisms as it occurs in soils were observed, underlining that the protective effect of minerals is highly dependent on the organic matter matrix and environmental conditions. Therefore, during composting and vermicomposting, the conditions are beneficial for 2:1 clay minerals to interact with organic matter and potentially protect carbon from mineralization.

Furthermore, our study opened new perspectives to close more knowledge gaps on the influence of worms and minerals and their interaction with the organic matter. We finely characterized the end-products of our different composting processes with complementary methods

(microscopic analyses, changes in their organo-mineral associations and in their nutrient composition and availability) and related their characteristics to the conditions (pH, EC, etc) during composting. Our results point to the relevance of the chosen parameters, which should be also monitored during composting: pH, organic compounds, microbial activity and microscopic analyses. Therefore, large composting and vermicomposting units must be implemented to allow samplings.

In addition, in soil, three categories of worms co-exist (anecic, epigeic and endogeic) which allow to transform organic matter rapidly into casts (Bouché, 1977). Due to the limited size of our composting units and the use of fresh organic matter, only epigeic species were chosen in this study. A future experiment may be done in larger composting units, allowing to use anecic and endogeic worms to investigate their influence on the formation of stable casts, according to minerals. In addition, we used similar fresh organic matter composition in all the treatments. Future studies could investigate the influence of the initial organic matter on the composting process.

One of the purposes of this study was to assess the protective effect of minerals, not only during composting but also after soil application of the end-products. Indeed, the raising interest in the production of composts and vermicomposts to recycle organic matter relies on their possible use as soil conditioner (**Chapter 4**) or as potting media (**Chapter 5**). The aim of the application of compost into soil is to increase/restore soil organic matter, and improve other soil properties as water retention, microbial activities or nutrient availability.

In **Chapter 4**, co-composts and co-vermicomposts produced with a 2:1 clay minerals (15% and 30% montmorillonite) and with a mixture of kaolinite and goethite (15%/15%) were applied to an arenic cambisol, poor in minerals and organic matter. After 3 months of a laboratory incubation, vermicomposted products showed a lower carbon mineralization than composts, but this effect was likely to occur only on the short-term. Only co-vermicompost with montmorillonite was predicted to still present a decrease of carbon mineralization after one year. Carbon protective effect of this mineral was likely persistent only if organic matter and minerals were associated through worm gut. Nevertheless, a soil microbial community and activity shift might have also played a role in this CO₂ evolution from soil. Mechanisms behind soil carbon mineralization of mineral containing casts needs further investigation to understand the interactions between exogenous and soil organic matter and microbial biomass. Implementing studies using isotopic methods could be one strategy to understand these interactions: the carbon of exogenous casts could be labelled with ¹³C to be distinguished from

soil carbon.

In view of stocking carbon into soil (4 per 1000 international initiative), the application of co-vermicompost may allow to increase soil carbon stocks (**Fig. 50**) and avoid CO₂ releases in the atmosphere. Previous studies suggested that hundred years after soil application of regular compost, 2 to 10% of the initial amendment C was still present in the soil (Favoino and Hogg, 2009). As discussed in **Chapter 1**, this estimation largely depends on the type of soil (Busby *et al.*, 2007), on the pedoclimatic conditions and on the type of compost (Peltre *et al.*, 2012). Based on our results and on a C mineralization model, we predicted that more than 10% of the initial C from co-vermicompost might remain in the soil, suggesting that mineral addition is able to increase potentially stabilized C. Our 3 month laboratory experiment using a specific soil allowed to suggest mechanisms explaining such changes and to estimate mineralization rates through modelling. Further experiments should be designed to precise the long term effects of worms and minerals on compost stability in different soils and environmental conditions, optimally in field studies. Such experiments should allow to optimize the application of co-composts and co-vermicomposts according to the soil and geographical area.

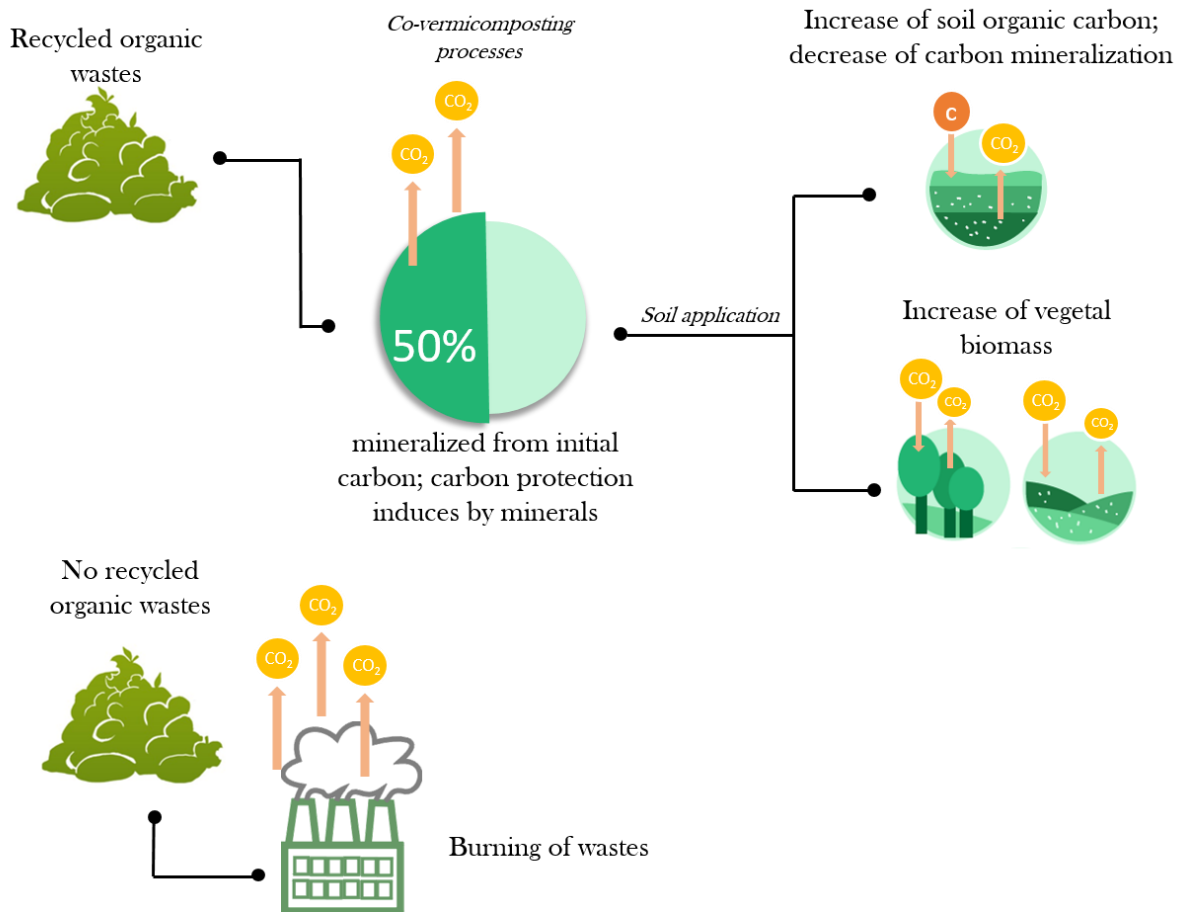


Figure 50 Potential carbon emissions and sequestration mechanisms related to co-vermicomposting processes.

2. Addition of minerals during composting processes: a negative aspect for co-compost maturity?

Another aspect studied here is the potential use of co-composts and co-vermicomposts as potting media. Few studies assessed the impact of co-compost on plant growth, let alone under field conditions (Chowdhury *et al*, 2014; Kuba *et al*, 2008). Generally, the effect of co-composts on plants is tested with the germination index, a biological indicator used to evaluate the toxicity and maturity of compost (Zucconi *et al*, 1981). However, this test does not consider all plant growth parameters affected by the organic fertilizer. The few field experiments carried out with co-composts showed that they might improve soil fertility and thus potential revegetation. Chowdhury *et al* (2014) observed that a co-compost (biowastes with alkaline amendment) improved soil fertility when used to revegetate an urban landfill soil. In our study, we evaluated our co-composts and co-vermicomposts as an alternative component to peat moss, in a

substitution rate of 7 % (v/v).

Before using co-composts and co-vermicomposts as potting media, their physico-chemical characteristics were evaluated (**Chapter 3**) and indicated that worms were beneficial in the process. Indeed, they increased nutrient availability (P and N) and counterbalanced the negative effects of minerals on the nutrient cycle. Finally, all treatments were considered as optimum to be used as organic fertilizer, except composts which presented high proportions of ammonium and thus may require a longer composting duration than vermicomposts.

Even if all physico-chemical characteristics of the co-composts were improved by worms (**Chapter 3**), plant growth was decreased with the co-vermicomposts compared to a non-substituted commercial potting media but increased with a regular vermicompost (**Chapter 5**). The addition of co-vermicomposts significantly increased some parameters as leaf area, particularly when no chemical fertilizer was added. Therefore, we showed that the parameters (Bernal *et al*, 2009) usually used to define a mature compost and thus predict plant growth were not fully applicable in the case of kitchen waste composts and vermicomposts with clay minerals. Different mechanisms might occur, delaying or inhibiting plant development which were not related to physico-chemical parameters of the organic fertilizer (*e.g.* hormone-likes; nutrient cycle in potting media).

The evaluation of co-composts and co-vermicomposts as organic fertilizer should be also assessed in soil and with different plant species. Such future experiments may allow to explain the mechanisms inherent to nitrogen and carbon accumulation from roots to shoots, as they appeared highly dependend on the substrates and plant species (**Chapter 5**). Moreover, it should cover the lack of knowledge on the interactions between roots and composts/vermicomposts and the differences induced in the rhizosphere mechanisms (root exudates; microbial activities; organic matter localisation...).

3. Feasibility of these processes at large scale? Economical and practical aspects of these new composting strategies

According to Lim *et al* (2016), composting and vermicomposting processes are both suitable systems but their economic potential is dependent on the initial cost of the installation, the production volume, the initial quality of the feedstock and the end-product prices at a particular location. They estimated that a composting facility annually costs about 4.37 USD millions (mainly related to initial investment and annual operation and maintenance costs) and generates 1.10 USD millions of benefits per year. Moreover, as underlined by Onwosi *et al* (2017),

composting is a time consuming process. Therefore, in order to reduce the processing time and thus the cost, composting facilities could shorten the duration by using additives.

Thus, to help to produce a high-quality end-product, the additives must be cheap and efficient, such as bentonite or allophane (Li *et al*, 2012; Bolan *et al*, 2012). Currently, the real cost of mineral application during composting is unknown, even if mineral additives raise increasing interests. Moreover, this cost depends on several factors such as the localisation of the composting platform or the required quantity. The optimal additive: organic waste ratio must be determined and adapted to each feedstocks in order to reduce the cost of composting.

In our study, in order to control and simplify the model system to understand the mechanisms, we used pure minerals which are expensively purified by industries. Thus, it should be interesting to test raw minerals in order to reduce the cost of these composting processes. For instance, we pointed out in **Chapter 6** that the use of red mud or even fly ash as additives should be further investigated as these industrial wastes are abundant and cheap (Gomes *et al*, 2016). Moreover, they appeared efficient to reduce carbon emissions without critically altering the end-products. Nevertheless, the transport costs of these products must be taken into account as they are produced on specific sites by coal combustion for electricity production (Wong *et al*, 1995; Fang *et al*, 1998; Chowdhury *et al*, 2014) and during the process of alumina extraction from bauxite, suggesting they should be used only in composting plat-forms located in the same area as these industries. Finally, selecting additives is a matter of trade-off. Taking into account the additive cost, spatial and temporal accessibility (e.g. agricultural residues collected seasonally) and abundance, the composting process with additives must be adapted to the region, the composting facility localisation and the season.

Our results completed the findings in the literature on the enhancement of the composting process by the presence of worms (*i.e.* vermicomposting), adding new beneficial aspects of worms beside those already described: avoiding mechanical turning, generating revenue with excess worm biomass and enhancing the beneficial aspects of the end-product compared to a regular compost (Gajalakshmi and Abbasi, 2002; Ngo *et al*, 2011; Doan *et al*, 2015). Our study further postulated that worm presence, combined with an additive, significantly improves the process by increasing the nutrient availability, carbon stabilisation, shortening compost duration and decreasing the total GHG emissions. In addition, co-vermicomposts may be used to decrease the consumption of chemical fertilizer and also may be a solution to restore degraded soils (Ngo *et al*, 2012). Therefore, this process may lead to higher return on investment and

lower annual cost than a regular composting with or without additives (Fig. 51). These observations must be confirmed by a cost analysis and a life cycle assessment of these new composting processes.

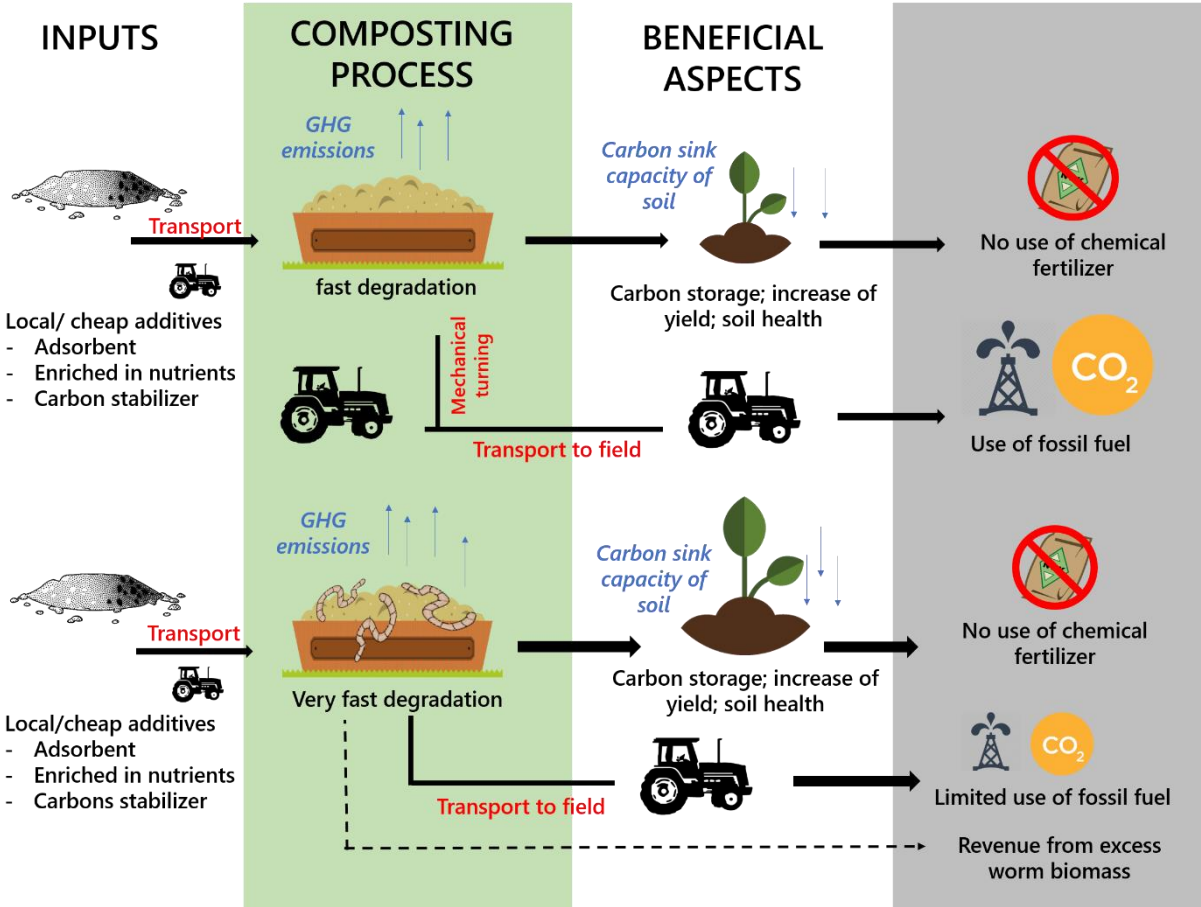


Figure 51 Main potential environmental differences between co-composting and co-fermentation processes.

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ANNEXES

Appendix 1: The different types of additives used during composting and their possible effects.

Feedstock	Additives	Effects of the additives	References
municipal waste	plastic tube, woodchip	aeration/porosity; CH ₄ emission reduction	Maulini-Duran <i>et al</i> , 2014
manure	minerals (oxide and clays)	carbon sequestration; reduction of CO ₂ emissions	Bolan <i>et al</i> , 2012
green-waste	biochar; clay minerals	carbon sequestration; reduction of CO ₂ emissions	Barthod <i>et al</i> , 2016
poultry manure	biochar	aeration	Czekala <i>et al</i> , 2015
manure	residual straw	aeration	Kulcu and Yaldiz, 2007
sewage sludge	wood ship, rice husk	regulation of moisture content	Morisaki <i>et al</i> , 1989; Eftoda and McCartney, 2004
kitchen waste	cornstalk; sawdust	absorption of leachate; reduction of CH ₄ emissions	Yang <i>et al</i> , 2013
food waste	sawdust	absorption of water	Chang and Chen, 2010
pig manure	bentonite	absorption of water; reduction of nutrient leachates	Li <i>et al</i> , 2012
green waste	ash	absorption of water; reduction of soluble P	Belyaeva and Haynes, 2009
green waste	phosphate rock / fish pond sediment	nutrient content increase; absorption of water; microbial activity	Zhang <i>et al</i> , 2017

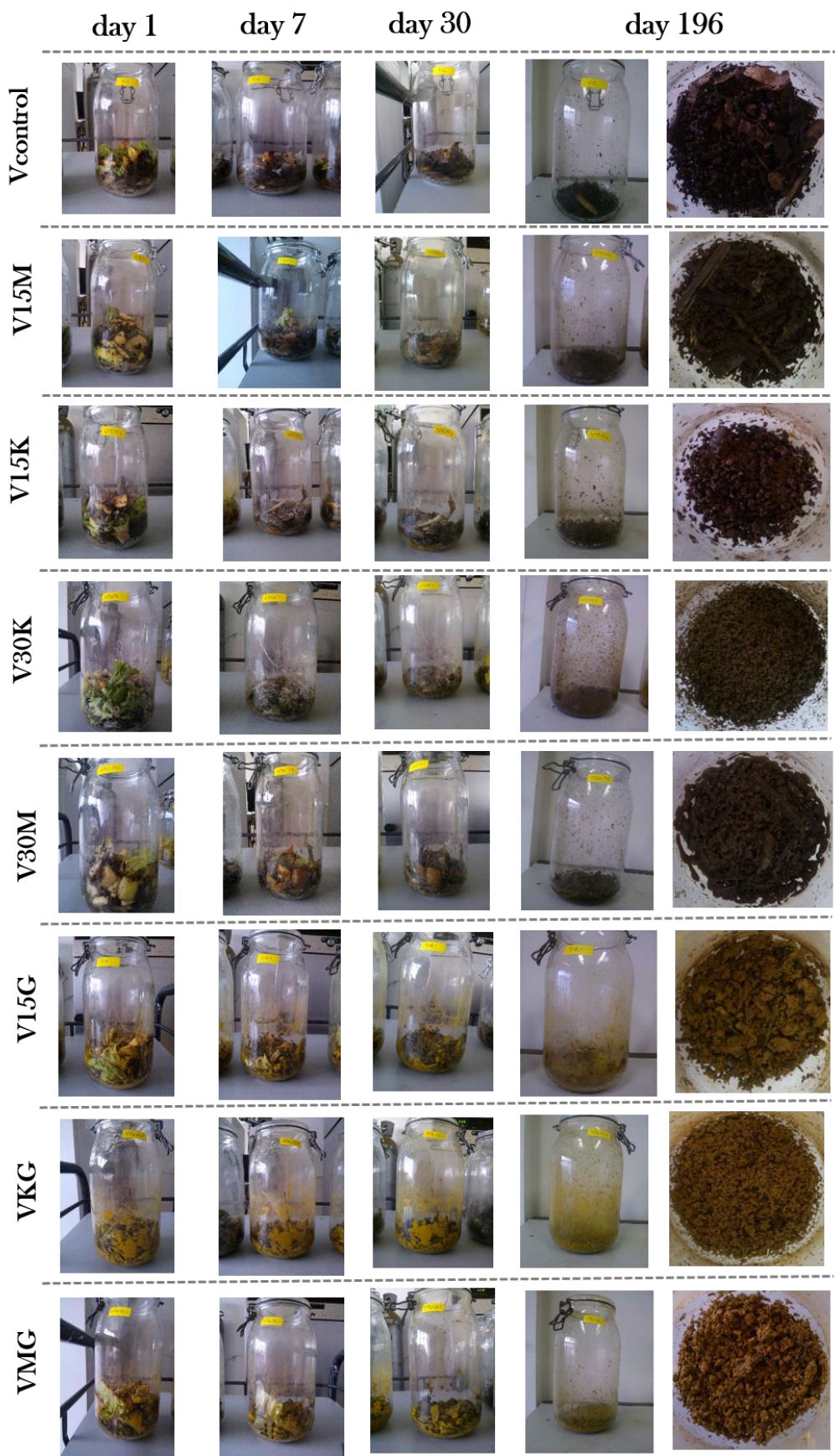
Feedstock	Additives	Effects of the additives	References
food waste	fly ash; lime	pH increase	Wong <i>et al</i> , 2009
food waste	inoculum consortium	pH increase; temperature increase; microbial activity increase; stability increase	Manu <i>et al</i> , 2017
municipal waste	bagasse, paper, peanut shell, sawdust	pH increase	Iqbal <i>et al</i> , 2010
sewage sludge	fly ash, red mud, lime	pH increase	Fang <i>et al</i> , 1997; Fang <i>et al</i> , 1999; Wong <i>et al</i> , 2009
pig manure	bamboo charcoal	nitrogen loss decrease / pH increase; reduction of heavy metal mobility; germination index increase	Chen <i>et al</i> , 2010
pig slurry	zeolite	pH increase	Venglosky <i>et al</i> , 2005
poultry manure	elemental sulphur	pH increase, reduction of ammonia and CO ₂ emissions	Mahimairaja <i>et al</i> , 1994
sewage sludge	rice husk	microbial activity increase	Nakasaki <i>et al</i> , 1996
manure	mature compost/ inoculum	composting duration shortening	Kato and Miura, 2008
green waste	jaggery, fly ash	microbial activity enhancement; nutrient content increase	Gabhane <i>et al</i> , 2012
green waste	biochar, spent mushroom	microbial activity enhancement	Zhang and Sun, 2014
food waste	sodium acetate	microbial activity enhancement	Yu and Huang, 2009
municipal waste	rice straw	aeration; reduction of odour emission	Shao <i>et al</i> , 2014
manure	ash	reduction of odour emissions	Koivula <i>et al</i> , 2003

