

Study of the effect of organic waste products amendments (OWPs) and microbial diversity on volatile organic compounds (VOCs) emissions by soil

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Sorbonne Université

Ecole doctorale 129 Science de l'environnement INRA UMR 1402 ECOSYS Écologie fonctionnelle et écotoxicologie des agroécosystèmes

Study of the effect of organic waste products amendments and microbial diversity on volatile organic compounds emissions by soil

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Thèse de doctorat de Science de l'environnement

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A mia madre, con tutto l'amore del mondo

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Background

Current investigation on climate science reveals that the air quality and global temperatures are positively or negatively affected by several biogeochemical processes. The biogeochemical processes involving greenhouse gases (CO_2 , CH_4 and N_2O) have been widely documented. However, another class of compounds, the volatile organic compounds (VOCs) family, has been widely investigated in the last decades. Despite the low VOCs concentration in the atmosphere (from part per billions - ppb to part per trillions - ppt) (Kesselmeier and Staudt, 1999), they are involved in several important reactions in the atmosphere such as: the reactions leading to the ozone (O_3) formation in the troposphere (Seinfeld and Pandis, 2016), the generation of large quantities of organic aerosols (Singh et al., 1995) or the production of the nitrates in the atmosphere (Atkinson, 2000). Those processes are directly responsible for atmospheric pollution. The consequences on the environment at a global scale, on human health and on ecosystems underline the importance, in Europe and more in general worldwide, of international agreements and investments in order to reduce and control the emissions of these pollutant gases in the atmosphere.

The purpose of this work is not to investigate how to reduce the impact of VOCs emissions in the atmosphere. Nevertheless, to reduce VOCs emissions, the study of the dynamics and the sources leading to the VOCs production is indispensable. Biogenic sources of VOCs are between 10 and 11 times higher than the VOCs emissions released from anthropogenic sources (Guenther, 1997). Amongst the most important biogenic sources of VOCs we can find plants and forests, which, at the moment are the widest studied sources of VOCs. However, recently, Bachy et al., (2016) highlighted the possibility of an overestimation of the VOCs derived from canopy compared to bare soils. Moreover, Potard et al., (2017) and Raza et al., (2017a) put forward the role played by the organic amendment on the VOCs emissions from soils. The use of organic amendment, and in particular of the organic waste products, has been widely encouraged in Europe (Europe commissions, 2010) during the last decades in order to recycle the exogenous organic matter, increase soil organic matter and partially substitute mineral fertilizers. The production of organic waste products in France is about 332 Mt per year, of which at least the 50 % return back to soil as organic amendment (ADEME, 2006). Furthermore, it has been reported that the emissions from organic waste products are potential precursors of the particulate matter formation in the atmosphere; this information underlines the need of a VOCs emissions characterization. In addition, several studies reported that VOCs emissions from soils are strongly related to the microbial activity (Bäck et al., 2010; Mayrhofer et al., 2006; McNeal and Herbert, 2009). Despite, VOCs sources such as soil and microorganisms, are still poorly explored due to the very complexity of the ecosystem. For instance, to our knowledge, no information has been reported concerning the relationship between VOCs emissions, organic waste amendment and microbial diversity in soil. Thus, the aim of this work will be to add small pieces to the complex world of the VOCs emissions by soil and microorganisms.

Chapter I General introduction

I. General introduction

I.1 What are Volatile Organic Compounds (VOCs)?

The article 2 of the European Council Directive 1999/13/EC of 11 March 1999 defines volatile organic compounds as:

"Volatile organic compound (VOC) shall mean any organic compound having at 293.15 K a vapour pressure of 0,01 kPa or more, or having a corresponding volatility under the particular conditions of use."

Volatile organic compounds are molecules containing atoms of carbon and hydrogen, often bonded with halogens, oxygen, sulfur, nitrogen, or phosphorous. The wide range of molecules they can form, with different functional groups, make the ensemble of these compounds difficult to be fully detected and identified. Indeed, a large number of VOCs have not been identified yet. The fact that they have different functional groups also implies different chemical and physical properties and thus differences in terms of toxicity (Cicolella, 2008). Several families of compounds are well-defined VOCs such as: ketones, aldehydes, alcohols, nitriles, hydrocarbons, diols etc...