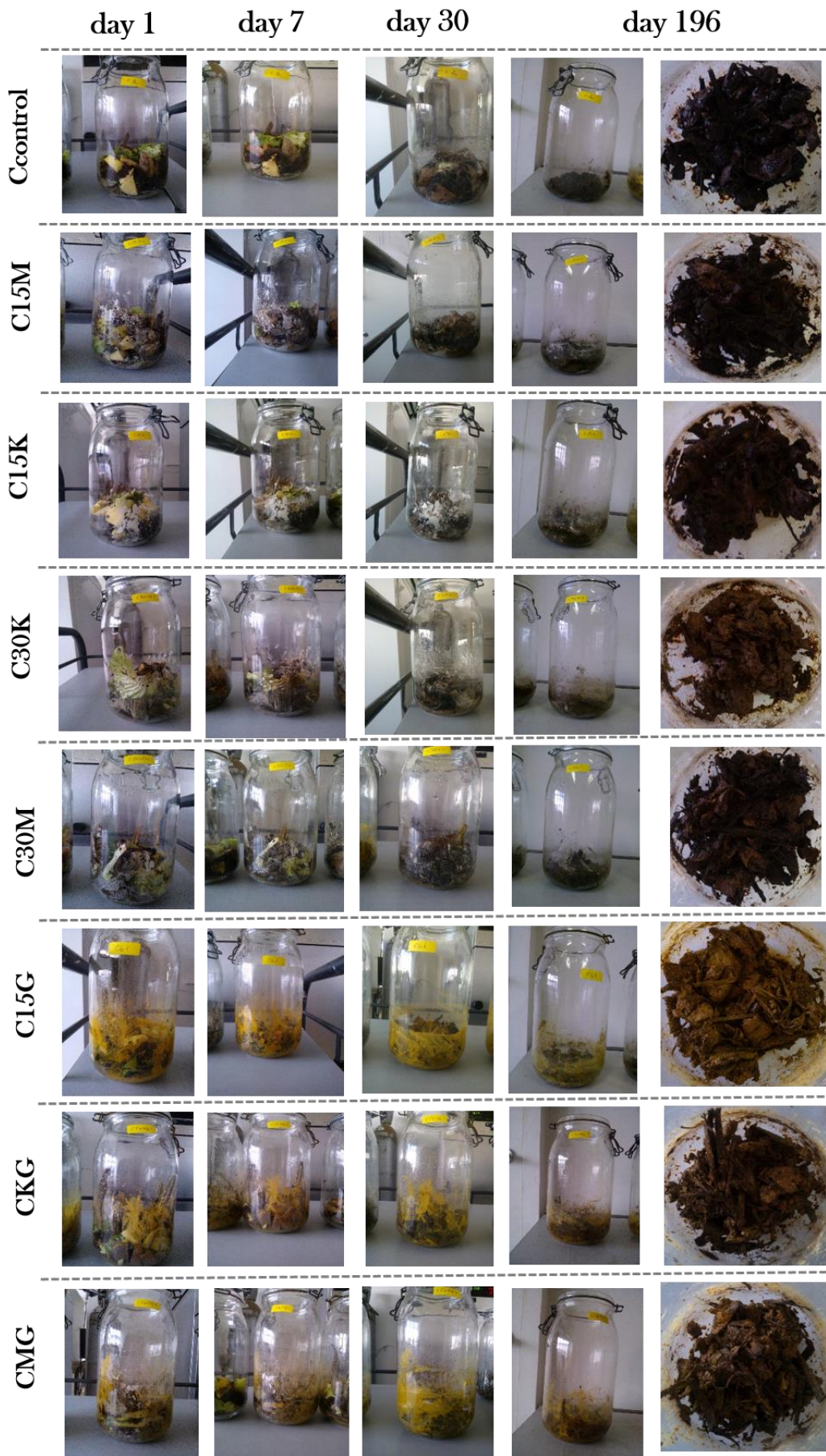
Feedstock	Additives	Effects of the additives	References
poultry manure	biochar	reduction of odour emissions	Steiner <i>et al</i> , 2010
sludge	bamboo charcoal	reduction of nitrogen loss; reduction of heavy metal mobility	Hua <i>et al</i> , 2009
chicken manure	biochar / sawdust	reduction of odour emissions	Khan <i>et al</i> , 2014
municipal solid waste	zeolite	reduction of ammonia emissions	Turan and Ergun, 2007
municipal solid waste	zeolite	reduction of ammonia emissions	Wang <i>et al</i> , 2014
dairy slurry	zeolite	reduction of ammonia emissions; reduction of soluble P	Lefcourt and Meisinger, 2001
pig manure	nitrogen electron acceptor, sodium nitrate and sodium nitrite	control of sulphur odours	Zang <i>et al</i> , 2017
pig manure	magnesium hydroxide and phosphoric acid	reduction of ammonia emissions	Ren <i>et al</i> , 2010; Jeong and Hwang, 2005
manure	phosphogypsum	reduction of GHG emissions	Hao <i>et al</i> , 2005; Luo <i>et al</i> , 2013
manure	biochar	reduction of CO ₂ emissions; carbon sequestration	Chowdhury <i>et al</i> , 2014
poultry manure	biochar	organic matter transformation increase	Dias <i>et al</i> , 2010
municipal solid waste	inoculum consortium	stability increase	Wei <i>et al</i> , 2007
			Lu <i>et al</i> , 2008
wastewater	fly ash	removal of phosphate in leachates	

Feedstock	Additives	Effects of the additives	References
manure	rock phosphate	Agricultural yield increase; high nitrogen and phosphorus content	Nishanth and Biswas, 2008; Billah and Bano, 2015
pig manure	rice straw	high nitrogen and phosphorus content	Lü <i>et al</i> , 2013
poultry manure	chestnuts, leaf litter	reduction of heavy metal content	Guerra-Rodriguez <i>et al</i> , 2006
pig manure	rock phosphate	reduction of heavy metal content	Lu <i>et al</i> , 2014
sewage sludge	lime	reduction of water-soluble heavy metals	Wong and Selvam, 2009
sewage sludge	lime	reduction of water-soluble heavy metals; pH increase	Fang and Wong, 1999
sewage sludge	zeolite	reduction of water-soluble heavy metals	Nissen <i>et al</i> , 2000; Zorpas <i>et al</i> , 2000; Villasenor <i>et al</i> , 2011
sewage sludge	red mud, fly ash	reduction of water-soluble heavy metals	Qiao and Ho, 1997; He <i>et al</i> , 2016; Wang <i>et al</i> , 2013
coffee grain trash/ manure	rock phosphate/ phosphogypsum	soil health improvement	Oliveira and Ferreira, 2013
biowastes/ green waste	wood ash	retention of Al in soil	Bougnom <i>et al</i> , 2009
rice straw	red mud	reduction of soil metal availability; soil health improvement	Zhou <i>et al</i> , 2017

Feedstock	Additives	Effects of the additives	References
separated organic waste	ash	soil microbial activity improvement; revegetation enhancement	Kuba <i>et al</i> , 2008
sewage sludge	zeolite, lime	reduction of GHG emissions	Awasthi <i>et al</i> , 2016

Appendix 2: The evolution of vermicompost and composts units during 196 days.





Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

Master 1 SDUEE

Mention Environnement

Continentaux et Hydrosience



Rapport de stage de M1

Margot CHAPUIS

CARACTÉRISATION DES LIGNINES

(QUANTITÉS ET QUALITÉS)

DANS LES COMPOSTS ET VERMICOMPOSTS ÉLABORÉS AU SEIN
DU PROJET LOMBRICOM'

Relié à la thèse de J. Barthod : « Vermicompostage en présence de
minéraux, un procédé innovant pour limiter les émissions de CO₂ :
compréhension des processus aux petites échelles »

Projet Lombricom' (CNRS, EC2CO)

2017

Maîtres de stage : Justine Barthod, Marie-France Dignac et Cornélia Rumpel

Laboratoire d'accueil : IEES

Date de soutenance : 30/06/2017

Résumé

L'augmentation continue de CO₂ anthropique dans l'atmosphère est de plus en plus un enjeu mondial. Le recyclage de la matière organique par le compostage est l'une des façons d'augmenter le stock de carbone tout en limitant les déchets de plus en plus abondants.

Dans cette étude, nous avons cherché à comprendre dans quelles mesures, la quantité et la nature de minéraux, et la présence de vers influent sur l'état de la matière organique lors de processus de compostage.

Pour ce faire, nous avons eu à quantifier les lignines qui sont des biomarqueurs de l'état de dégradation de la matière organique, grâce à la méthode d'oxydation cuivrique en milieu alcalin avec détection des monomères de lignine par chromatographie gazeuse.

Nous avons trouvé que l'action des minéraux n'était pas la même selon leur nature et leur quantité. Et que l'action des vers nuancait cet effet des minéraux. Ainsi les minéraux semblaient moins efficaces pour limiter la dégradation des lignines en présence de vers.

II. Matériel et méthode

II.1. Caractéristiques du compost utilisé

Les composts et vermicomposts ont été produits en laboratoire en conditions contrôlées (20°C, à l'obscurité, humidité des substrats maintenue à 70% (poids sec)).

Le compostage et vermicompostage a été élaboré à partir de matières organiques fraîches, composées dans les proportions suivantes ; en poids sec : 1.2% de laitue, 19.6% de pomme, 39.1% de marc de café, 10.1% de maïs et 27.9% de carton. Les vers ajoutés pour les vermicomposts, sont *Eisenia andrei* et *Eiseina foetida*. Quatre réplicats ont été réalisés pour chaque traitement. Le processus a duré 196 jours, au bout desquels les différents substrats obtenus ont été séchés à l'air libre puis broyés avant d'être stockés. Dans le cas des vermicomposts, les vers ont été préalablement enlevés, ainsi que leurs cocons avant le séchage des substrats.

II.2 Caractéristiques des minéraux ajoutés

Les minéraux ajoutés à la matière organique fraîche, dès le début du compostage et du vermicompostage sont, la montmorillonite (type 2:1) en proportion 15% et 30% et la kaolinite

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

en association avec la goethite (oxyde de fer) en proportion 15%-15% a été utilisée. Les pourcentages sont exprimés par rapport au poids sec de déchet initial. Les témoins sont le compost et vermicompost classique, sans ajout de minéraux (Tableau 1).

Tableau 1 : Abréviations des différents traitements et explication

Ccontrôle	Compost
Vcontrôle	Vermicompost
C15M	Compost avec 15% de montmorillonite
V15M	Vermicompost avec 15% de montmorillonite
C30M	Compost avec 30% de montmorillonite
V30M	Vermicompost avec 30% de montmorillonite
CKG	Compost avec 15% de kaolinite et 15% de goethite
VKG	Vermicompost avec 15% de kaolinite et 15% de goethite

II.3 Principe

Les lignines ne peuvent pas être analysées à partir du compost directement. Une méthode couramment utilisée pour déterminer les lignines du sol est l'oxydation alcaline par CuO (Thevenot et al., 2010). Par ce procédé, des monomères phénoliques sont isolés et quantifiés par Chromatographie en phase Gazeuse avec Détection par Ionisation de Flamme (GC-FID). La somme des monomères phénoliques est un indicateur de la quantité de lignines du sol, nous allons l'appliquer au compost.

Cette méthode a été développée pour des sols ou des végétaux, or comme nous sommes dans le cas de composts et vermicomposts il a fallu l'adapter. Une série dure deux jours et permet d'analyser 8 échantillons et un sol de référence.

L'explication de la méthode dans les paragraphes suivant est tirée de l'article de (Thevenot et al, 2010).

II.3.1 L'oxydation

La quantité d'échantillon pesée a été de 25mg à 200mg (voir annexe 1) selon les échantillons pour adapter la méthode aux différents composts et vermicomposts. L'oxydation des échantillons s'est faite par ajout de CuO, d'Ammonium ferrous sulfate hexahydrate

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

$\text{FeH}_8\text{N}_2\text{O}_8\text{S}_2\text{6H}_2\text{O}$, de glucose et d'hydroxyde de sodium. L'air, qui est un oxydant non sélectif est chassé par injection d'azote avant de mettre les échantillons à chauffer. La réaction d'oxydation se fait à 172°C pendant 2h30.

II.3.2 Précipitation des acides humiques

Nous ajoutons un standard externe, l'éthylvanillin (EV) pour estimer le rendement après l'oxydation. Les rendements finaux d'EV doivent être compris entre 0,5 et 0,7. Nous centrifugeons la solution pour éliminer la phase solide par gradient de densité. Le surnageant est ensuite acidifié jusqu'à un pH de 1,8 à 2,2 pour permettre aux acides humiques de précipiter.

II.3.3 Purification des monomères phénoliques

Nous centrifugeons la solution pour passer 30 ou 60mL (voir annexe 1) du surnageant sur des colonnes d'extractions en silice (Interchim, Octadecyl (C18), 1g/6mL). Le conditionnement des colonnes se fait respectivement avec de l'éthylacétate, du méthanol et de l'eau déminéralisée.

II.3.4 Séchage et élution des monomères phénoliques

Les colonnes sont séchées, puis nous ajoutons de l'éthylacétate pour récupérer notamment les monomères de lignines. Cet éluat est séché puis solubilisé par l'éluant du chromatogramme, la pyridine. On ajoute un standard interne : l'acide phénylacétique (PAA) à 0,4 mM et du N,O-bis(triméthylsilyl)trifluoroacétamide (BSTFA) pour dériveriser.

II.3.5 Préparation du standard et de la gamme étalon

La solution mère est composée de 12 molécules standards : Le p-hydro benzaldéhyde, le p-hydro acetophenon, la vanilline, l'éthyl vanillin, l'acide p-hydro benzoic, l'acetovanillone, le syringaldéhyde, l'acide vanillique, l'acétosyringone, l'acide syringique, l'acide p-coumarique et l'acide férulique (figure 1).

La gamme étalon est constituée de cinq à sept dilutions selon les séries d'échantillons pour les encadrer au mieux. La gamme est composée du standard interne (PAA), de la solution mère et de BSTFA.

II.3.6 Chromatographie gazeuse

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

1 microL d'éluat contenant les échantillons ainsi que les standards sont passés au GC-FID (agilent, HP GC 6890, 6890N(G1530N), USA) contenant une colonne SGE BPX-5(65.0m*320microm OD, 0.25microm ID). L'injecteur à 280°C à une pression de 12,24 φ et un flux d'hélium à 14.0 mL/min. La température est programmée pour rester à 100°C pendant 2 minutes, puis subit une augmentation de 8°C/min jusqu'à 172°C, puis de 4°C/min jusqu'à 184°C puis 10°C/min jusqu'à 310°C et reste à cette température pendant 5 minutes.

II.4. Expression des résultats

Nous réalisons une gamme d'étalonnage qui permet de convertir les aires obtenues en concentration.

Nous calculons air du standard / air PAA et quantité standard gamme étalon/ quantité PAA. Cela nous permet de connaître les limites de la gamme étalon et de vérifier que la gamme est linéaire et donc valide. On utilise les coefficients directeurs et les ordonnées à l'origine pour le calcul suivant :

$$\frac{\text{mg de monophénol}}{\text{g de carbone}} = \frac{\frac{\text{mg de monophénol}}{\text{g de substrat}} * 1000}{\frac{\text{g de carbone}}{\text{g de substrat}}}$$

Nous avons calculé la somme (V+S+C) et les différents rapports caractéristiques, tels que les rapports acides sur aldehyde (Ad/Al) et C/V et S/V (avec V : la vanilline, l'acetovanillone et l'acide vanillique ; S : le syringaldéhyde, l'acétosyringone et l'acide syringique ; C : l'acide p-coumarique et l'acide férulique (Fig.3))

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

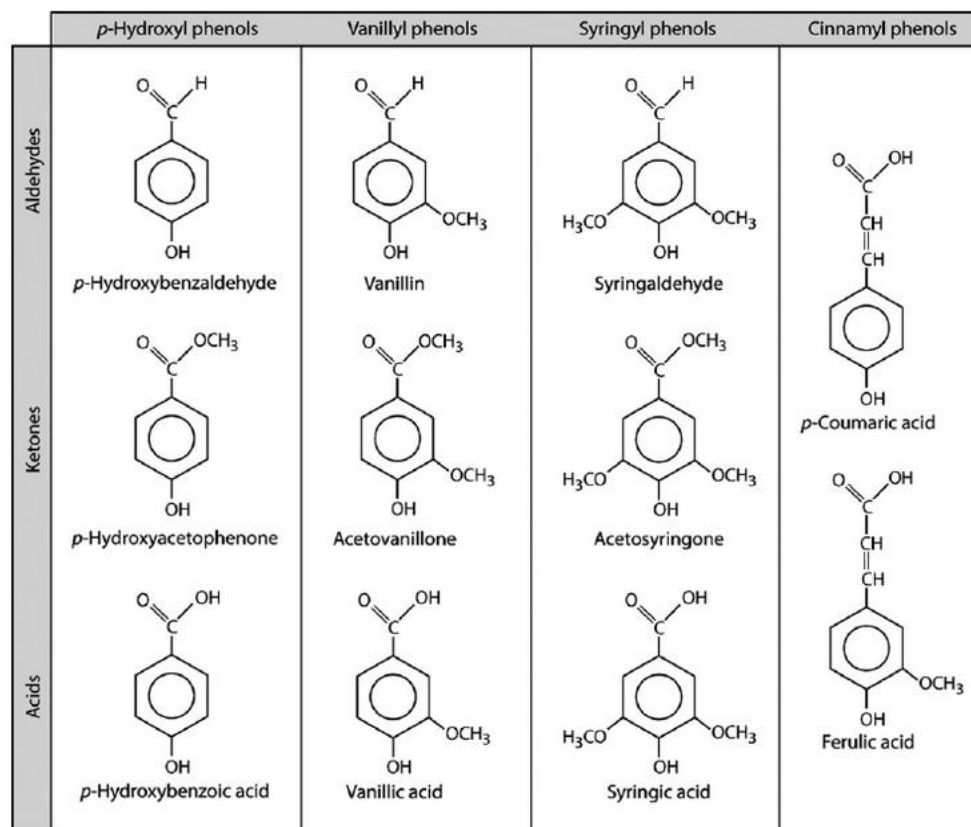


Figure 352 : Monomères de lignines, après oxydation alcaline CuO : de type H, V, S et C. (Thevenot et al., 2010)

II.5. Analyses statistiques

Les données ont été analysées à l'aide du logiciel de statistique R Version 3.4.1 (interface Rstudio). Un test en Analyse en Composantes Principales (ACP) a été réalisé sur l'ensemble des résultats obtenus pour distinguer les différents traitements selon leur teneur en lignines (Annexe 2). De plus, un test de Kruskal-Wallis a été effectué pour désigner les différences statistiques de lignines entre les traitements. Les résultats obtenus sont considérés statistiquement différent pour un $\alpha = 0,05$. Ces différences sont signalées à l'aide des lettres a, b, c, d et e dans les résultats suivants. Différentes lettres indiquent donc une différence significative dans le cas d'une p -value < 0.05 .

III. Résultats

Au vu des teneurs en carbone différentes de nos échantillons (Figure 4) et du fait que les lignines sont des composés carbonés, nous avons normalisé nos résultats par rapport à cet élément. En effet, l'ajout de matière minérale a pour effet de diluer la teneur en carbone dans l'échantillon

final.

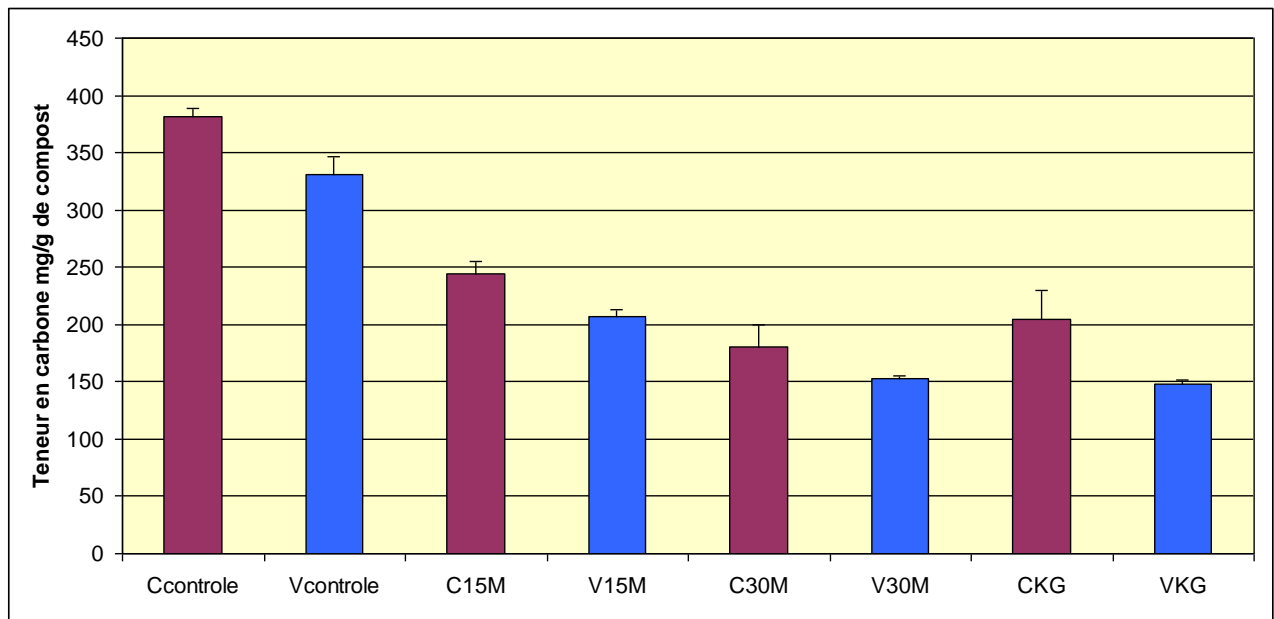


Figure 453 : Teneur en carbone des différents traitements analysés au bout de 196 jours de compostage et vermicompostage. Les barres d'erreur représentent l'erreur standard (nombre de répliqués=3)

III.1. Teneur en lignine totale et analyse des ratios

Sur les diagrammes suivants (figures 3 à 8), les composts ont été mis en violet, et les vermicomposts en bleu pour faciliter la lecture. Les différents traitements avec des minéraux sont caractérisés par des motifs.

III.1.1. Analyse de la teneur totale en lignine

Nous avons réalisé l'étude de la somme des lignines V+S+C qui s'apparente à la lignine totale de l'échantillon (Ngo et al., 2012) et permet une mesure quantitative de la lignine dans notre compost (figure 5). La teneur de lignines totales est comprise entre 3,55 et 36,16 mg/g de carbone. Le processus de compostage classique Ccontrôle, aboutit à une concentration de 8,75 mg/g de carbone alors qu'en présence de minéraux, cette valeur augmente significativement lors de l'ajout de 15% de montmorillonite C15M et du mélange argile oxyde de fer en proportion 15%-15% CKG. Inversement, la teneur en lignine a tendance à diminuer avec 30% de montmorillonite C30M.

La présence de vers lors du compostage (Vcontrôle) tend à augmenter la teneur en lignine dans le produit final comparé à Ccontrôle. De plus, en présence d'argile, cette teneur augmente selon

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

l'ordre suivant : $V30M < V < V15M < VKG$, sans toutefois montrer des différences significatives.

Enfin, l'ajout de vers lors du compostage ne permet pas d'aboutir à des différences significatives entre traitements avec et sans vers. En revanche, nous pouvons observer plusieurs tendances. Ainsi, nous pouvons voir que le compost (Ccontrôle) semble contenir moins de lignines que le vermicompost (Vcontrôle). C30M et V30M présentent la même tendance, alors que C15M et V15M ainsi que CKG et VKG ont une tendance inverse.

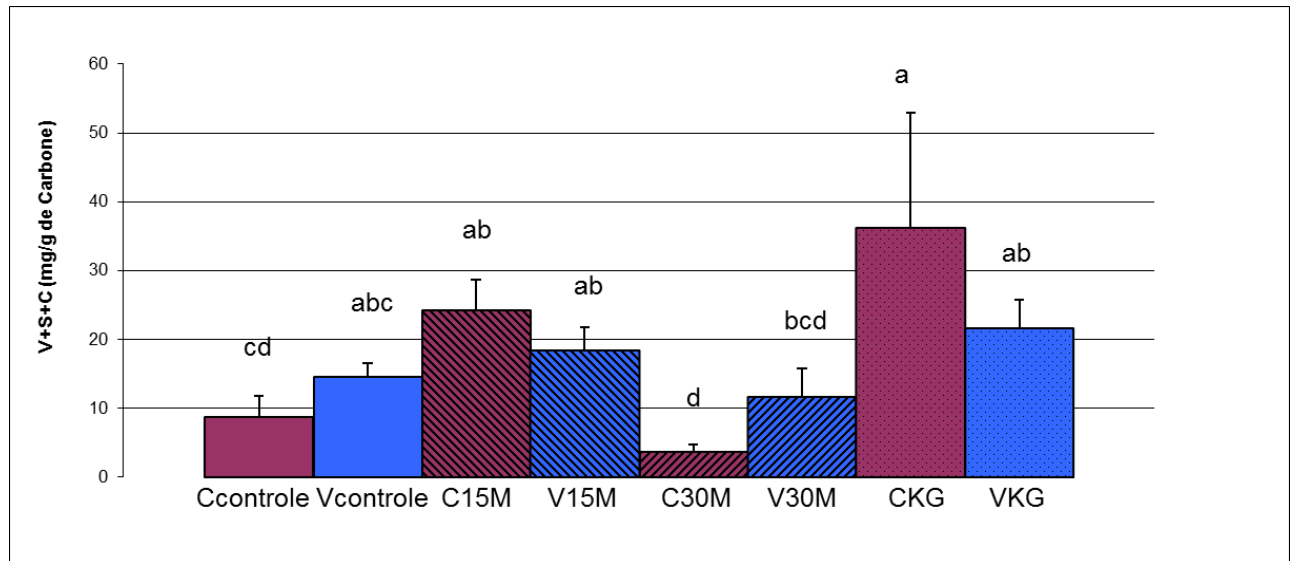


Figure 5 : Somme des lignines totale VSC selon les différents composts et vermicomposts. Test kruskal wallis avec $\alpha=0,05$ et une $p < 0.05$. Les barres d'erreurs correspondent aux erreurs standards (nombre de répliqués=3).

III.1.2. Analyses des ratios (Ac/Al)V

Nous avons calculé les rapports acides sur aldéhyde en fonction du groupe de lignine V pour chaque traitement (figure 6). Comme la p-value obtenue est plus grande que 0.05, nous ne pouvons pas parler de différences significatives, mais de tendances. Les valeurs de ce rapport sont comprises entre 0,6 et 11,18. Le compostage classique C_{contrôle}, à une valeur moyenne de 0,97. La présence de minéraux lors du compostage induit une diminution de ce ratio, excepté pour C30M de telle sorte que $CKG < C15M < C_{contrôle} > C30M$. Le vermicompostage (V_{contrôle}) à une valeur moyenne de 0,71, plus faible que C_{contrôle}. De plus, contrairement au compostage, l'ajout de minéraux lors du vermicompostage, a tendance à augmenter le rapport de sorte que $V_{contrôle} < VKG < V15M < V30M$. Enfin, nous remarquons que la présence de vers induit soit une diminution soit une augmentation de ce ratio comparé aux composts similaire. Ainsi, dans les contrôles et avec 30% de montmorillonite, la présence de vers tend à diminuer ce ratio. A l'opposé, dans les traitements avec 15% de montmorillonite et le mélange kaolinite-goethite,

leur présence tend à l'augmenter.

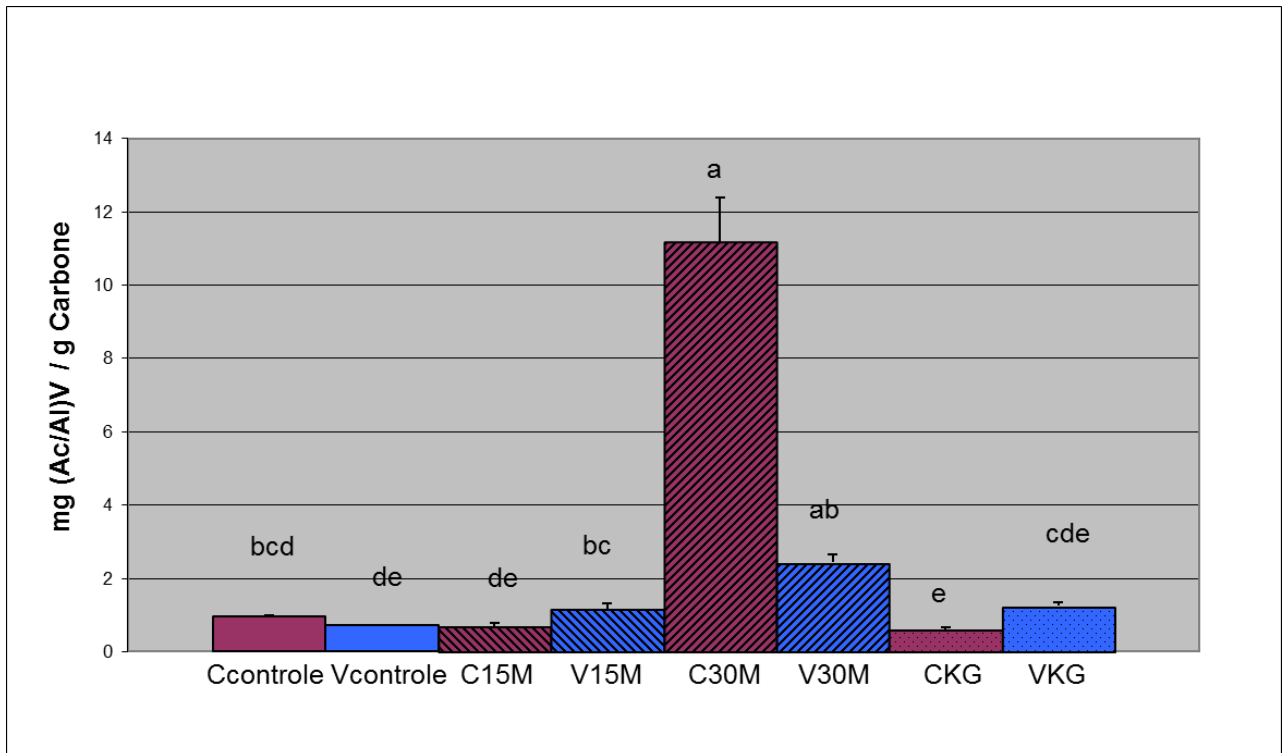


Figure 6 : Ratio (Ac/Al)V selon les différents composts et vermicomposts. Test kruskal wallis avec $\alpha=0,05$ et une $p > 0,05$. Les barres d'erreurs correspondent aux écartypes / racine (nombre de réplicats=3).

III.1.3. Analyses des ratios (Ac/Al) S

De la même manière, nous avons calculé les rapports acides sur aldéhydes en fonction de S pour chaque traitement (figure 7). Les valeurs de ce ratio sont comprises entre 0,63 et 2,12. Le compost classique ($C_{\text{contrôle}}$) présente la valeur la plus basse, suivie par le vermicompost avec une valeur de 0,64 ($V_{\text{contrôle}}$). L'ajout de minéraux au compost semble avoir tendance à augmenter le ratio, voire le doubler en présence de 30% de montmorillonite ($C < CKG < C15M < C30M$). Cette observation est similaire dans le cas des vermicomposts ($V < V15M < V30M \leq VKG$). Toutefois, l'augmentation de ce ratio est différente selon la présence de vers ou non, et le type de minéraux. En effet, le vermicompostage conduit globalement à un ratio plus élevé, que le compostage, particulièrement en présence de minéraux kaolinite et goethite (VKG et CKG).

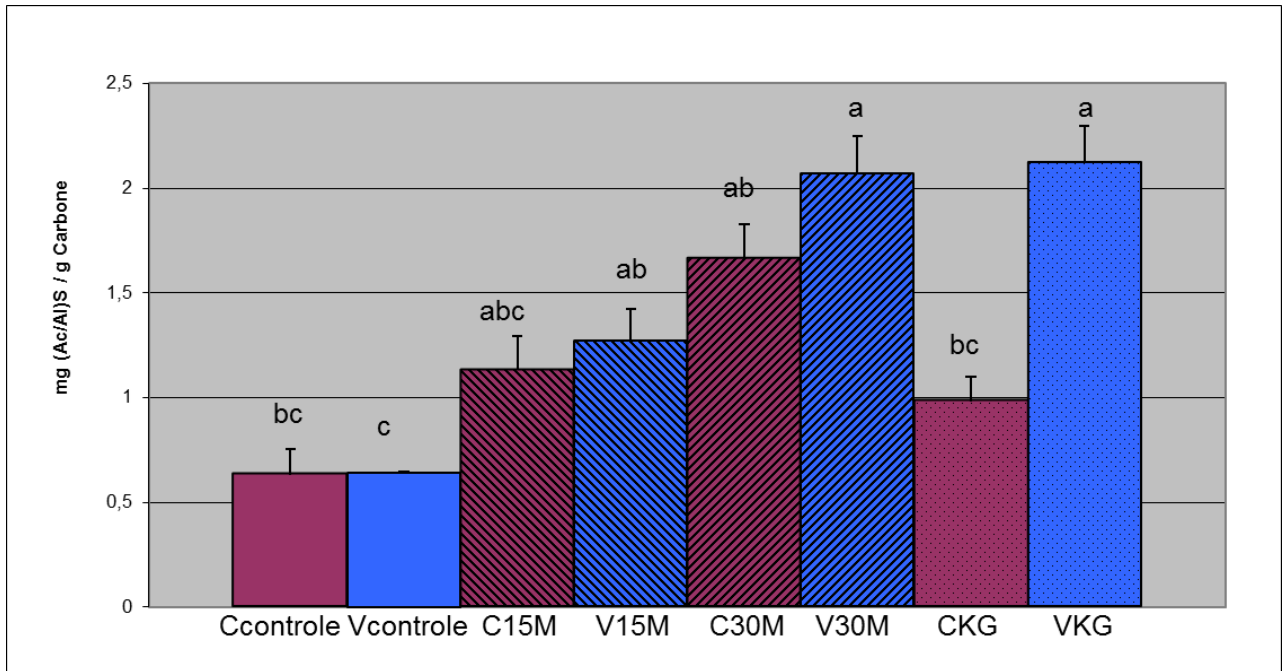


Figure 7 : Ratio (Ac/Al)S selon les différents composts et vermicomposts. Test de Kruskal Wallis avec $\alpha=0,05$ et une $p\text{-value}>0,05$. Les barres d'erreurs correspondent aux erreurs standard (nombre de réplicats=3).

III.1.4. Analyses des ratios C/V et S/V

Nous avons calculé les rapports C/V pour chaque traitement (figure 8). Les valeurs de ce rapport sont comprises entre 0,19 et 2,65. Bien qu'il n'y ait pas de différence significative entre les contrôles compost et vermicompost, la présence de vers induit une baisse de ce ratio (Ccontrôle a une valeur de 0,98 et Vcontrôle de 0,20.). De plus, cette baisse du ratio en présence de vers s'observe aussi dans les traitements avec minéraux. Enfin, l'ajout de minéraux lors du compostage et du vermicompostage tend à augmenter ce ratio comparé à nos deux contrôles. Cette augmentation est d'autant plus forte dans le cas du compostage avec montmorillonite, en particulier avec 30%, que ce soit pour le vermicompostage V30M avec une valeur de 1,31 et le compostage C30M avec une valeur de 2,65.

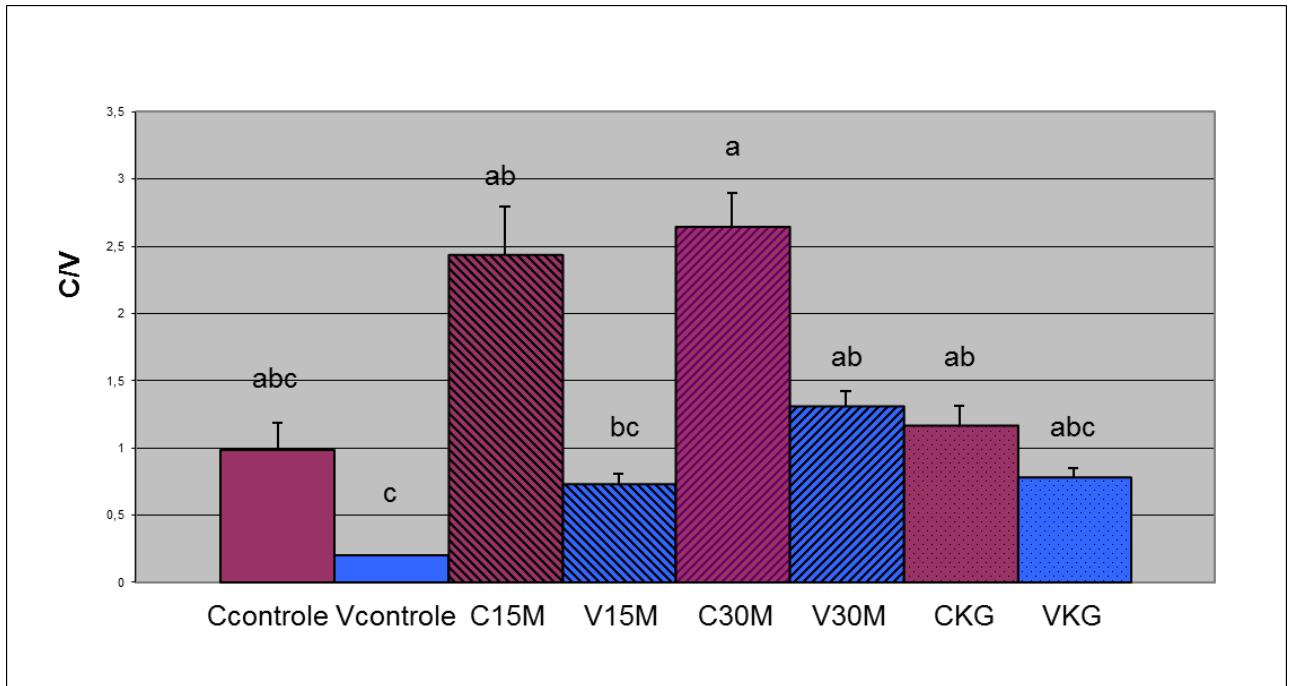


Figure 8 : Rapport C/V e selon les différents composts et vermicomposts. Test de Kruskal Wallis avec $\alpha=0,05$ et une $p\text{-value}>0,05$. Les barres d'erreurs correspondent aux erreurs standards (nombre de répliqués=3).

Nous avons calculé les rapports S/V pour chaque traitement (figure 9). Les valeurs de ce rapport sont comprises entre 1,34 et 6,42. Le compost classique, Ccontrôle à une valeur de 1.66. L'ajout de minéraux au compost montre une augmentation significative du rapport S/V. Bien qu'aucune différence significative ne soit mise en évidence selon le type de minéraux, la montmorillonite semble être le minéral conduisant à l'augmentation la plus importante de ce ratio (C15M et C30M). Le vermicompost V_{contrôle} a une valeur de 1.34, soit un ratio légèrement plus faible, mais non significatif que pour le compost. Nous pouvons voir, de la même manière qu'avec le compost, que les traitements avec ajout de minéraux en présence de vers sont significativement plus grands que V_{contrôle} de plus d'une unité. Il n'y a pas de différence significative entre les traitements avec et sans vers, mais nous pouvons voir une tendance des composts et co-composts à avoir un plus fort ratio qu'en présence de vers, excepté pour les traitements (CKG/VKG) qui ont la tendance inverse.

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

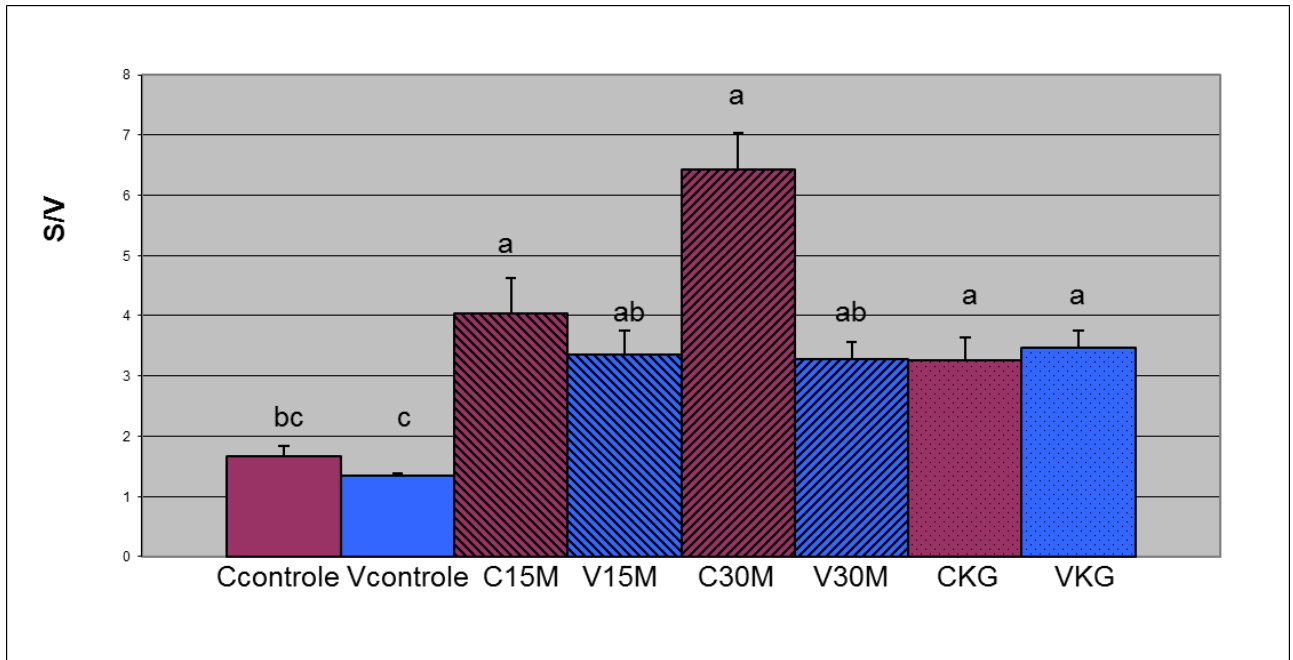


Figure 9 : Rapport S/V selon les différents composts et vermicomposts. Test de Kruskal Wallis avec $\alpha=0,05$ et une p -value $< 0,05$. Les barres d'erreurs correspondent aux erreurs standards (nombre de réplicats=3).