Sources of VOCs can be gathered in two important groups: anthropogenic sources and biogenic sources. Within the anthropogenic sources, the main ones are transportation, gas, bio-fuel, industrial and domestic solvents, house painting, glue and emissions from wastes (waste water and solid waste) (Wei et al., 2008). Anthropogenic sources contribute up to 10% of the total emissions of VOCs in the atmosphere. Emissions from biogenic sources contribute to the rest (Atkinson, 2000). Biogenic VOCs (bVOCs) are released from plants, litter, forests, animals, soil, and microorganisms. The complexity of studying bVOCs is also related to the difficulty to discriminating sources in a very complex system such as the biosphere.

It is important to underline that even if methane can be defined as VOC, the dynamics and the properties of this compound are not similar to the other compounds of the family. For this reason, generally, methane is not taken into account in VOCs studies. Therefore, in this project, we will talk about non-methane VOCs (NMVOCs). For simplicity, we will use the term VOCs for NMVOCs in this manuscript.

I.2 The regulatory context

I.2.1 VOCs affect human health and crop productions

Volatile organic compounds (VOCs) are an essential component of atmospheric chemistry that contributes to the production of pollutant harmful to the human health and the environment: ozone (O3) and secondary organic aerosols (SOA). It has been widely demonstrated that the increase in the atmosphere of O₃ (Lippmann, 1993) and SOA (Davidson et al., 2005) have significant consequences on human health and crop production. For instance, the tropospheric concentration of O₃ in 2050 has been estimated to increase by 23% (Ehhalt et al., 2001, IPCC) with a consequence of a 17% decrease on soybean crop production (Morgan et al., 2006). With the global human population currently over 6 billion and expected to reach 8 billion by 2050 (Lutz et al., 2001), assessing the impact of changing atmospheric O_3 concentration on crops is crucial to maintaining food security. Moreover, in Europe, the consequences of the SOA concentration in the atmosphere on human health has been reported as 42000 premature deaths in 2000 (CAFE-AEAT/ED51014/Baseline Issue 5, 2005) while O_3 incease in the troposphere caused 21,000 premature deaths and 14,000 respiratory diseases in Europe (EU25) in 2000 (Enarson, 2013). The worrying scenarios just described led to the need for law enforcement measures to reduce the impact of O₃ and SOA. Since VOCs are precursors of the SOA and O_3 they represent a source of pollution as well.

I.2.2 The regulatory context

The regulatory context in France regarding VOCs emissions directly is not very restrictive. The most important regulations, resulting from the 1999 Gothenburg Protocol (ratified by France in 2007 and modified in 2012), concerned the limitation of VOCs emissions from industries and of the VOCs concentration in consumer products. However, fine particles (PM 2.5) are subject to very restrictive regulations. France must ratify the limitation imposed by the European "air quality" directive (2008/50/EC). To achieve these objectives, France has implemented the plan to reduce emissions of atmospheric pollutants (PREPA, established in 2003) supplemented by the Particulate Matter Plan (2010). The plan has the purpose of reducing particulate matter and is complemented at a local level by atmospheric protection plans and regional climate-air-energy schemes.

I.3 The role of VOCs in air pollution and climate change

I.3.1 The atmosphere composition

The atmosphere is divided into four layers of varying thickness. The boundaries between these layers have been set according to temperature discontinuities and altitude (**Figure I-1**). From the Earth's surface, the atmosphere is composed of the troposphere (0-10 km), stratosphere (12-50 km), mesosphere (50-80 km) and thermosphere (above 90 km altitude). The O₃ layer is found within the stratosphere. O₃ layer absorbs high-energy ultraviolet (UV) (λ <290 nm) light from the Sun, converting the UV energy into heat. The troposphere is the densest layer and contains up to 75% of the mass of the atmosphere. The troposphere is composed of 78% N₂, 21% O₂, 1% Ar, a varying concentration of water vapour, CO₂ (between 350 and 450 ppm), and trace gases. The presence of O₃ in the troposphere is due to the net downward of O₃ by eddy diffusion from the stratosphere and to the interaction of VOCs, NO_x, and sunlight (Roelofs and Lelieveld, 1997). Furthermore, within the troposphere, there is as a suspension of fine solid or liquid particles named particulate matter or aerosols. To our knowledge, all reactions involving VOCs occurs within the troposphere.



Figure I-1. Layers of the atmosphere (Source: https://courses.lumenlearning.com/geophysical/chapter/layers-of-the-atmosphere/).

I.3.2 Tropospheric air pollution

Air pollution is defined as a condition in which substances resulting from anthropogenic activities have tropospheric concentrations higher than a certain threshold, and that has measurable and undesirable effects on humans, animals, vegetation, or more generally on ecosystems. VOCs contribute to air pollution through several cycles where they interact with other elements within the troposphere. The purpose of this paragraph is to describe the different cycles producing harmful air pollutants that involve VOCs.

I.3.2.1 VOCs lifetime

VOCs compounds range from low to highly reactive molecules. Once in the atmosphere, their lifetime can be quite short. **Table I-1** reports the lifetime of the most abundant VOCs in the atmosphere due to the reaction with OH, NO₃ radicals, O₃ and as a result of photolysis. We see in **Table I-1** that while terpenoids and acetaldehyde are very reactive with OH and NO₃ and have lifetimes smaller than a few hours, acetone and methanol are quite stable with regards to reaction with OH, NO₃, and O₃. We should, however, stress that methanol and acetone are very soluble gases and can readily be adsorbed to wet surfaces including cloud droplets.

		Life time due to			
VOCs	Biogenic Emissions TgC yr ⁻¹ ± sd	OH ¹	NO ₃ ²	O ₃ ³	Photolysis ⁴
Isoprene	594 ± 34	1.4h	50 min	1.3 day	
Monoterpenes	95 ± 3	2.7h	5 min	1.9h	
Acetone	37 ± 1	53 day	>11 years		~60 day
Acetaldehyde	19 ± 1	8.8 h	17 days	>4.5 years	6 day
Methanol	130 ± 4	12 day	1 year		

Table I-1. Lifetime of the most abundant VOCs in the atmosphere reacting with OH radical, NO₃ radical O₃ and due to the photolysis. NA= not available, sd=standard deviation (Atkinson, 2000; Guenther et al., 2006; Sindelarova et al., 2014).

 $^{^{1}}$ For a 12-h daytime average OH radical concentration of 2.0 x 10^{6} molecule cm⁻³

 $^{^{2}}$ For a 12-h nighttime average NO₃ radical concentration of 5.0 x 10⁸ molecule cm⁻³

³ For a 24-h average O₃ concentration of 7.0 x 10¹¹ molecule cm⁻³

⁴ For overhead sun

I.3.2.2 The role of VOCs in ozone formation

The key reactive species in the troposphere is the hydroxyl radical (OH). Since it does not react with O_2 , its lifetime in the troposphere is long enough to allow the reaction with all organic molecules excluding chlorofluorocarbons and halons not containing H atoms. OH radical is the primary oxidizing species in the troposphere. The following equations describe the tropospheric mechanisms leading to OH formation.

Thanks to the photolysis of O_3 excited singlet oxygen, O (¹D), or ground states O are formed:

a) $0_3 + hv \to 0_2 + O({}^1D)$

or

b) $O_3 + h\upsilon \rightarrow O_2 + O_3$

The ground state O combines with O_2 to form O_3 :

$$O_2 + O + M \rightarrow O_3 + M$$
 (Equation 2)

The equation 1b and 2 lead to no O_3 production. Moreover, O (¹D) most often reacts with N_2 or O_2 removing the excited state of the singlet and becoming a ground state O.

$$O(^{1}D) + M \rightarrow O + M$$
 (Equation 3)