III.2. Analyse des résultats obtenus par ACP

Nous avons réalisé une Analyse en Composante Principale (ACP) pour comprendre la relation entre les variables (ratios $(AC/Al)V$, $(AC/Al)S$, S/V , C/V et la somme des lignines VSC) et les individus (réplicats des composts et vermicomposts).

Globalement, l'ACP contenant les valeurs normalisées par le carbone (figure 9 b) nous montre que la majorité des échantillons ne se distingue pas par les lignines, du fait de leur regroupement.

Comme la somme des dimensions 1 et 2 est environ égale à 80% (supérieure à 50%), elle explique 80% de la variabilité des échantillons par les variables utilisées. Nous pouvons voir sur la figure 9a que les variables C , S , V et CSV sont corrélés, c'est-à-dire par exemple que si V augmente, C augmentera aussi. Les variables $(AC/Al)V$, $(AC/Al)S$, S/V et C/V sont de la même manière corrélées entre elles, toutefois, la variable $(AC/Al)S$ est proche de 0.5, elle a donc moins de poids dans l'explication des différences entre individus. Nous pouvons voir que le rapport $(AC/Al)V$ est fort quand les composts ont 30% de montmorillonite (Fig. 9b).

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

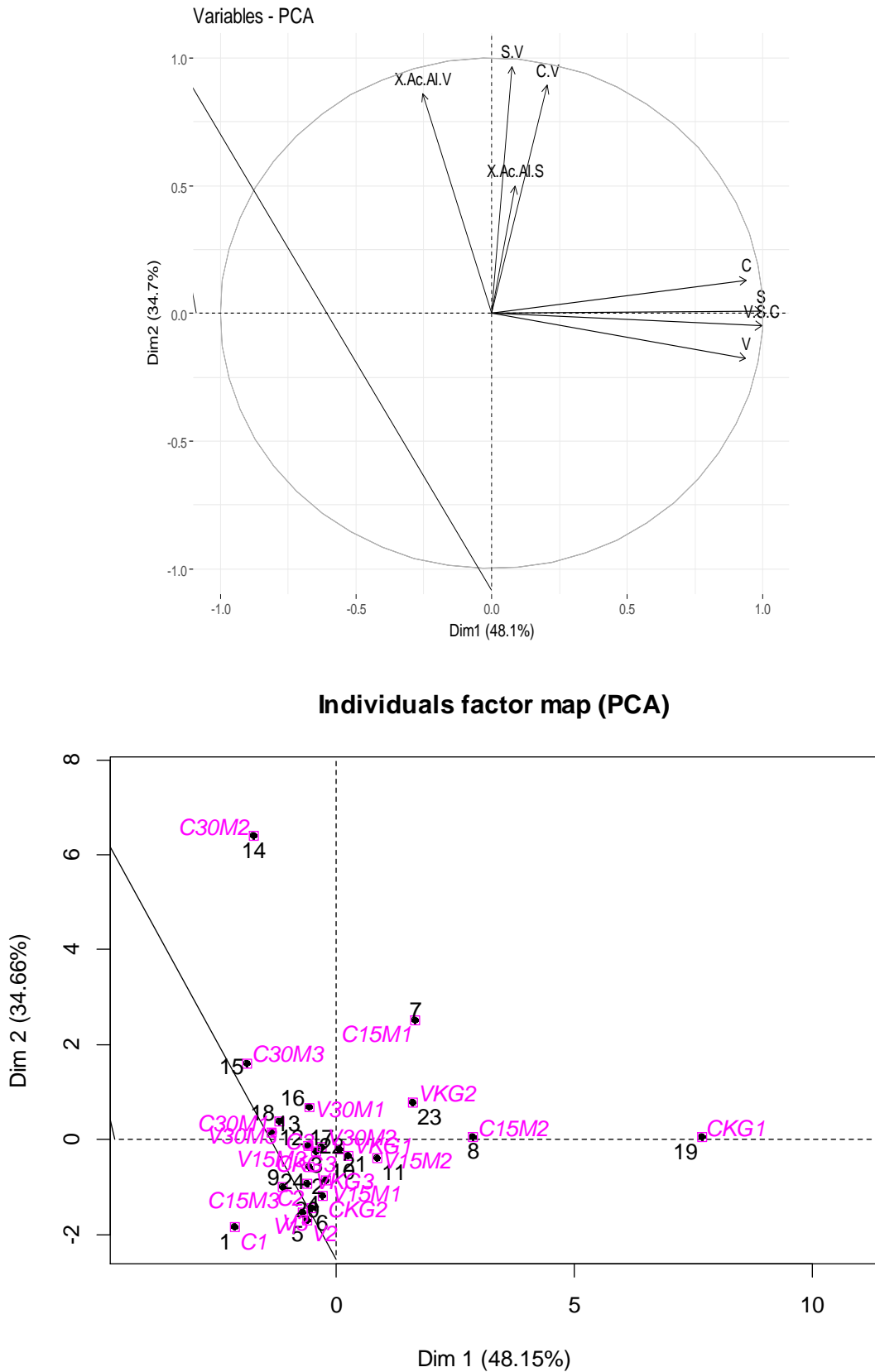


Figure 9 a et b : ACP des répliqués normalisés par le carbone total, en fonction des groupes de lignines et des ratios des échantillons.

IV. Discussion

L'oxydation de CuO produit plusieurs composés phénolytiques à un seul cycle : la vanillyl (V), la syringyl (S) et la cinnamyl (C) avec leurs substitutions (aldéhyde, cétone et acide) (figure2). Les produits dérivés de lignine obtenus après oxydation de CuO peuvent être utilisés comme biomarqueurs environnementaux et varient également selon le degré de dégradation de la molécule (Thevenot et al, 2010). . Nous utilisons ces composés pour trouver la quantité de lignine et son état de dégradation et donc celui de la matière organique du compost. La somme (V+S+C) et les différents rapports caractéristiques, tels que les rapports acides sur aldéhyde (Ad/Al) et C/V ou S/V ont été calculés. La somme V+S+C est généralement considérée comme une mesure quantitative de la lignine du sol, plus le rapport est élevé, plus la décomposition de ces molécules est faible. Alors que les rapports (Ad/Al), C/V et S/V sont des indicateurs de l'état de la dégradation de la lignine dans le compost. Ainsi, quand les rapports acides/aldhéydes sont élevés ou que les rapports C/V et S/V sont faibles, l'état de dégradation de la lignine dans nos composts et vermicompost est important. (Ngo et al.,2012).

IV.1. Effets des minéraux sur les lignines suite au compostage

La teneur totale en lignine mesurée dans le compost est de moins de 10 mg/g carbone. Cette valeur est trois fois plus faible que la valeur observée par Ngo et al (2011) suite au compostage de fumier de buffle. Ces différentes valeurs peuvent principalement s'expliquer par des différences de matières organiques initialement compostées, ainsi que par les conditions environnementales (contrôlées en laboratoire vs. non contrôlées en extérieur). En effet, comme mentionné dans l'introduction, le matériau parental est un facteur primordial dans la teneur en lignine dans le compost final. Ainsi, nous pouvons supposer que le compostage de matières organiques proches des déchets ménagers aboutit à de plus faible teneur en lignine qu'une matière organique prédigérée/transformée telle que le fumier.

L'ajout de minéraux semble impacter significativement la teneur totale des lignines VSC pour les composts (figure 5). En effet, la présence de montmorillonite (C15M) augmente la teneur en lignine, ainsi que le mélange kaolinite/goethite et inversement, 30% de montmorillonite diminue cette teneur, Ceci nous laisse supposer que non seulement le type de minéral mais aussi sa teneur influe grandement sur la dégradation de la lignine. Plusieurs hypothèses peuvent expliquer ces différences de teneurs par rapport à un compostage classique : (1) les lignines sont protégées par les minéraux par adsorption sur la surface minérale et on donc plus de difficulté à être décomposées par les micro-organismes qui y ont moins accès ; (2) les mécanismes d'adsorption ne sont pas les mêmes selon les minéraux (montmorillonite et kaolinite) et (3) la quantité de montmorillonite ajouté (15 ou 30%) influence l'état de décomposition des lignines. Ces hypothèses semblent en accord avec certains mécanismes se

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

déroulant dans le sol. En effet, dans le sol, les minéraux sont capables d'adsorbées les molécules carbonées, les rendant ainsi difficilement décomposable ou mineralisable par les micro-organismes (Sollins et al, 1996). De plus, les minéraux peuvent aussi limiter l'accès de ces molécules aux microorganismes. Enfin, de fortes teneurs en minéraux semblent favoriser le développement microbien, en particulier dans des technosols et donc augmenter indirectement la minéralisation des molécules carbonées (Wei *et al*, 2014).

Toutefois, ces hypothèses ne peuvent être confirmées par nos résultats sur les ratios (figure 6, 7, 8 et 9). En effet, la présente action des minéraux a tendance à augmenter les deux rapport acides/aldéhydes et donc l'état de dégradation des molécules de lignines. Cela nous laisse supposer que les minéraux, contrairement à ce que nous pourrions penser, ne permettent pas de ralentir la dégradation des molécules carbonées en particulier les lignines. Ce qui est contraire à l'hypothèse 1 du paragraphe ci dessus. Nous pouvons donc penser que la présence de minéraux tend à accélérer cette dégradation. Nous pouvons faire l'hypothèse que lors d'ajout d'argile et d'oxyde de fer, les espèces de micro-organismes capable de dégrader la lignine changent (augmentation de la population et du métabolismes des microorganismes), ou que leurs conditions environnementales soient améliorées (meilleure accessibilité, localisation, etc).

De plus, il faut prendre en compte le fait que la dégradation des molécules carbonées lors du compostage tend à augmenter le pool de carbone représenté par les lignines, en tant que molécules difficilement dégradables. Par conséquent, de faible teneur en lignines peuvent aussi informer sur une faible dégradation des autres composés carbonés, eux aussi potentiellement adsorbés par les minéraux. Pour confirmer nos hypothèses, une mesure de la teneur en lignine initiale aurait été nécessaire, ainsi que des autres composés carbonés tels les sucres.

IV.2. Effets des vers sur les lignines du vermicompost

Le vermicompost, pourtant issu de matières organique initiales identiques au compost, ne présente pas les mêmes teneurs en lignine. En effet, la présence de vers semble induire une augmentation de la teneur en lignine dans le produit final (Fig. 5). Toutefois, des tendances similaires sont obtenues par Ngo *et al* (2011) avec du compost et vermicompost de fumier. De plus, les ratios Ac/Al et les rapports C/V et S/V ne sont pas significativement différent pour le vermicompost de ceux du compost. Cela laisse supposer que les vers n'impactent pas ou peu la dégradation des molécules de lignines. Ces résultats semblent en contradiction avec la littérature (Stevanovic et al, 2009), suggérant que la digestion et ingestion de la matière organique des vers favorisent sa dégradation, et donc celle les lignines. De ce fait, la quantité de lignines dans le substrat final devrait être inférieure à celle du compost.

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

Deux principales hypothèses peuvent expliquer ces observations : (1) le compost dégrade mieux les lignines que le vermicompost ; (2) les lignines sont plus concentrées dans les vermicomposts car les autres molécules de carbone, comme les sucres, ont été dégradées de façon beaucoup plus efficaces et rapides que dans un compost. Ainsi, le carbone restant est principalement représenté par les lignines dans ces substrats transformés par les vers. Cette dernière hypothèse semble la plus probable au vue des différentes observations effectuées au sein du projet Lombricom⁷. En effet, les observations effectuées sur les composts et vermicomposts non broyés en fin de compostage (Figure 10 ; Barthod *et al*, en préparation) montrent des résidus de déchets ligneux encore très présents dans les composts et qui ont pratiquement disparut dans les vermicomposts. Enfin, ce résultat nous permet de supposer que les vermicomposts sont donc plus stables, d'un point de vue moléculaire, que les composts, du fait d'une plus grande proportion de molécules difficilement dégradables. Cette conclusion semble être en accord avec Vincelas-Akpa et Loquet (1997) qui montrent que le vermicompost est à un stade plus avancé dans l'humification et donc la dégradation des produits carbonés que le compost.

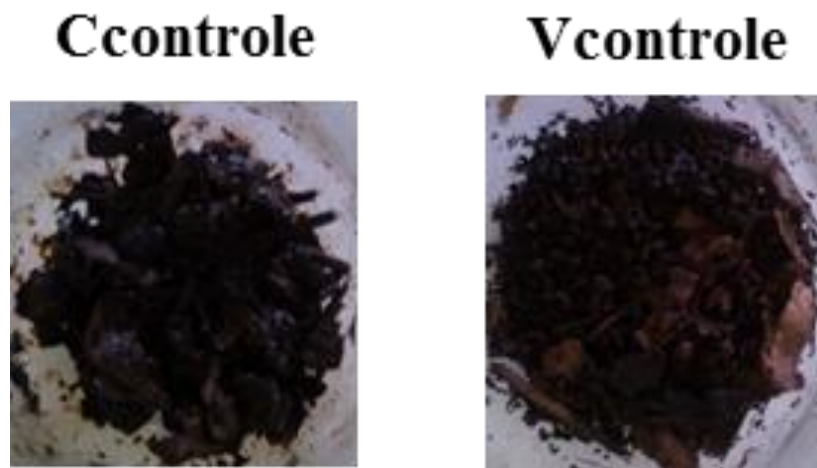


Figure 10 : Etat de la matière organique non broyée au bout de 196 jours de compostage et vermicompostage (Barthod *et al*, en préparation)

IV.3. Effet(s) des vers combinés à l'action des minéraux ?

Les teneurs en lignines dans les co-vermicomposts sont légèrement plus faibles que dans les co-composts, excepté lorsque 30% de montmorillonite est utilisé comme additif lors du compostage. De ce fait, nous pouvons faire l'hypothèse que la présence de vers limite l'augmentation de la teneur en lignine comparé aux composts analogues et donc que ces dernières sont plus facilement dégradables, même si potentiellement adsorbées par les minéraux comparés à un vermicompost classique. De plus, au vue des résultats, nous pouvons supposer que l'ajout de minéraux a plus d'influence sur la teneur en lignine

que l'ajout de vers. La présence de vers ne permettrait que de diminuer ou augmenter l'impact des minéraux.

Concernant l'état de dégradation des lignines, représenté par les différents ratios, ce dernier présente une tendance similaire à celui observé pour les co-composts. Ainsi, nous pouvons supposer que, même en présence de vers, les minéraux semblent augmenter l'état de dégradation des lignines, probablement du à des changements environnementaux favorables pour les micro-organismes (Wei et al, 2014), en particulier pour ceux décomposant les lignines. De plus, cette dégradation est augmentée dans les co-vermicomposts par rapports aux co-composts, mettant en évidence le rôle des vers dans la dégradation des molécules dites « récalcitrantes » (Fox *et al*, 2006).

Conclusion

Cette étude avait pour objectif de voir dans quelles mesures, le type de minéraux, leur quantité et la présence de vers influent sur l'état de la matière organique lors du processus de compostage.

Pour ce faire, nous avons eu à quantifier les lignines qui sont des biomarqueurs de l'état de dégradation de la matière organique, grâce à la méthode d'oxydation cuivrique en milieu alcalin avec détection des monomères de lignine par chromatographie gazeuse.

Nous avons pu montrer que l'action des minéraux n'était pas la même selon leur nature et leur quantité. La décomposition des lignines est plus importante en présence de 30% de montmorillonite, et au contraire, elle est plus faible avec 15% de montmorillonite. Nous avons fait l'hypothèse que la décomposition était plus ou moins efficace selon l'adsorption des monomères par les minéraux ; ou bien que les conditions environnementales et les espèces de microorganismes décomposeurs de lignines changeraient avec des ajouts de minéraux (argiles et oxyde de fer). Nous avons trouvé que l'action des vers nuançait cet effet des minéraux. Ainsi les minéraux semblaient moins efficaces pour limiter la dégradation des lignines en présence de vers. Enfin, pour expliquer les fortes teneurs en lignines en présence de vers, nous avons aussi fait l'hypothèse que la lignine était plus concentrée dans les vermicomposts car toutes les autres molécules étaient à un stade de dégradation plus avancé, voire minéralisées. Ainsi, le vermicompost serait plus stable d'un point de vue moléculaire.

V. Perspective/ Critiques

Des tendances sont observées dans nos résultats, cependant peu de différences significatives sont obtenues du fait d'une grande hétérogénéité dans les substrats. En effet, les répliqués analysés sont des répliqués d'expérimentation et non d'analyses. Afin de valider nos tendances, il serait envisageable de réaliser de nouveaux répliqués d'expérimentation. De plus, cela permettrait de palier à une potentielle hétérogénéité au sein du substrat final mais aussi de tester la répétabilité de la méthode sur nos échantillons.


En effet, la méthode a été développée pour des sols, or comme nous sommes dans le cas de composts et vermicomposts, nous ne connaissons pas le domaine d'application de la méthode pour nos échantillons. Par exemple, nous avons remarqué que la précipitation d'acide humique était beaucoup plus importante que dans le cas d'un sol, il est donc possible qu'ils n'aient pas tous précipité. La gamme étalon, les pesées et les volumes à prendre ont été créés pour des sols ou des végétaux, lors de ce stage, nous les avons donc adaptés pour nos substrats. Cette adaptation explique que certains répliqués ont donc dû faire l'objet de plusieurs dilution/concentration dans des séries différentes pour rentrer dans la gamme étalon, et ainsi être interprétables.

Toutefois, pour certaines molécules, nous avons dû faire des extrapolations car les aires ne rentraient pas dans la gamme étalon. Ces extrapolations ont pu être faites car les coefficients de corrélations des standards de molécules de la gamme étaient très bons ($>0,99$ ou $>0,999$)

Enfin pour réussir à valider nos différentes hypothèses, nous pourrions faire des mesures complémentaires, comme la mesure des sucres, ou des composés carbonés. Une autre idée pourrait être de quantifier les lignines à différentes étapes du compostage. Ces compléments permettraient de conclure sur l'état de dégradation de la matière organique carbonée de manière générale dans ces composts et vermicomposts.

Annexe : Extrait du protocole du stage

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

	Protocole de recherche / expérimental	Nom du fichier: lignines-lombricom'_20170501 Version : 1 Date de création : Date de mise à jour Page 1 sur 7
	<i>Extraction des lignines-projet Lombricom'</i>	

Notes importantes :

Informations sur ce document

Titre du Projet, code Projet :

Thèse J.BARTHOD : « ~~Vermicompostage~~ en présence de minéraux, un procédé innovant pour limiter les émissions de CO2 : compréhension des processus aux petites échelles ». Projet LOMBRICOM (CNRS, EC2CO)

⇒ Caractérisation des lignines (qualités et quantités) dans les composts et vermicomposts élaborés au sein du projet Lombricom'.

Suivi des modifications de ce document :

=> Toute modification devra être notée afin d'améliorer le protocole et que son contenu aide à la rédaction des rapports, des articles...

Date	Nom du fichier	Modifications apportées / Justification	Intervenant
01/05/2017	Sucre Lignines-Lombricom'	Création	JB
22/06/2017	Lignines-Lombricom'.V2.2	Modification de fin de stage	Margot Chapuis

Où trouver ce document : *Lieu physique (site, bâtiments, pièce) ou Chemin d'accès à l'espace informatique où se trouve la dernière version de ce protocole.*


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4	Liste des opérations et planification.....	3
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	Rédacteur(s)	Vérificateur(s) éventuel(s)	Approbateur(s) éventuel(s)
Nom :			
Fonction :			Animateur Qualité
Date du visa :			

Trame1_Protocole_v1.3 du 13/01/2016

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.