So most of the time null cycles are produced (Eqns 1b and 2 or 1a, 3 and 2). However, occasionally, the excited singlet $O(^{1}D)$ collides with a water molecule producing 2 OH:

$$O(^{-1}D) + H_2O \rightarrow 2OH$$
 (Equation 4)

The OH radical is involved in the formation of O_3 by reacting with VOCs. Two possible pathways for the O_3 formation exist: the NO_x reactions with O_x and the NO_x reaction with O_x in the presence of VOCs, both are represented in **Figure I-** 2. The equilibrium of the reactions between NO_x and O_x species do not lead to a net formation of O₃ (**Figure I-** 2**a**). However, the presence of VOCs produces a shift in the equilibrium of the O₃ cycle as represented in

(Equation 1)

Figure I- 2**b**. The degradation reactions of VOCs start with the oxidation of the VOCs by the OH radical, leading to the formation of RO_2 and HO_2 radicals as intermediate products (**Figure** I- 2**b**). The intermediate products RO_2 and HO_2 convert NO in NO₂ following the same scheme as in **Figure I-** 2**a** (Atkinson, 2000). Hence, the result of this second pathway is a net formation of O_3 in the atmosphere.



Figure I- 2. (a) Reactions involving NOx and Ox in the ozone formation. (b) Reactions between NOx and Ox in the presence of VOC (Atkinson, 2000).

Net photochemical formation of O_3 also depends on the VOC/NO_x ratio. There is strong competition for the OH radical between VOCs and NO_x. A rate of VOCs/NOx of 5.5/1 leads to an equal reaction rate of VOCs and NO_x with OH. If the concentration of NO_x in the atmosphere increases and the VOC/NO_x ratio becomes lower than 5.5/1, the reaction between OH and NO_x is predominant. The consequence is that OH radical reacts less in the VOCs oxidation cycle (since there is less availability of OH radical due to the higher rate of the OH-NO_x reaction), reducing the O₃ production. However, if the VOC/NO_x rate is higher than 5.5/1 the OH-VOCs reactions are predominant and the production of O₃ increases. In conclusion, VOCs are not only involved in the tropospheric ozone production but they are able to shift the balance of the null cycle described in **Figure 2a** leading to a net O₃ formation (Seinfeld and Pandis, 2016)

I.3.2.3 VOCs as precursor of particulate matter (or secondary organic aerosol – SOA)

Within the troposphere, we can find aerosol particles. These aerosols play a crucial role in many atmospheric processes, both regarding climate and atmospheric reactivity. Aerosol particles are classified into two categories, according to their formations: (i) primary particles, which are emitted directly into the atmosphere in solid or liquid form and (ii) secondary particles, which are formed by reactions between precursors in the atmosphere. According to their size, particles are classified into three categories (Figure I-3): ultrafine particles, having an aerodynamic diameter less than 0.01 µm; fine particles, having an aerodynamic diameter between 0.01 and 1 µm; large particles, with an aerodynamic diameter greater than 2.5 µm. Particle nucleation from gaseous precursors is the base mechanism of the formation of new atmospheric particles. The size of the particles formed is in the order of 10^{-3} to $10^{-2} \,\mu\text{m}$. The lifetime of these particles is usually less than five hours. These particles will quickly increase in size to give birth to fine particles by coagulation or condensation processes (Figure I-3). They are still too small to sediment and their lifetime in the atmosphere is about a few days. They are eliminated mainly by washing or by incorporation in liquid droplets. Largest particles are mainly emitted by soil erosion, as well as by plants (pollens and spores). They are generally removed by sedimentation.



Figure I-3. Production, growth, and removal of atmospheric aerosols. (Jacob, 2000)

VOCs oxidation in the troposphere leads to the formation of lower volatile organic products. The addition of oxygen and nitrogen to organic compounds reduce their volatility

and so do the addition of alcohol, aldehydes, ketone, and nitro groups; in these cases, the volatility can be reduced by several orders of magnitude. These semi-volatile organic compounds can efficiently initiate particle formation (Lee et al., 2006). The reactions explained in paragraph 2.1 between VOC, OH and NOx can potentially all lead to the secondary organic aerosol formation. The ability of a VOC to form SOA depends on its concentration in the atmosphere, its chemical reactivity and on the volatility of its oxidation products (Williams and Koppmann, 2007).