	Protocole de recherche / expérimental	Nom du fichier: lignines-lombricom'_20170501
	<i>Extraction des lignines-projet Lombricom'</i>	Version : 1 Date de création : Date de mise à jour Page 2 sur 7

Contenu de ce document :

1 Contexte - Objectif général

Objectif scientifique :

L'objectif scientifique général du projet **Lombricom'** est de **déterminer et caractériser l'influence des vers et des minéraux sur la dégradation de matières organiques fraîches (salades, pommes, cartons, résidus maïs, marc de café), en particulier sur la stabilisation du carbone.**

Différents composts et **vermicomposts (*Eisenia andrei* et *foetida*)** ont été réalisés en amont avec présence de minéraux. Ces minéraux présentent différentes propriétés physiques et chimiques : la montmorillonite (type 2:1), la kaolinite (type 1:1) et la goéthite (oxyde de fer). Ces minéraux ont été ajoutés dès le début du processus de dégradation de la matière organique, en différentes concentrations, mélangés ou non. La matière organique « stable » issue de ces procédés est dénommée compost (control sans vers), vermicompost (control avec vers) et, **co-composts et co-vermicomposts** (avec minéraux). Des analyses précédentes sur ces produits ont montré que la présence de minéraux influence la minéralisation du carbone au cours de la dégradation, ainsi que la teneur en alkyl, composés aromatiques, **etc** (résultats de RMN). De plus, la présence de vers semble accélérer le processus de dégradation et la combinaison vers-minéraux n'aboutit pas à des résultats similaires à ceux obtenus lors du compostage avec minéraux.

L'objectif principal de cette partie du projet (stage) est donc **d'évaluer l'état de dégradation de la matière organique présente dans les composts, vermicomposts, co-composts et co-vermicomposts.** L'état de dégradation de la matière organique peut être déterminé **par mesures quantitative et qualitative des composés carbonés tels que les lignines** restantes dans le produit final.

Champs thématique :

Compost/ vermicompost ; minéraux ; lignines ; état de dégradation

L'objectif principal de cette partie du projet (stage) est donc **d'évaluer l'état de dégradation de la matière organique présente dans les composts, vermicomposts, co-composts et co-vermicomposts.** L'état de dégradation de la matière organique peut être déterminé **par mesures quantitative et qualitative des composés carbonés tels que les lignines** restantes dans le produit final.

Champs thématique :

Compost/ vermicompost ; minéraux ; lignines ; état de dégradation

Hypothèse & Principe du protocole :

Plusieurs hypothèses sont formulées pour ce projet :

- Le processus de vermicompostage classique permet un état de dégradation plus avancé de la matière organique, en particulier les lignines, que le compostage.
- La présence de minéraux lors du compostage protège la matière organique de la minéralisation et de la dégradation. De ce fait, l'état de dégradation des matières organiques est moins avancé. Toutefois, cet état est dépendant de la quantité de minéral ajouté et de la minéralogie (2:1 ; 1:1 ou oxyde de fer).
- La présence de vers, combinée à celle des minéraux, modifie l'état de dégradation de la matière organique. Les vers accélèrent la dégradation mais cette dernière est limitée par la protection de la matière organique par les minéraux.

Les **problématiques soulevées sont donc les suivantes :**

- Quel est le degré de dégradation des composts et vermicomposts, et comment les vers influencent-ils sur la dégradation ?
- Quelle est l'origine principale des molécules restantes dans les composts et vermicomposts ?
- La présence de minéraux permet-elle, tout comme dans le sol, de retarder la dégradation des molécules, en particulier des lignines ?
- Les propriétés intrinsèques des minéraux influencent-elles sur la dégradation de la matière organique ?
- La combinaison vers/minéraux est-elle efficace pour stabiliser le carbone ? sous quelle forme ?

Le principe du protocole est le suivant :

Appendix 3: Extract of the internship report of Margot Chapuis (UPMC, M1 SDUEE) on lignin contents in co-compost and co-vermicompost.



Hydrosciences

Master 1 SDUEE

Mention Environnement Continentaux et



Rapport de stage de M1

EFFET DES VERS ET DES MINÉRAUX SUR LA DEGRADATION DES SUCRES DANS LES COMPOSTS

2017

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Laboratoire d'accueil : IEES

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MATERIELS ET METHODES

1. Elaboration des composts et vermicomposts

Les composts étudiés dans ce stage ont été réalisés préalablement à partir de matière organique fraîche (laitues et des pommes issues de l'agriculture biologique, marc café, de morceaux de carton (2x3cm) et de maïs résiduel issus d'un champ de Grignon). Les différents minéraux sont ajoutés dès le début du compostage et mélangés à la matière organique fraîche de manière à obtenir une pile de déchets homogène.

Le processus de compostage s'est déroulé en conditions contrôlées à 20°C à l'obscurité pendant 196 jours. Au terme de ces processus, les vers des vermicomposts ont été enlevés manuellement ainsi que leurs cocons puis la matière organique résultante a été séchée à l'air libre puis finement broyée avant d'être stockée.

2. Espèces de vers utilisées

Les espèces des vers qui ont été utilisées lors du processus de vermicompostage sont *Eisenia andrei* et *Eisenia foetida*, en raison de leur fort taux de consommation, de digestion et d'assimilation de la matière organique, en plus de leur tolérance aux facteurs environnementaux, leur court cycle de vie et leur forte reproduction et leur résistance aux manipulations (Dominguez *et al*, 2013).

3. Caractéristiques des échantillons utilisés

Dans le cadre du projet Lombricom', de nombreux minéraux ont été testés, ainsi que des teneurs différentes (20 traitements, 4 réplicats par traitement). Pour ce stage, seuls certains composts et leur équivalent en vermicompost ont été analysés. De ce fait, j'ai travaillé sur 8 traitements dont les caractéristiques sont résumées dans le **Tableau 1**. Brièvement, ces composts et vermicomposts ont été obtenus en présence de (1) montmorillonite, argile (type 2:1); (2) kaolinite, une argile (type 1:1) et (3) de goethite (un oxyde de fer), seuls ou combinés avec différents caractéristiques (Barthod *et al*, 2017*en préparation*). La proportion de minéral est donnée par rapport au poids sec de matière organique initialement compostée.

Tableau 1 : Caractéristiques principales des échantillons analysés au cours de ce stage

Appendix 4: Extract of the internship report of Oumayma Taoussi (UPMC, M1 SDUEE) on sugar contents in co-compost and co-vermicompost.

Type de l'échantillon	Nom donné	Composition
Compost	C	Compost classique
Vermicompost	V	Compost+ vers utilisés
Co-compost	C15M	Compost avec 15% de montmorillonite
Co-vermicompost	V15M	Vermicompost avec 15% de montmorillonite
Co-compost	C30M	Compost avec 30% de montmorillonite
Co-vermicompost	V30M	Vermicompost avec 30% de montmorillonite
Co-compost avec deux types de minéraux	CKG	Compost avec 15% de kaolinite et 15% de goethite
Co-vermicompost avec deux types de minéraux	VKG	Vermicompost avec 15% de kaolinite et 15% de goethite

4. Analyses des sucres

Pour déterminer la concentration en sucres dans les différents échantillons, les sucres non-cellulosiques sont hydrolysés par acide trifluoroacétique (TFA), et ensuite analysés par chromatographie en phase gazeuse après purification et transformation en acétates d'alditol selon la méthode décrite par Rumpel et Dignac, 2006. Trois répliques ont été analysés pour chaque traitement.

Brièvement, environ 70 à 500 mg des échantillons sont hydrolysés en utilisant 10 ml d'acide trifluoroacétique 4 M (TFA) à 105°C pendant 4h. Il existe d'autres méthodes pour hydrolyser les sucres non celluloses, mais l'utilisation de TFA permet d'avoir un rendement supérieur pour les unités de monosaccharides, et le TFA peut être évaporé directement (Amelung *et al*, 1996).

Après refroidissement des échantillons, 0,5 ml de methylglucose (3 g/L) est ajouté, puis la solution est filtrée (filtre à fibres de verre Whatman GF/C) et évaporée dans un speedvac pendant 12h.

Les monosaccharides sont alors purifiés et dérivatisés, dans des tubes à essai à vis, par l'ajout de 0,5 mL de myoinositol. Le myoinositol est le standard interne permettant par la suite de corriger la réponse du détecteur lors de la chromatographie en phase gazeuse. Le pH de la solution est ré-équilibré par l'ajout de la solution Na₂-EDTA à 0.2M. Après ajout de 1 mL de la solution NaBH₄-DMSO, la réduction des aldoses aux alditols est effectuée dans un bain marie (40°C) pendant 1h30.

Appendix 4: Extract of the internship report of Oumayma Taoussi (UPMC, M1 SDUEE) on sugar contents in co-compost and co-vermicompost.

Dès la sortie du bain marie la réaction est stoppée en mettant les tubes dans l'eau glacée. La transformation en acides d'alditols est effectuée par l'ajout de l'acide acétique pure, 1-méthyl imidazole (catalyseur) et l'acide anhydrique.

Ces sucres dérivés sont enfin extraits par séparation liquide-liquide, en utilisant du dichlorométhane pour récupérer une aliquote dans la phase apolaire.

Par la suite les échantillons sont analysés à l'aide d'un chromatographe (HP GC 6890N AGILENT (USA)) équipé d'un détecteur d'ionisation de flamme, d'une colonne type SGE BPX-70 (60m x 320 µm OD, 25µm ID), et un mode Split. 1µl d'échantillon est utilisé lors de l'injection. La programmation de la température est la suivante : 200°C à 250°C à 8°C/min, 250°C maintenu pour 15min. Afin d'étalonner les échantillons, ces derniers sont passés au GC avec une gamme étalon préparée en même temps que les échantillons (**Tableau 2**). La solution mère contient environ 72 mg de rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose, glucose.

Tableau 2 : Etendue et préparation de la gamme étalon :

Solution (µl)	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Sugar mix	0	100	250	500	750
H ₂ O	750	650	500	250	0
Myoinositol	500	500	500	500	500
Na ₂ -EDTA	900	900	900	900	900

5. Expression des résultats

Les résultats d'analyses (standards et échantillons) sont exprimés en aire pour chaque sucre puis convertie en mg de sucre grâce à la gamme d'étalonnage. La concentration de chaque sucre est exprimée en mg de sucre par g⁻¹ de carbone, après normalisation des aires par rapport au myoinositol. Les détails de calcul sont les suivants :

$$\text{Sucre (mg/g carbone)} = a(\text{sucre considéré}) * \frac{\text{aire du sucre considéré}}{\text{aire du myoinositol}} + b(\text{sucre considéré})$$

Les valeurs de a et b sont obtenues à partir des gammes d'étalonnage, propre à chaque sucre extrait. Ces résultats sont par la suite réajustés par rapport aux rendements de l'extraction, basée

Appendix 4: Extract of the internship report of Oumayma Taoussi (UPMC, M1 SDUEE) on sugar contents in co-compost and co-vermicompost.

sur le rendement du méthylglucose.

6. Analyses statistiques

Les données ont été analysées statistiquement par le logiciel R 3.12 sous Windows en utilisant le test de Kruskal-Wallis (package *agricolae*) pour déterminer les différences significatives entre les traitements.

D'autres tests de corrélation et d'Analyse en Composantes Principales(ACP), ont été utilisés pour vérifier la corrélation entre certaines variables, ainsi pour différencier les traitements.

RESULTATS

L'état de dégradation de la matière organique peut être déterminé par mesure quantitative des composés carbonés tels que les sucres restants dans les produits finaux. L'ensemble des résultats est normalisé par rapport au carbone total présent dans les différents traitements. Cette normalisation est nécessaire du fait d'une grande variabilité en teneur en carbone due à la présence de minéraux (effet de dilution).

1. Teneurs en sucre totaux et proportions de sucres analysés

La **figure 4** représente les résultats d'analyse des sucres totaux en mg/ g de carbone. Bien que ces résultats ne soient pas significativement différents entre eux ($p\text{-value} > 0.05$) du fait d'une grande hétérogénéité entre les réplicats, nous pouvons constater certaines tendances. En effet, la teneur en sucre dans les composts et vermicompost classiques (C et V) est la plus faible par rapport aux autres traitements. Cette teneur est par ailleurs proche entre C et V, avec 242,2 mg/g de carbone à 222,6 mg/g de carbone respectivement. Lorsque des minéraux sont présents lors du compostage, la teneur en sucre total a tendance à augmenter.

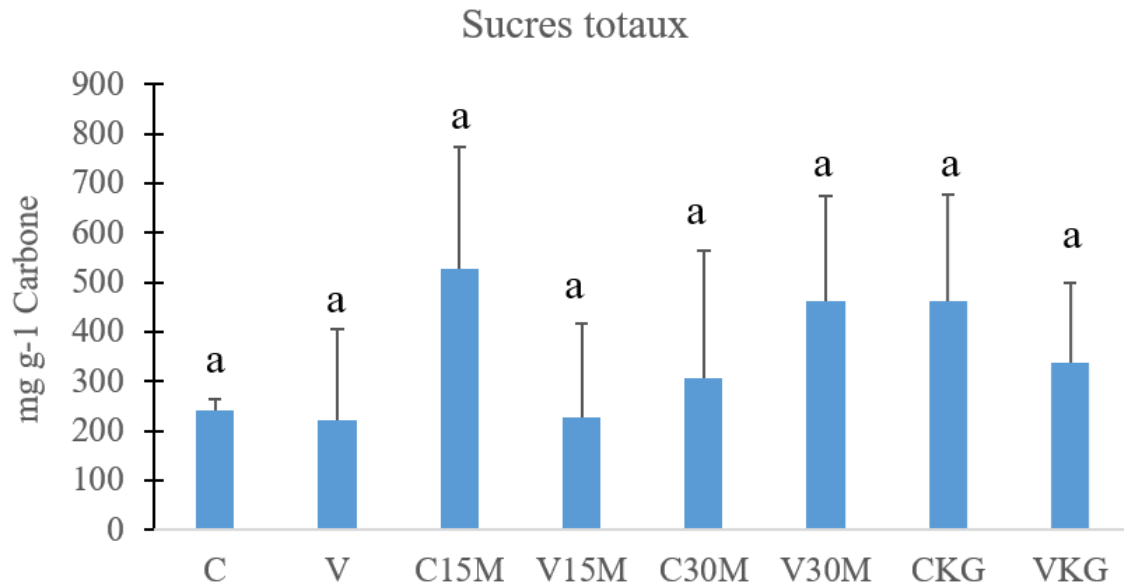


Figure 4 : Concentration en sucres totaux en mg/g de carbone dans les différents traitements. (Kruskal-Wallis, p-value >0.05)

Les composts contenant des minéraux présentent une teneur en sucres de telle sorte que C15M>CKG>C30M> C. Ainsi les composts réalisés avec 15% de montmorillonite (C15M) ont une teneur en sucre total de 528.4 mg/g Carbone, soit deux fois plus que dans un compost classique. En revanche, les composts produits avec 30% de montmorillonite présentent peu de différence comparés aux composts normaux. Enfin, la présence combinée de vers et de minéraux aboutit à des teneurs en sucres totaux suivant cet ordre V30M>VKG>V15M ≥V, avec V30M ayant une teneur de 463,7 mg/g de Carbone. De plus, comparés aux différents composts, les vermicomposts ont une teneur en sucres totaux plus faible, excepté dans le cas des composts avec 30% de montmorillonite.

L'analyse fine de ces teneurs en sucres (Figure 5) nous indique que la quantité de chaque différent monomère n'est pas le même entre chaque traitement. Globalement, les sucres les plus importants dans le compost et vermicompost (C et V) sont le galactose et le mannose, avec environ 24% et 23% respectivement, suivi du glucose (18%), de l'arabinose et du xylose (10-15%), puis du rhamnose, fucose et ribose. Dans le cas du vermicompost, le rhamnose, fucose et ribose sont présents en plus faible proportion que dans le compost, toutefois ces différences ne sont pas significatives.

En présence de minéraux, ces proportions sont modifiées. En effet, en présence de 15% de montmorillonite, C15M et V15M, la proportion de mannose, galactose, ribose et fucose

Appendix 4: Extract of the internship report of Oumayma Taoussi (UPMC, M1 SDUEE) on sugar contents in co-compost and co-vermicompost.

diminue, et de manière significative dans le cas du mannose. Inversement, la proportion de rhamnose, arabinose, xylose et glucose augmente. Ces changements semblent plus importants entre C et C15M, qu'entre V et V15M. En revanche, l'ajout de 30% de montmorillonite entraîne une augmentation de la proportion en mannose et galactose comparé à C et V. Les proportions des autres sucres étant inchangées (rhamnose, arabinose) ou diminuées (fucose, ribose, xylose, glucose). De même qu'avec 15% de montmorillonite, les différences sont relativement plus importantes entre C et C30M qu'entre V et V30M. Enfin, l'observation des résultats de CKG et VKG montre que la combinaison de kaolinite et de goethite modifie significativement la proportion de chaque sucre. Dans ces traitements, le sucre dominant est le glucose (et non plus le mannose ou le galactose) et aucun fucose et ribose n'a été détecté.

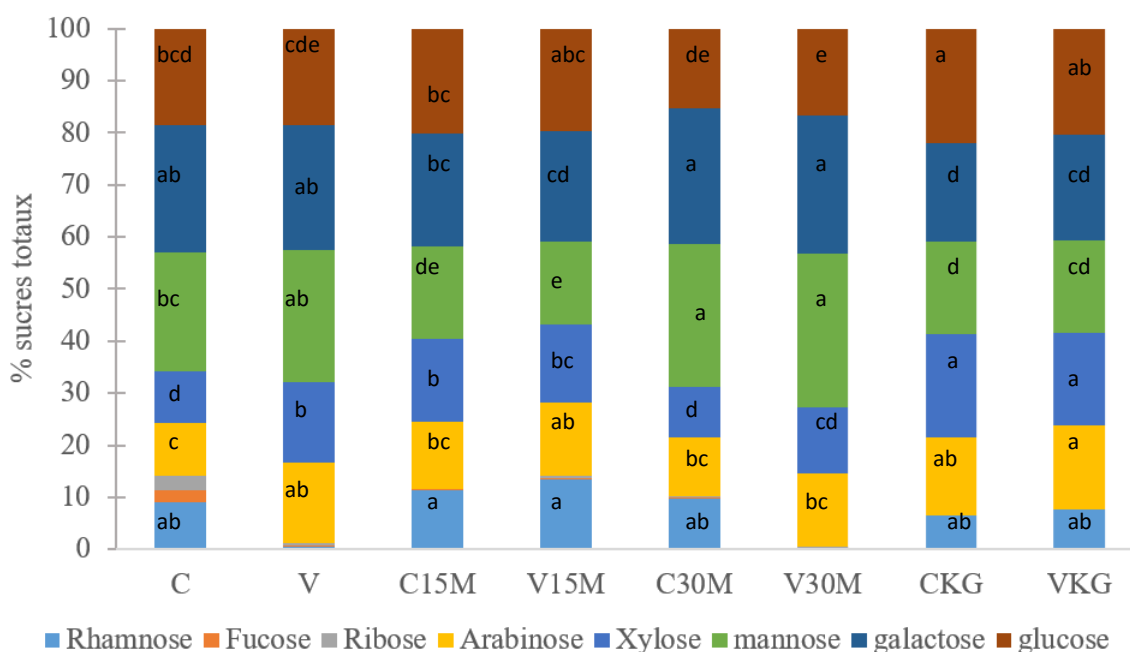


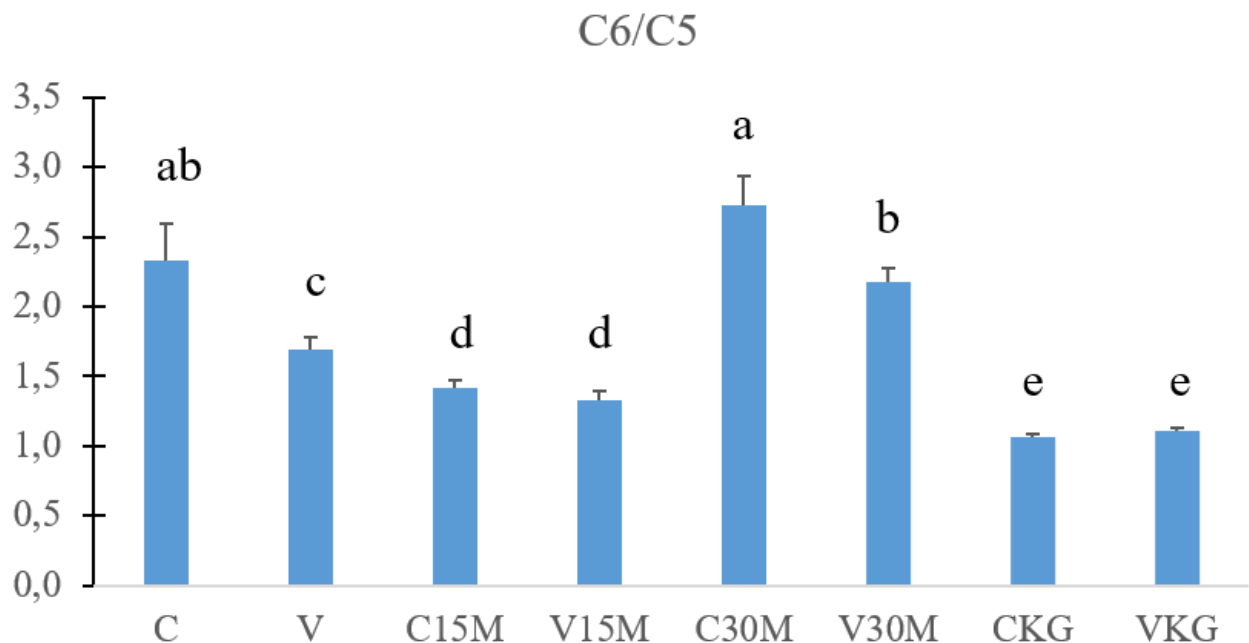
Figure5 : Pourcentage des différents sucres extraits (% des sucres totaux) dans les traitements. Les différentes lettres indiquent des différences significatives entre traitement pour un même sucre.

2. Ratio C6/C5 et ratio desoxy/C5

Les ratio C6/C5 (soit (mannose + galactose) / (xylose + arabinose)) des différents traitements sont indiqués dans la figure 6. Dans le cas des composts ce ratio, augmente dans l'ordre suivant : CKG < C15M < C ≤ C30M et est compris entre 1 et 3. La présence de minéraux dans les composts diminue significativement ce ratio, excepté avec 30% de montmorillonite,

Appendix 4: Extract of the internship report of Oumayma Taoussi (UPMC, M1 SDUEE) on sugar contents in co-compost and co-vermicompost.

comparé au contrôle. Bien que les ratios dans les vermicomposts soient significativement plus faibles que pour les composts, des observations similaires aux composts peuvent être établies



concernant le rapport C6/C5 (VKG < V15M < V < V30M).

Figure 6 : Rapport C6 / C5 (mannose + galactose) / (xylose + arabinose). Les différences significatives sont mentionnées par des lettres différentes. Les barres d'erreurs représentent l'erreur standard. (Kruskal-Wallis, p-value < 0.05)

Le rapport C6 / C5 a été défini pour discriminer les glucides principalement dérivés des végétaux (faible rapport, inférieur à 0,5), ou bien d'origine microbienne (rapport élevé, supérieur à 2) (Murayama, 1984, Oades, 1984, Guggenberger et Zech, 1994). Selon les résultats que nous avons obtenus (**figure 6**), seulement deux traitements présentent des rapports dépassant la valeur 2, le C30M (C6/C5 = 2,725) et le V30M (C6/C5 = 2,175), donc nous pouvons supposer que les sucres sont plutôt d'origine microbienne dans ces traitements, et par ailleurs nous pouvons conclure la même chose pour le compost car il tend vers 2 (C6/C5 = 1,960). Par contre, nous ne pouvons pas conclure sur l'origine des sucres pour le reste des traitements, leur ratio étant compris entre 0.5 et 2.

D'après la **figure 7**, nous observons des différences entre les rapports desoxy /C5 (soit (rhamnose + fucose) / xylose + arabinose) entre traitements. Toutefois, ces dernières ne sont

Appendix 4: Extract of the internship report of Oumayma Taoussi (UPMC, M1 SDUEE) on sugar contents in co-compost and co-vermicompost.

pas significatives ($p\text{-value} > 0.05$). L'ensemble des ratios est compris entre 0.1 et 0.6. Comme nous l'observons, il existe une différence entre le compost et le vermicompost où ce ratio diminue, passant de 0,5 à 0,1. Dans le cas des composts, l'ajout de minéral tend à diminuer ce ratio, et ce de manière plus importante avec 15% de kaolinite et 15% de goethite ($CKG < C15M < C30M < C$). Inversement, dans le cas des vermicomposts, ce ratio tend à augmenter de sorte que $V < VKG < V30M < V15M$. Ainsi, la présence de vers diminue ce ratio, excepté pour le traitement avec 15% de montmorillonite.

Tout comme le rapport C6/C5, le ratio désoxy / C5 a été défini pour distinguer les glucides principalement dérivés des végétaux (faible rapport, inférieur à 0,5), ou bien dérivés des micro-organismes (rapport élevé, supérieur à 2) (Murayama, 1984, Oades, 1984, Guggenberger et Zech, 1994). Selon la **figure 7** les traitements qui ont un ratio supérieur à 0,5 sont le compost, le V15M et le C30M, le reste des échantillons est inférieur à 0,5.

Nous pouvons alors supposer que le compost et le C30M et le V15M contiennent des sucres d'origine bactérienne, et inversement, les autres traitements, ainsi que les contrôles, présentent une majorité de sucres d'origine végétale.

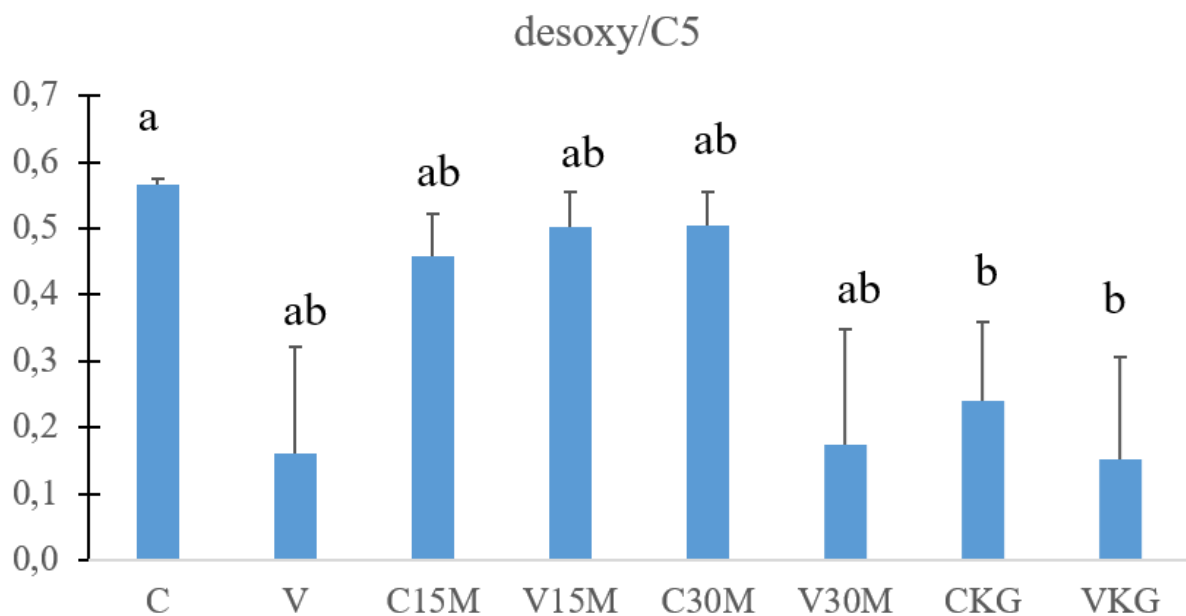


Figure 7 : Rapport désoxy / C5 (Rhamnose + fucose) / xylose + arabinose). Les barres d'erreurs représentent l'erreur standard. (Kruskal-Wallis, $p\text{-value} > 0.05$)

DISCUSSION

La méthode d'analyse utilisée dans ce rapport a été développée pour étudier les polysaccharides dans les sols ou les végétaux. L'application de cette méthode avec un substrat composté en présence ou absence des vers, contenant des proportions relativement importante en minéraux comparé à un sol, nous oblige à rester critique vis-à-vis des résultats. En effet, il est possible que l'extraction des sucres ne soit pas totale ou bien encore, que la matrice minérale a eu une influence sur la quantité de sucre extrait. Tandis que nous l'avons utilisé pour des composts et des vermicomposts, ce qui explique probablement les différences non significatives entre les résultats obtenue en utilisant les tests statistiques. Alors il faut rester critique par rapport à l'interprétation des résultats, car la méthode utilisée est seulement adaptée à nos échantillons. Ce qui explique aussi que certains échantillons ne rentraient pas dans la gamme étalon, même en changeant les pesées après chaque série selon chaque traitement. Nous avons été obligés à faire appel aux extrapolations.

La méthode d'analyse des sucres employés au cours de ce stage permet de caractériser l'origine de ces sucres, en plus de leur concentration. En effet, le xylose est un indicateur des polysaccharides structuraux d'origine végétale alors que le mannose est considéré comme le marqueur des excréments polysaccharidiques microbiennes (M. Karroum *et al*, 2004 et S. Murayama, 1984). De plus, comme mentionné dans l'introduction de ce stage, les sucres sont généralement des molécules facilement dégradées lors du processus de compostage, ce qui laisse supposer qu'un état de dégradation avancé du compost diminue leur quantité (Lynch, 1992 ; Castaldi *et al*, 2005).

1. Effet(s) des vers sur le compost

Contrairement à ce qui est observé dans les sols (Zhang and Schrader, 1993), la présence de vers lors du compostage sans minéraux n'augmente pas la teneur en sucre total (Fig.4). Ces résultats soulignent l'effet de l'espèce de vers sur la concentration en sucre (dans notre cas, *Eisenia andrei* et *foetida* et dans les sols, *Lumbricus terrestris*). En revanche les vers diminuent les ratios C6/ C5 et desoxy/C5 (**Fig. 6 et 7**). Ainsi, nous pouvons supposer que les sucres en présence de vers sont principalement d'origine végétale, et donc dérivés des matériaux initiaux, alors que dans les traitements sans vers, les sucres ont une origine plus microbienne. Cette

observation nous permet de faire plusieurs hypothèses : (1) les vers ne permettent pas une dégradation plus rapide des sucres ; (2) le compost, ayant des sucres d'origines microbiennes, a une activité microbienne plus importante que le vermicompost. Toutefois, ces deux hypothèses semblent contradictoires avec la littérature soulignant l'efficacité des vers pour dégrader la matière organique (Kaviraj and Sharma, 2003 ; Khwairakpam and Bhargava, 2009 ; Suthar and Singh, 2008) et augmenter l'activité microbienne (Aira *et al*, 2007). Ainsi, nous pouvons supposer que la faible présence de sucre microbien est due à une consommation et ingestion des micro-organismes par les vers. De récentes études (Dominguez *et al*, 2010 ; Gomez-Brandon *et al*, 2013) montrant que les vers ingèrent préférentiellement les micro-organismes avant la matière organique, semblent confirmer cette hypothèse.

2. Effet(s) des minéraux sur le compost

D'une façon générale, l'addition de minéraux lors du compostage semble augmenter la concentration en sucres totaux, par contre cette augmentation n'est maximale qu'avec un pourcentage faible en montmorillonite. L'augmentation de la teneur en sucres suggère que les composts produits en présence de minéraux sont plus riches en molécules facilement dégradables que le compost classique. De plus, le ratio C6/C5 tend à diminuer dans ces composts soulignant la présence de sucres d'origine végétales et donc du matériau initial. L'ensemble de ces résultats nous suggèrent que les minéraux influencent sur la dégradation des sucres, notamment en les protégeant d'une potentielle dégradation. De plus, ces minéraux ne semblent pas avoir la même influence sur le niveau de protection, cette dernière apparaissant optimale avec 15% de montmorillonite, suivi de 30% de montmorillonite. Ainsi, les différences de structures des minéraux et leur capacité d'adsorption des molécules carbonées jouent un rôle primordial dans la stabilisation/protection des sucres lors du compostage. Des mécanismes similaires se produisent dans les sols, due à la présence de minéraux (Amato et Ladd, 1992 ; Gonzalez et Laird, 2003 ; Nelson et al, 1997). De plus, selon Nelson *et al*, 1997, l'accessibilité de la matière organique et donc des sucres, aux microbes du sol peut être limitée par la formation d'agrégats. Dans le cas de nos composts avec minéraux, l'étude des agrégats n'ayant pas été réalisée, nous ne pouvons pas confirmer cette hypothèse.

3. Effet(s) de la combinaison des vers et des minéraux

De même, le co-vermicompostage semble augmenter la concentration en sucres totaux surtout avec un pourcentage de 30% de montmorillonite (V30M), par contre avec seulement 15% il

n'y a quasiment pas de différence par rapport au vermicompost. Ce qui nous permet de supposer que l'ajout d'une grande proportion de minéraux est nécessaire en présence des vers pour avoir une protection/ stabilité plus efficace des sucres dans le compost. De plus, le traitement V30M diminue la valeur du ratio desoxy/C5 (Fig :7) par rapport à celui du compost classique, ce qui nous permet de dire que les sucres dans le traitement avec les vers et minéraux sont d'origine végétale principalement issus du matériau initial.

Toutes ces suppositions nous mènent à faire l'hypothèse que la combinaison des vers et des minéraux ralentit la dégradation rapide des sucres, autrement dit cette combinaison stabilise les sucres dans le compost, en changeant les conditions environnementales favorables pour le développement des micro-organismes responsables de la dégradation des monosaccharides.

CONCLUSION ET PERSPECTIVES

Le projet Lombricom' a pour objectif de tester l'effet des vers et des minéraux, seuls ou combinés, sur la dégradation de la matière organique ainsi que la limitation d'émissions de CO₂. Ce stage a porté sur l'analyse des sucres dans ces différents traitements.

L'ajout des vers seuls n'a pas d'effet positif sur l'augmentation des sucres totaux et les sucres dans le vermicompost sont plutôt d'origine végétale, issus du matériau parental. Inversement, le traitement sans vers, contient plus de sucres d'origine microbienne. La présence de minéraux semble augmenter la concentration des sucres dans les composts, avec un maximum dans le cas de V30M. Nous avons tiré ainsi plusieurs hypothèses, dont celle (1) que les vers ingèrent et minéralisent les sucres d'origine microbienne. Ainsi, lors du vermicompostage, les microorganismes consomment une partie des sucres végétaux, libérant des sucres microbiens, ces derniers sont finalement consommés par les vers ; (2) L'ajout des minéraux seuls ou combinés aux vers influence positivement la stabilisation des sucres dans les composts. Ainsi, la combinaison vers plus minéraux ralentit dégradation des sucres et les stabilise dans les composts, en diminuant potentiellement la croissance habituelle des micro-organismes due à des conditions défavorables à leur développement. Toutefois, ces hypothèses doivent par ailleurs être confirmées par l'analyse des micro-organismes dans les substrats.

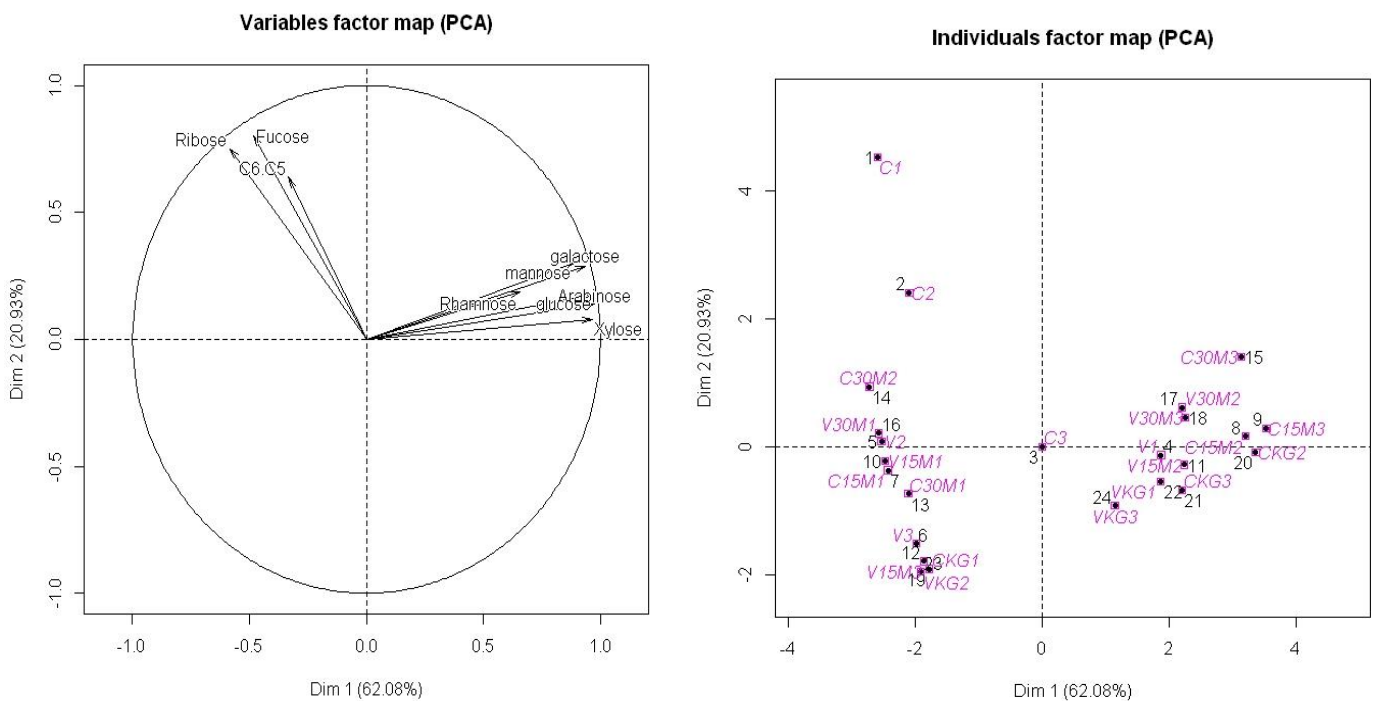
En perspective, il serait intéressant de réaliser plus de réplicats pour confirmer nos résultats. De plus le facteur temps peut jouer un rôle important sur la dégradation des sucres, alors le fait de mesurer les sucres à différents pas de temps serait souhaitable pour bien comprendre par

Appendix 4: Extract of the internship report of Oumayma Taoussi (UPMC, M1 SDUEE) on sugar contents in co-compost and co-vermicompost.

exemple l'effet des vers et des minéraux sur le compost. De plus, pour suivre l'état de dégradation de la matière organique, d'une façon générale, dans le compost sous différents traitements, il faut tenir compte d'autres molécules carbonées, qui ne sont pas forcément facilement dégradables dans une courte durée, tel que les lignines

ANNEXES

Figures 1 et 2 : résultats de l'Analyse en Composantes Principales sous R



Appendix 4: Extract of the internship report of Oumayma Taoussi (UPMC, M1 SDUEE) on sugar contents in co-compost and co-vermicompost.

Résumé de la thèse en français

Introduction et objectifs de la thèse

L'augmentation de la population humaine mondiale, couplée à une forte consommation de biens et services, a abouti à une importante production de déchets, aussi bien ménagers que urbains, industriels et agricoles (Hoorweg *et al*, 2013). Cette situation engendre de graves problèmes environnementaux, nécessitant de trouver de nouvelles stratégies pour traiter les déchets de manière durable et écologique, et ce, de leur production jusqu'à leur recyclage ou élimination.

En parallèle à ce problème, le changement d'usage des terres et la mise en place de pratiques agricoles intensives sont responsables en partie de l'augmentation constante de CO₂ dans l'atmosphère, avec l'activité industrielle et l'utilisation des énergies fossiles. Dans ce contexte, une perte de matière organique au niveau des sols est observée, aboutissant au déclin de nombreux services écosystémiques rendus par les sols dont la fertilité, la fonction support de culture et le stockage de carbone (Smith *et al*, 2015). Ces changements globaux ont des conséquences majeures sur l'environnement, mais aussi sur l'agronomie, l'économie et la société. D'après, le rapport de l'IPCC (2014), ces changements pourraient être atténués par la restauration des stocks de matière organique dans les sols. Ainsi, l'augmentation du carbone dans les sols est envisagée et possible avec l'utilisation d'amendements organiques (Barral *et al*, 2009 ; Ngo *et al*, 2012), pouvant ainsi diminuer les émissions de carbone dans l'atmosphère tout en séquestrant le carbone dans les sols (Lashermes *et al*, 2009).

Résoudre ces deux problèmes environnementaux (augmentation des déchets/ déclin de la matière organique des sols) est l'un des enjeux majeurs de l'agriculture et de la société. Ainsi, deux procédés de décomposition de la matière organique basés sur la bio-oxydation, le compostage et le vermicompostage, permettent de transformer les déchets organiques en amendements. De plus, ces deux procédés requièrent peu d'investissements et d'énergie pour recycler de manière efficace la matière organique. Le compostage est une méthode traditionnelle de bio-oxydation des déchets, divisé en 3 phases (phase active, thermophile et mésophile), aboutissant à une matière organique stable et hygiénisée, pouvant présenter des propriétés fertilisantes. Le vermicompostage repose aussi sur des mécanismes de bio-oxydation mais la présence de vers induit une aération continue résultant en une dégradation et

stabilisation plus rapide de la matière organique (Lazcano *et al*, 2008 ; Paradelo *et al*, 2009, 2010). Malgré les avantages de ces deux procédés, de larges quantités de gaz à effet de serre sont produits lors de la décomposition de la matière organique tels que CO₂, CH₄ ou encore N₂O (Hobson *et al*, 2005 ; Chan *et al*, 2011 ; Thangarajan *et al*, 2013). De plus, l'incorporation des composts et vermicomposts au sol entraîne aussi des émissions de gaz à effet de serre (Cambardella *et al*, 2003 ; Bustamante *et al*, 2007), ayant pour principale origine la minéralisation du carbone des (vermi)composts apportés ainsi que celle de la matière organique du sol due à une augmentation de l'activité microbienne, mécanisme connu sous le nom de « priming effect » (Bustamante *et al*, 2010).

Le compostage est un procédé se déroulant en aérobie, qui peut être effectué à grande échelle sous forme d'andain (Hobson *et al*, 2005) mais aussi à l'échelle du particulier à l'aide de composteur (Andersen *et al*, 2011). Traditionnellement, le compostage permet de recycler une grande variété de matières organiques telles que du fumier, des déchets verts, des boues de station d'épuration, etc. (Goyal *et al*, 2005). De même, le vermicompostage peut traiter des déchets organiques similaires (Chan *et al*, 2011 ; Garg *et al*, 2006 ; Ngo *et al*, 2011) mais ne peut toutefois être pratiqué que sur de faibles épaisseurs, afin d'éviter les montées en température et donc éviter la perte des vers. Certains déchets, tels que les boues doivent être compostés en présence d'additifs qui sont soit organiques (Gabhane *et al*, 2012), soit minéraux (Wong *et al*, 1995) ou soit biologiques (inoculation de micro-organismes, Wakase *et al*, 2008). Ces additifs sont employés pour améliorer le procédé de compostage, notamment en réduisant les pertes de nutriments (Steiner *et al*, 2010) et augmentant leur biodisponibilité pour la plante (Gabhane *et al*, 2012), en limitant les odeurs (McCroory *et al*, 2001), en favorisant l'aération, voire en accélérant la stabilisation de la matière organique (Sanchez-Garcia *et al*, 2015). Néanmoins, peu d'études portent sur l'utilisation d'additifs lors du compostage pour stabiliser le carbone et donc limiter les émissions de CO₂ (Bolan *et al*, 2012 ; Chowdhury *et al*, 2016) et aucune avec le procédé de vermicompostage. Une des principales raisons expliquant ce manque d'informations dans la littérature est le fait que les émissions biogéniques de CO₂ ne sont pas prises en compte dans les émissions de gaz à effet de serre totales de ces procédés de décomposition de la matière organique (IPCC, 2014). Malgré tout, environ 50% du carbone initial du déchet est minéralisé sous forme de CO₂ lors de la bio-oxydation (Tiquia *et al*, 2002), impactant de la même manière l'environnement que les émissions non-biogéniques (**Fig. 3**).

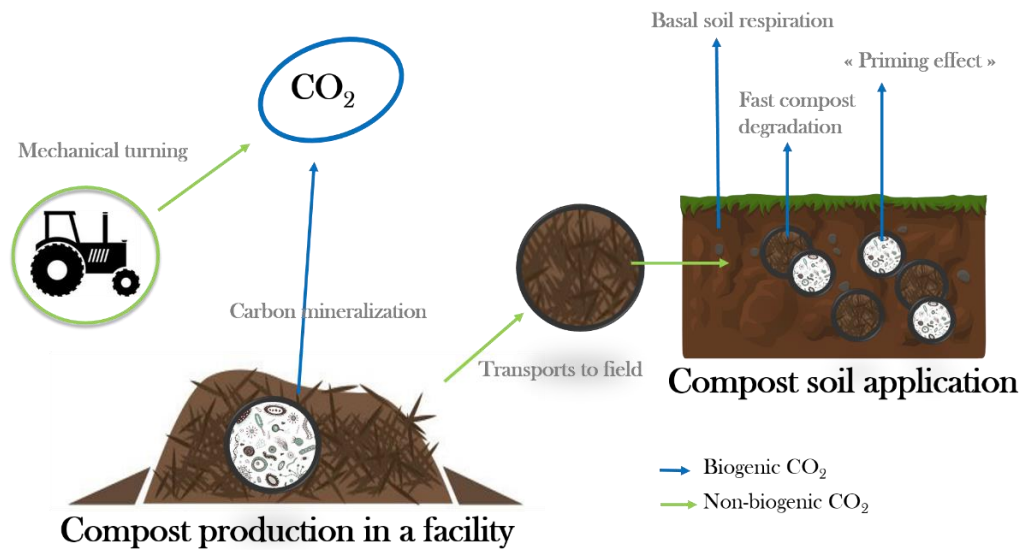


Figure 3 Origines des émissions de CO₂ (biogéniques et non biogéniques) lors du procédé global de recyclage de la matière organique par le compostage

De plus, les mécanismes de stabilisation de la matière organique, en particulier du carbone, lors du compostage sont peu connus, alors que ces derniers sont largement étudiés dans les sols (Sollins et al, 1996). Ainsi, par analogie avec les sols, la stabilisation du carbone peut s'effectuer à travers (von Lützow et al, 2006) : l'inaccessibilité spatiale du carbone pour les micro-organismes, la récalcitrance chimique des molécules carbonées et la formation d'associations organo-minérales. Parmi ces mécanismes, les associations organo-minérales (**Fig.8**) sont les plus efficaces pour stabiliser le carbone sur des échelles de temps importantes (Kleber et al, 2015).

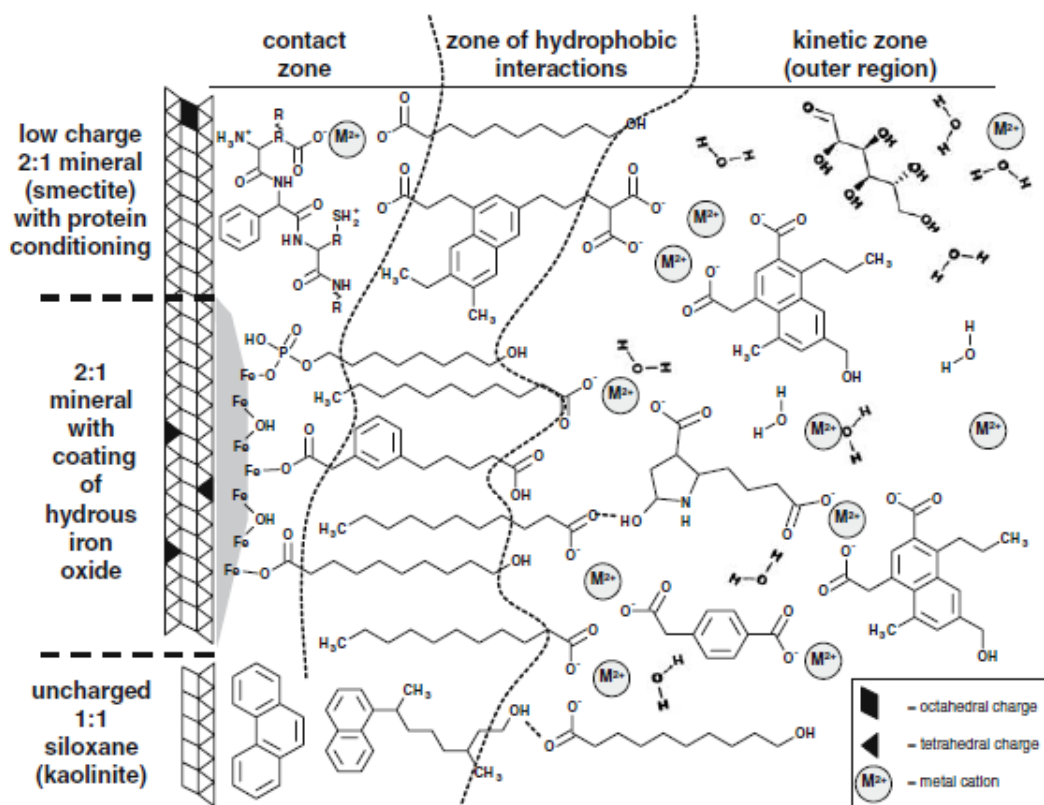


Figure 8 Modèle d'associations organo-minérales selon Kleber et al (2007).

Par conséquent, l'objectif de ce travail est de déterminer l'influence des minéraux sur la décomposition de matières organiques compostables et donc « fraîches » lors du compostage et vermicompostage, ainsi que les effets des minéraux sur la stabilisation du carbone sur le court et moyen terme. En tant qu'espèces ingénieuses, les vers peuvent jouer un rôle significatif dans la stabilisation du carbone. Plusieurs minéraux, présentant diverses propriétés, ont été testés en tant qu'additifs, seuls ou combinés : la montmorillonite (argile 2 :1), la kaolinite (argile 1 :1) et la goéthite (oxyde de fer). D'autres minéraux, considérés comme déchets industriels, ont aussi été testés, le red mud et le fly ash. Cette étude se concentre tout particulièrement sur les potentielles associations organo-minérales formées selon le type de minéral et la présence de vers ou non (*Eisenia andrei* et *Eisenia foetida*). Outre la stabilisation du carbone lors du compostage, les changements physico-chimiques induits par la présence de minéraux et/ou des vers au niveau du produit final sont aussi étudiés, ainsi que leur(s) potentiel(s) effet(s) en tant qu'amendements sur un sol (cambisol). Enfin, l'autre objectif de ce travail est de vérifier si ces nouveaux composts et vermicomposts peuvent être utilisés en tant que substrat de culture pour

favoriser la croissance des plantes. La qualité et la maturité des co-composts et co-vermicomposts obtenus sont donc évaluées dans ce projet.

Pour répondre à ces objectifs, cette thèse est divisée en 6 parties principales dont l'une consiste en un état de l'art sur les différents additifs actuellement utilisés (**Fig. 4**) lors du compostage et leurs effets associés sur le procédé, la qualité et la maturité des composts, et enfin leurs effets en tant qu'amendements et fertilisants.

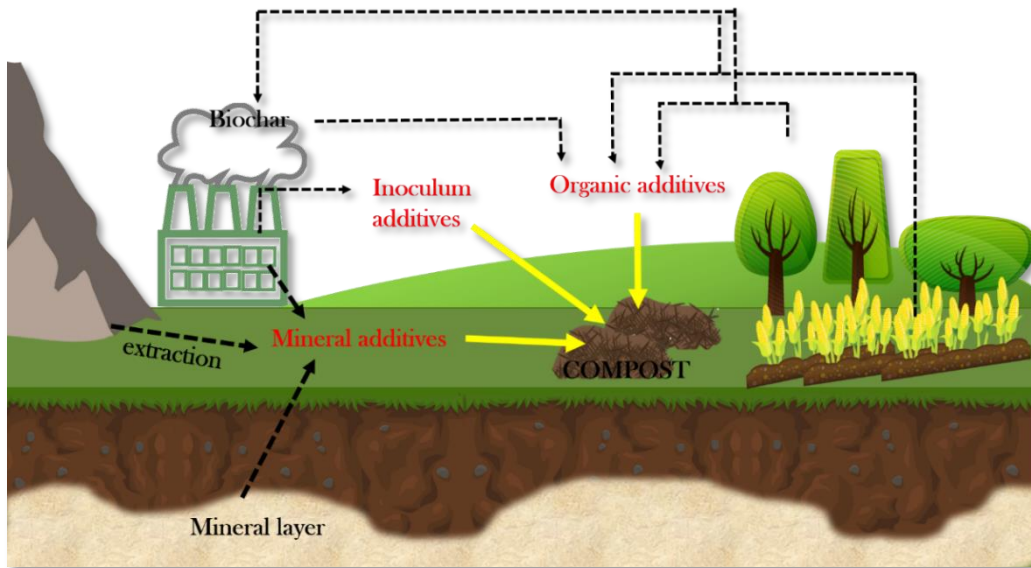


Figure 4 Les différentes origines des additifs utilisés lors du compostage. Les additifs minéraux peuvent provenir des couches minérales du sol ou extraits des carrières, les additifs organiques peuvent être directement issus de l'agriculture (résidus agricoles) ou de déchets biologiques (urbains, ménagers, etc.) et peuvent être au préalable transformés en biochar. Enfin, les additifs qualifiés de biologiques (micro-organismes) sont généralement issus de procédés industriels.

Les différentes problématiques soulevées par ce travail et reprises dans les parties suivantes sont (**Fig. 13**) :

- Quel est le minéral le plus « efficace » et en quelle quantité pour limiter les émissions de CO₂ et stabiliser le carbone lors du compostage ? (**Parties 2 et 6**)
- -Quel est le rôle et l'effet des vers sur la stabilisation du carbone ? (**Parties 2 et 6**)
- La stabilisation du carbone par les minéraux lors du compostage est-elle persistante une fois le co-compost/co-vermicompost incorporé au sol ? Les propriétés physico-chimiques du sol sont-elles modifiées par cet apport ? (**Partie 4**)
- Dans quelles mesures les minéraux et les vers (combinés ou non) influencent-ils la qualité et la maturité des produits obtenus ? (**Parties 3 et 6**)
- Quelle est leur influence sur la croissance des végétaux ? (**Partie 5**)

Partie 2 et 6: Suivi de la minéralisation du carbone et analyses des potentielles associations organo-minérales formées lors du compostage et vermicompostage. Evaluation des effets des vers et des minéraux lors du compostage.



Co-compostage • Co-vermicompostage

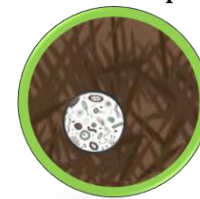
Partie 3 et 6: Evaluation de la qualité (biodisponibilité des nutriments) et des caractéristiques microbiennes des produits obtenus

Co-compost

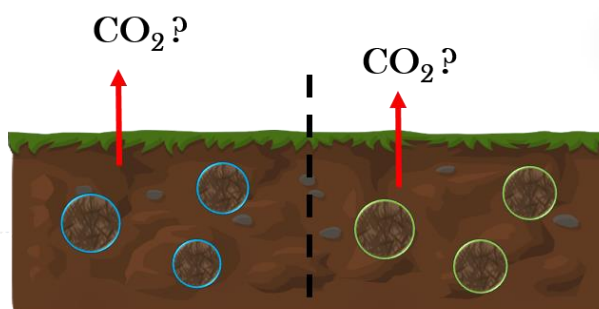


- Fractionnement du phosphore
- Acides phospholipidiques (PLFA)
- Biomasse microbienne
- Concentration en azote
- Mesures physico-chimiques, ...

Co-vermicompost



Partie 4: Evaluation du changement des propriétés d'un sol induit par l'incorporation de co-composts et co-vermicomposts



Cambisol (sableux)

- Suivi minéralisation sol+amendement
- Répartition du carbone (fractionnement Zimmerman)
- Modélisation/ prédiction sur le moyen terme



- Suivi de croissance (graines à graines) de *A. Thaliana* en conditions contrôlées
- Suivi de la concentration en carbone et azote dans les tissus de la plante

Partie 5: Influence des co-composts et co-vermicomposts sur le développement des végétaux en tant que substrat de culture

Figure 13 Approche générale du sujet et des méthodes utilisées. MET : microscope électronique à transmission.

Partie 2 et 6 : Influence des vers et des minéraux sur la dégradation et la stabilisation de la matière organique compostable

Dans cette partie, nous nous sommes intéressés à la formation potentielle des associations organo-minérales, mais aussi à la minéralisation du carbone issu de la matière organique fraîche lors du compostage avec différentes quantités et type de minéraux, avec ou sans vers. L'ensemble des traitements et leurs abréviations correspondantes sont mentionnés dans le **tableau 9**.

Tableau 9 Résumé des différents traitements réalisés au cours de cette thèse

	Minéraux	Quantité de minéral ajouté (% en poids sec de la matière organique initiale)	Présence de vers	Nom du traitement
Absence de minéral	-	-	oui	V _{control}
			non	C _{control}
Minéraux seuls	Kaolinite	15%	oui	V15K
			non	C15K
		30%	oui	V30K
			non	C30K
	Montmorillonite	15%	oui	V15M
			non	C15M
		30%	oui	V30M
			non	C30M
	Goethite	15%	oui	VG
			non	CG
Combinaison de minéraux	Montmorillonite + Goethite	15%-15%	oui	VMG
			non	CMG
	Kaolinite + Goethite	15%-15%	oui	VKG
			non	CKG

A l'issue de cette expérience, nos résultats suggèrent que lors du compostage, les minéraux ont la capacité de protéger le carbone et d'éviter ainsi sa minéralisation sous forme de CO₂. Cette capacité, tout comme dans le sol, semble dépendante des propriétés intrinsèques des minéraux (surface spécifiques, minéralogie, etc). Ainsi, la montmorillonite apparaît comme le minéral le plus efficace pour remplir notre objectif de réduction des émissions de carbone. Néanmoins, contrairement à ce qui se produit dans les sols, nous n'avons pas mis en évidence de capacité

d'adsorption du carbone, en particulier labile, par les oxydes de fer. Ceci peut s'expliquer par une différence de l'état et des caractéristiques de la matière organique (sol vs. compostable, plus grand pool de carbone labile dans la matière organique compostable, etc.). De plus, la combinaison de minéraux (oxyde et argiles) ne semble pas diminuer la minéralisation du carbone et donc améliorer la protection du carbone, et au vue des résultats, les minéraux seuls mais en proportion élevée semblent plus efficaces. Toutefois, la capacité théorique de rétention maximale de carbone par les minéraux n'est pas atteinte dans le cas de notre étude, et cette rétention est parfois même inférieure à celle observée dans les sols. Ainsi, des différences entre les mécanismes de stabilisation de la matière organique du sol et ceux avec la matière organique « fraîche » sont observées, suggérant l'importance de l'état de la matière organique mais aussi, le temps nécessaire pour la stabilisation du carbone. Enfin, la présence des vers lors du compostage semble maximiser la formation d'association organo-minérales, en particulier avec la montmorillonite. De plus, la matière organique associée aux minéraux est soit dégradée, soit intacte dans le cas du vermicompostage alors que lors du compostage, seule la matière organique partiellement dégradée semble être associée (**Fig. 18**). Dans le cas de minéraux issus de déchets industriels (red mud et fly ash), les mécanismes de rétention du carbone sont différents, faisant probablement intervenir la formation de carbonates. Cependant, tout comme avec les autres minéraux, ces mécanismes semblent favorisés par la présence de vers. En conclusion de cette partie, nous pouvons dire que les vers jouent un rôle fondamental dans la stabilisation du carbone de la matière organique compostable permettant d'homogénéiser la répartition des minéraux et donc de favoriser les interactions.

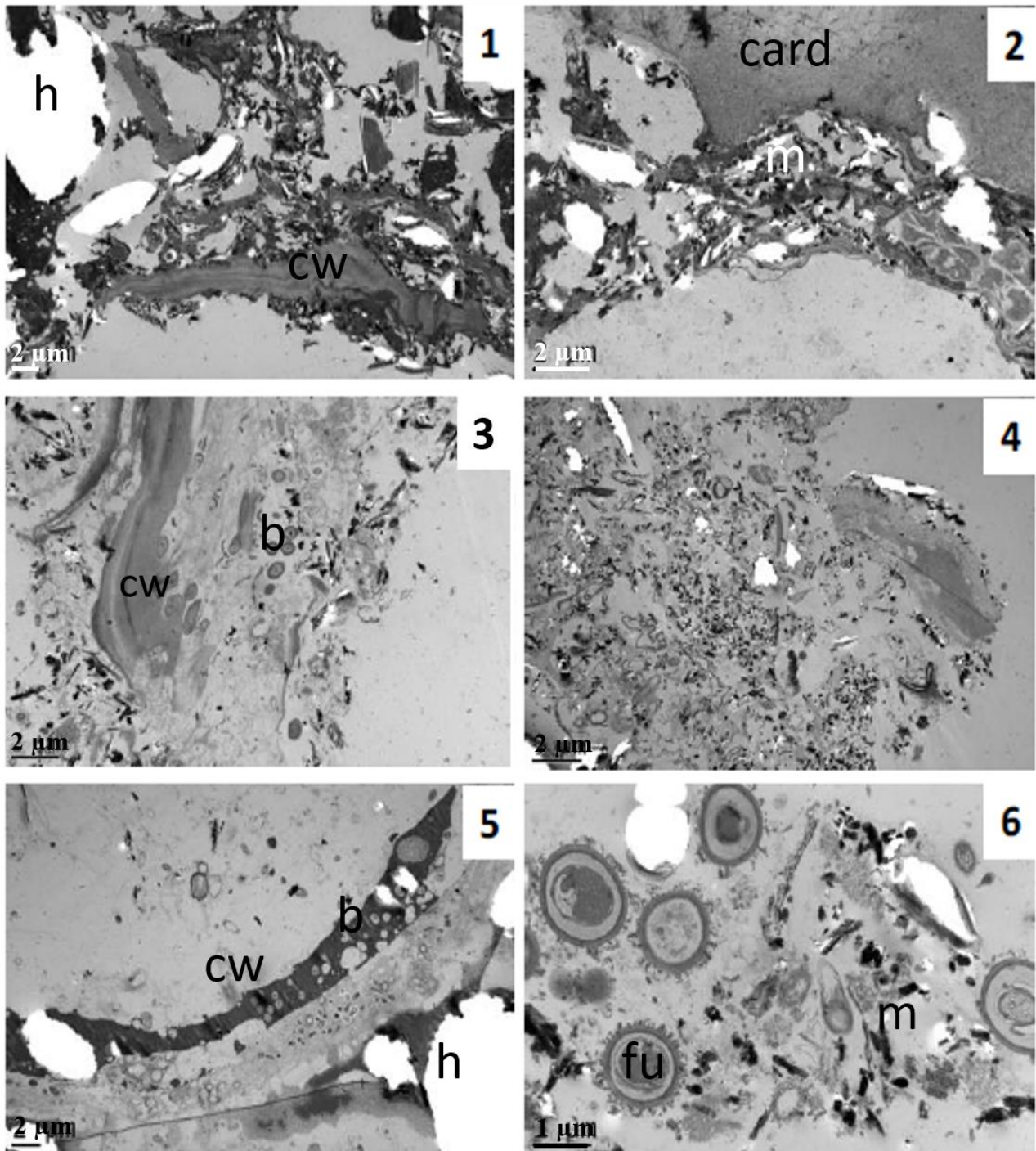


Figure 18 Observations au MET de la matière organique vermicompostée en présence de kaolinite et goethite (VKG). 1] associations minérales avec des cellules dégradées ; 2] associations minérales avec des résidus de cartons ; 3] associations minérales avec la matière organique dégradée (non identifiable) ; 4] vue d'ensemble de la répartition homogène des minéraux ; 5] dégradation de parois par des bactéries ; 6] champignon intact. b: bactérie; card: carton; cw: paroi cellulaire; fu: champignon; h: artefact (trou); m: minéral.

Partie 3 et 6 : Influence des vers et des minéraux sur la biodisponibilité des nutriments et les caractéristiques physico-chimiques des composts obtenus

Les principaux résultats obtenus suite à la caractérisation physico-chimique des composts et vermicomposts obtenus, avec et sans minéraux, sont présentés dans le **tableau 10**. Il en ressort que la présence de minéraux modifie la qualité du compost. Ainsi, la présence de minéraux diminue la teneur en carbone organique totale du compost et vermicompost, du fait d'un effet de dilution. Néanmoins, les minéraux semblent diminuer significativement la proportion de carbone sous forme labile (*i.e.* carbone organique dissous) suggérant une potentielle stabilité de ces composts une fois incorporés au sol. Malgré ces aspects positifs dus aux minéraux, ces derniers diminuent la biodisponibilité des nutriments, probablement par adsorption de molécules azotées ou phosphatées sur leur surface (Shen *et al*, 2011 ; Hinsinger *et al*, 2001) modifiant ainsi le cycle des nutriments. Cette modification de biodisponibilité est plus marquée dans le cas d'argile tel que la montmorillonite. Un des moyens permettant de réduire la rétention des nutriments par les minéraux est d'effectuer ce type de compostage en présence de vers. En effet, d'après nos résultats, les vers semblent jouer un rôle important dans le cycle des nutriments : l'azote se retrouve principalement sous forme directement assimilable par les plantes, et ce même en présence de minéraux ; et la biodisponibilité du phosphore est augmentée.

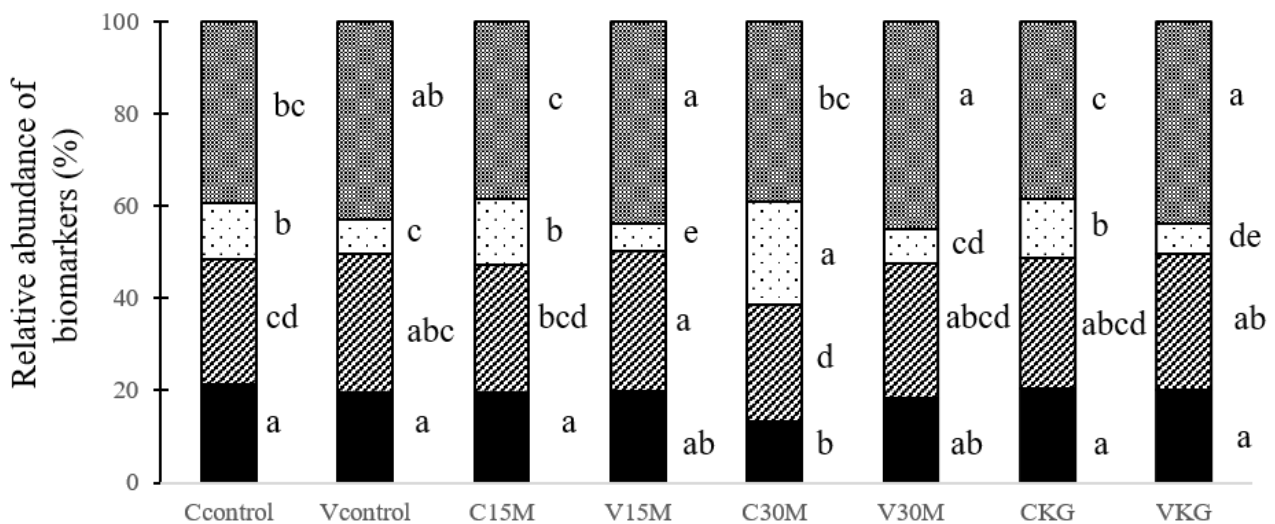


Figure 10 Abondance relative des biomarqueurs des bactéries et champignons (PLFA) présents dans les co-composts et co-vermicomposts. Différentes lettres entre mêmes biomarqueurs pour des traitements différents indiquent une différence significative ($p < 0.05$ Kruskal-Wallis).

Enfin, il convient de souligner que les minéraux ont aussi un impact sur les communautés microbiennes et leur activité (**Fig. 25**), notamment en favorisant le développement de communautés fongiques. De plus, la présence de vers lors du compostage tend à diminuer ces communautés, suggérant que les vers se nourrissent non pas de la matière organique directement mais de micro-organismes (Curry and Schmidt, 2007).

Tableau 25 Caractéristiques physico-chimiques des co-composts et co-vermicomposts obtenus en présence de minéraux.

Treatment	Total N (mg g ⁻¹ compost)	Total OC (mg g ⁻¹ compost)	C:N	pH	EC (dS m ⁻¹)	DOC (% OC)
C	34,8 ^a	381,2 ^a	11,1 ^{abcd}	8,3 ^c	2,0 ^{bc}	9,7 ^{bc}
C15K	20,3 ^b	273,1 ^{ab}	13,6 ^a	9,2 ^a	1,6 ^{cd}	11,5 ^{ab}
C15M	20,2 ^{bc}	244,5 ^{bc}	12,2 ^{ab}	8,2 ^{cd}	1,2 ^{def}	8,3 ^c
C30K	15,9 ^{def}	177,5 ^{efg}	11,3 ^{ab}	9,0 ^a	1,1 ^{ef}	13,3 ^a
C30M	15,4 ^f	179,9 ^{efg}	11,8 ^{bcde}	8,2 ^{cd}	1,2 ^{ef}	2,8 ^{de}
CG	23,1 ^b	261,0 ^{bc}	11,3 ^{ab}	8,7 ^{ab}	1,2 ^{def}	12,1 ^{ab}
CMG	16,5 ^{def}	182,7 ^{ef}	11,1 ^{abc}	8,2 ^c	0,8 ^f	4,1 ^{de}
CKG	17,3 ^{de}	204,5 ^{de}	11,8 ^{ab}	8,8 ^a	1,0 ^f	11,4 ^{ab}
V	35,2 ^a	331,2 ^{ab}	9,4 ^{ef}	7,6 ^{ef}	3,1 ^a	3,9 ^d
V15K	22,2 ^b	211,1 ^{cd}	9,5 ^{cdef}	7,6 ^f	1,9 ^{abc}	10,3 ^{bc}
V15M	23,1 ^{ab}	206,5 ^{cde}	9,0 ^{fg}	7,8 ^{ef}	1,9 ^c	2,9 ^{de}
V30K	15,5 ^{ef}	145,2 ^h	9,4 ^{def}	7,9 ^{de}	1,8 ^c	10,0 ^{bc}
V30M	16,5 ^{def}	152,5 ^{fgh}	9,3 ^{efg}	7,6 ^f	1,3 ^{def}	2,3 ^e
VG	22,8 ^b	201,7 ^{de}	8,9 ^{fg}	7,8 ^{ef}	2,9 ^{ab}	7,9 ^c
VMG	17,6 ^{cd}	151,8 ^{fgh}	8,6 ^g	7,5 ^{ef}	2,1 ^{abc}	2,6 ^{de}
VKG	16,4 ^{def}	147,7 ^{gh}	9,0 ^{fg}	8,3 ^{bc}	1,6 ^{cde}	10,0 ^{bc}

Partie 4 : Influence des co-composts et co-vermicomposts sur les propriétés physico-chimiques du sol et sa capacité de stockage du carbone

Dans cette partie, nous nous sommes intéressés à l'effet des co-composts et co-vermicomposts produits en tant qu'amendement. Pour cela, ces différents substrats ont été incorporés à un sol sableux (cambisol). Bien que les minéraux apparaissent efficaces dans la protection du carbone lors du compostage, l'application des co-composts ne permet pas de diminuer les émissions de CO₂ du sol amendé comparé à un sol amendé avec du compost classique. La principale hypothèse pouvant être émise sur ce point est que l'application de ces co-composts peut stimuler l'activité microbienne du fait de (1) l'ajout de source de carbone potentiellement assimilable par les micro-organismes et (2) la présence de minéraux qui peut faciliter la croissance microbienne en tant que support de croissance et en favorisant les contacts avec la matière organique (Wei *et al.*, 2014). Ainsi, les communautés microbiennes du sol semblent favorisées par ce nouvel environnement. De ce fait, l'effet positif des minéraux sur la rétention du carbone n'est plus valable une fois les co-composts appliqués au sol. De plus, la répartition du carbone est modifiée par cet ajout, augmentant la quantité de carbone potentiellement minéralisable du sol sur le moyen terme (**Fig. 33**). Au contraire, la présence de vers durant le compostage semble aboutir à un produit plus stable que le compost. En effet, l'application de co-vermicompost au sol montre des émissions de carbone inférieures à celles des sols amendés avec les co-composts. Néanmoins, tout comme pour les co-composts, la présence de minéraux augmente ces émissions. Sur le moyen terme, l'application de co-vermicompost (avec montmorillonite en particulier) est préférable car ce produit semble augmenter de manière significative le stock de carbone du sol, ainsi que sa teneur en azote disponible, tout en limitant les émissions de CO₂.

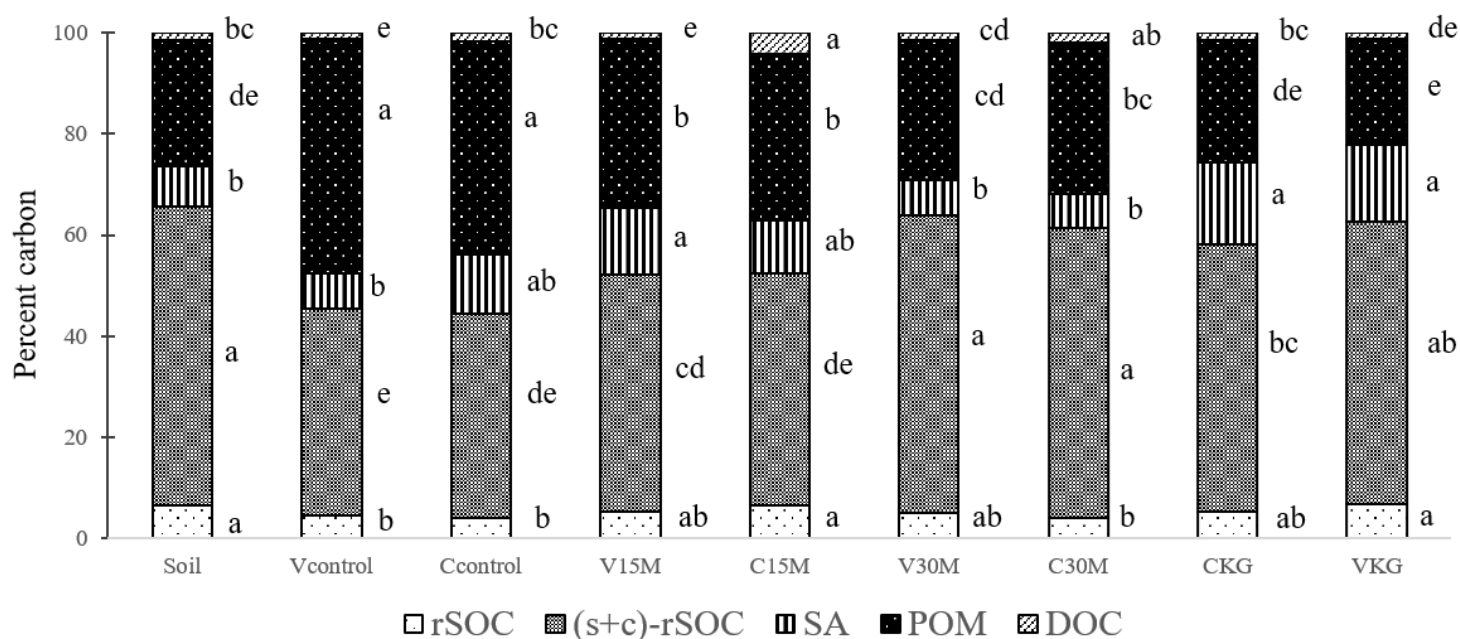


Figure 33 Proportion relative (% carbone total du sol) du carbone organique pour chaque fraction analysée, des sols amendés et du sol non amendé. Différentes lettres entre même fractions pour des traitements différents indiquent une différence significative ($p < 0.05$ Kruskal-Wallis). POM: matière organique particulaire; SA: agrégat stables ; (s+c)-rSOC: fraction argileuse; rSOC: fraction récalcitrante chimiquement; DOC: fraction représentant le carbone organique dissous.

Partie 5 : Influence des co-composts et co-vermicomposts sur la croissance des végétaux. Cas de *A. Thaliana*

Cette dernière partie consiste à évaluer les co-composts et co-vermicomposts en tant que substrat de culture et donc leurs qualités en tant que fertilisant. L'expérience réalisée consistait à mettre en culture des plants d'*A. Thaliana*, de graines à graines, sous conditions contrôlées et de mesurer leur surface foliaires, nombre de feuilles, biomasse racinaire et aérienne, etc. Nous avons observé que la substitution d'un terreau commercial, à hauteur de 7% (v/v) par du compost et du vermicompost classique ne permet pas d'améliorer la croissance des plantes, bien que ces derniers présentent des caractéristiques physico-chimiques optimales en tant que fertilisant. De plus, la présence de vermicompost semble inhiber la croissance alors que celle du compost ne ferait que la ralentir, au vu des résultats sur les rendements de graines et la longueur de l'inflorescence maximale (**Fig. 42**). Cette inhibition par le vermicompost peut s'expliquer en partie par un changement de structure du milieu de culture (pH, conductivité électrique, etc.) mais aussi par une potentielle et forte concentration en auxine dans ce substrat.

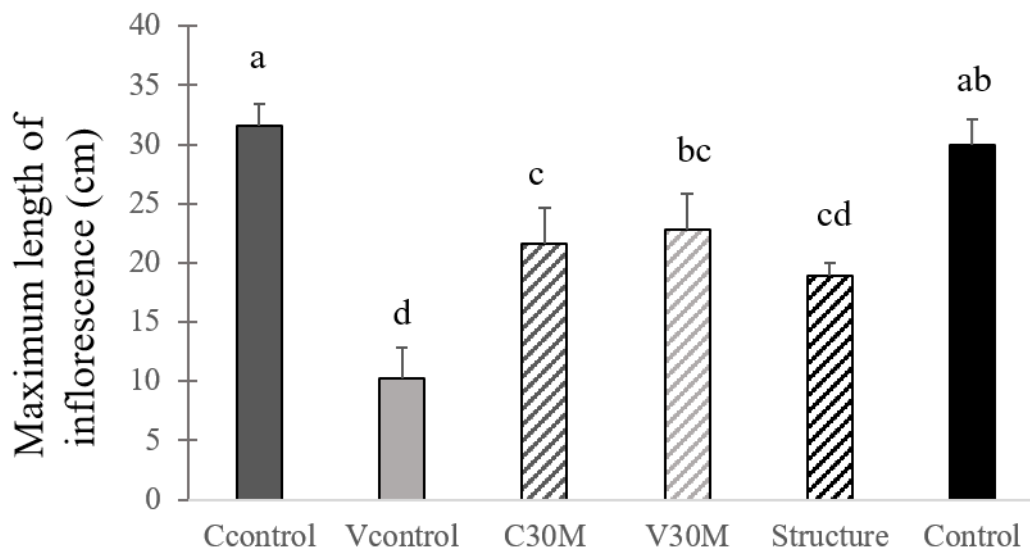


Figure 42 Longueur maximale de l'inflorescence observée pour *A.Thaliana*, cultivée sur différents substrats et sans ajout de fertilisation chimique. Les barres d'erreur indiquent l'erreur standard. Différentes lettres indiquent une différence significative entre traitement (n=4)

Enfin, l'ajout de co-compost et co-vermicompost semble améliorer la croissance de *A.Thaliana*, en particulier le co-vermicompost, comparé aux composts classiques. Les changements de pH et de conductivité électrique induits par les minéraux peuvent expliquer ces observations, et la présence de minéraux peut par ailleurs favoriser la rétention de l'eau et des nutriments, sans que la plante ne présente de difficultés pour utiliser ces ressources. Finalement, cette partie nous permet de montrer que les indicateurs classiques employés pour caractériser la capacité fertilisante des composts n'est pas complètement applicable dans le cas des vermicomposts, soulignant l'intervention de mécanismes autres que nutritionnels (phytohormone ?). La présence de minéraux au sein du vermicompost permet de diminuer les effets négatifs sur la plante, voire d'augmenter sa croissance, en particulier aérienne.

Conclusion et perspectives

Le traitement des déchets organiques pose problème environnemental mais économique et sociétal. Actuellement, de nombreuses techniques de gestion des déchets existent dont deux, le compostage et le vermicompostage, permettent de recycler ces déchets en matière organique amendante et fertilisante. Bien que ces deux procédés apparaissent comme écologiques, la dégradation biologique de la matière organique produit une quantité non négligeable de gaz à effet de serre, dont le CO₂.

Pour limiter l'empreinte carbone du compostage et du vermicompostage, des additifs peuvent être utilisés lors de la dégradation de la matière organique. Récemment des minéraux (argiles, oxydes) sont utilisés en tant qu'additifs pouvant stabiliser le carbone et donc limiter les émissions de CO₂ (Bolan *et al*, 2012). Dans le cadre de ce travail, nous avons étudié l'effet de l'ajout de certains minéraux non seulement lors du compostage mais aussi du vermicompostage. Ainsi, il en ressort, que l'utilisation de minéraux tels que la montmorillonite peut permettre de diminuer les émissions de CO₂ lors de ces deux procédés, mais que la qualité des produits obtenus est meilleure dans le cas du co-vermicompostage. Les vers semblent donc jouer un rôle fondamental dans ce procédé en (1) stabilisant la matière organique sous différentes formes et favorisant les interactions organo-minérales ; (2) améliorant la qualité des co-composts en limitant la rétention des nutriments nécessaires pour la plante par les minéraux ; (3) en formant des « turricules » susceptibles d'augmenter le stock de carbone dans les sols sur le court et moyen terme et d'améliorer la fertilité des sols d'un point de vue nutritif et microbien et (4) en améliorant la croissance des parties aériennes de la plante en tant que substrat, en remplacement de la tourbe. Malgré ces observations positives, l'aspect économique et applicable à plus grande échelle de ce procédé de vermicompostage en présence de minéraux doit être déterminé, afin de minimiser au mieux son empreinte carbone et de l'ajuster selon la région concernée et l'utilisation future de ces produits (type de sol, plantes, etc.).

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RESUME

Procédé de compostage innovant en présence de vers et de minéraux : effets sur le stockage de carbone, les propriétés du sol après amendement et la croissance des végétaux.

Basés sur la bio-oxidation de la matière organique, le compostage et le vermicompostage émettent des quantités non négligeables de CO₂. Ainsi, ce travail s'intéresse (1) à la stabilisation potentielle du carbone de la matière organique compostable due à l'ajout de minéraux, avec et sans vers, et (2) l'influence des minéraux sur la maturité et la qualité du compost final. La première expérience consista en une incubation de matière organique, permettant d'obtenir différents composts et vermicomposts, avec et sans minéraux, de suivre les émissions de carbone et d'analyser les produits obtenus. Une deuxième incubation a été menée pour étudier l'effet de ces produits en tant qu'amendement sur un sol. Enfin, le suivi de la croissance de *A.Thaliana* en présence de co-composts et co-vermicomposts en tant que fertilisant fut réalisé.

La présence de minéraux lors du compostage induit une baisse des émissions de carbone, probablement due à la formation d'associations organo-minérales ou bien par un changement des communautés microbiennes. De plus, les co-composts, en tant qu'amendement semblent augmenter les stocks de carbone du sol, comparé à un compost classique. Enfin, bien que l'aspect fertilisant des co-composts obtenus soit optimum, ils n'ont pas amélioré la croissance d'*A.Thaliana*. La présence de vers lors du co-compostage a augmenté les émissions de carbone mais l'effet positif des minéraux sur la minéralisation était toujours observable. De plus, les vers ont augmenté la biodisponibilité des nutriments et le co-vermicompost a permis d'améliorer les propriétés du sol ainsi que la croissance des végétaux comparé à un (vermi)compost classique et un co-compost.

Keywords : compost; vers; minéraux; carbone; croissance des plantes; sol; stock de carbone; amendement; fertilisant; analyses physico-chimiques; analyses microscopiques.

ABSTRACT

Innovative waste treatment by composting with minerals and worms: effects on carbon storage, soil properties and plant growth

Due to bio-oxidative mechanisms, composting and vermicomposting produce a large amount of CO₂ emissions. Therefore, in this study we aimed to assess (1) the potential stabilization of carbon of fresh organic matter due to mineral presence, with and without worms, and (2) the influence of minerals on maturity and quality of the finished products. The first experiment was based on a laboratory incubation of fresh organic matter, which allowed to obtain composts and vermicomposts, with and without minerals, to monitor carbon emissions and to analyse the final products. A second incubation was carried out to investigate the effect of these products as organic amendment on an arenic cambisol. Finally, the last experiment was carried out to assess the use of co-composts and co-vermicomposts as potting media, by following the growth of *A.Thaliana*.

The presence of minerals during composting induced a decrease of carbon emission, likely due to the formation of organo-mineral associations or shift of microbial communities. Moreover, the use of co-compost as soil conditioner may allow to increase soil carbon storage, compared to a regular compost. Although the physico-chemical characteristics of the end-products were optimum for its use as potting media, no beneficial aspects on *A.Thaliana* growth has been observed. In presence of worms during co-composting, the carbon emissions were increased due to worm activities but the positive effect of minerals was still observed. In addition, worms improved the nutrient availability in the end products. Therefore, co-vermicompost tended to improve soil properties and to enhance plant growth compared to a regular (vermi)compost.

Keywords : compost; worms; minerals; carbon; plant growth; soil; carbon storage; amendment; organic fertilizer; physico-chemical analyses; microscopic analyses.