I.3.2.3 VOCs effects on air quality and climate change

It has been widely demonstrated that high ozone concentration in the troposphere, SOA formation and the methane lifetime augmentation due to the presence of VOCs (William et al. 2013) all contribute significantly to the earth radiating balance. Hence, VOCs emissions are directly and indirectly involved in climate change as well as air pollution and thus on human and ecosystem health. All the reactions involving VOCs emissions explained in paragraph I.3 have different consequences on air quality.

First, tropospheric O_3 is a greenhouse gas which anthropogenic origin accounts for 0.25 W m⁻² (IPCC 5th report). Furthermore, several consequences on human health have been reported. O_3 in the troposphere also leads to adverse health effects such as throat and eye irritation, headache, fatigue, and nausea (Lippmann, 1993).

SOA are precursor of cloud condensation nuclei $(CCN)^5$, responsible for scattering and absorbing solar and terrestrial radiation. The formation of clouds influences the radiation budget of the earth by absorbing direct solar radiations leading to a cooling effect (IPCC 5th report). Aerosols also scatter solar radiation leading to an increased diffusive radiation which can increase CO₂ fixation by canopies (Niyogi, 2004). Consequences of PM2.5 (particulate matter with a diameter smaller than 2.5 μ m) on human health have been reported. These small particles can irritate the respiratory system and promote respiratory diseases responsible for increased mortality (Atkinson et al., 2014).

At first, VOCs emissions were studied as compounds emitted from anthropological activities. During the last decades, literature underlined the importance of the biological

 $^{^{5}}$ Cloud condensation nuclei (CCN) are small particles (0.2 μ m) on which water vapor condenses and they are key players on clouds formation.

sources of VOCs, which contribute up to 90% of the total emission rate. Next paragraph will be focused on the description of the biogenic type of VOCs emissions in the atmosphere.

I.4 Biogenic Volatile Organic Compounds

The biosphere produces biogenic gases including volatile compounds other than carbon monoxide, carbon dioxide, and methane, called biogenic Volatile Organic Compounds (bVOCs). This classification allows differentiating the VOCs released from biogenic sources to the anthropogenic ones. BVOCs emissions represent the largest VOC source with an estimated annual emissions amount of 760 Tg (C) yr⁻¹. If we consider that global total carbon monoxide emissions (CO) is 90 Tg (C) yr⁻¹, we can notice that the impact of the VOCs emissions in the atmosphere is between 8 and 9 times higher than CO emissions (Sindelarova et al., 2014). Nevertheless, bVOCs fluxes contribute up to 5-10% of the total net carbon exchange between the biosphere and the atmosphere (Peñuelas, 2003). Chemical compounds included in the BVOCs family are: isoprenoids such as isoprene (contributing up to 69% total BVOCs emissions) and monoterpenes (contributing up to 11% total BVOCs emissions), methanol (6%), acetone (3%), sesquiterpenes (2.5%) and other BVOC species each contributing less than 2% (Sindelarova et al., 2014).

I.4.1 Main sources and sinks

The predominant source of biogenic VOCs emissions is the terrestrial vegetation, which includes plant, forests, and grassland as well as anthropogenically induced vegetation such as crops. Forests contribute up to 55% of the total bVOCs emissions (Karl et al., 2009), while crops contribute up to 27% in Europe (Karl et al., 2009). Another bVOCs source is the ocean. The total bVOCs emissions estimation from oceans is between 37 and 148 Tg y⁻¹ (Abbatt, 2000). Oceans can be considered as sinks as well; they have the capacity to store acetone and methanol with an estimated deposition rate of 0.10 cm s⁻¹ and 0.08 cm s⁻¹ respectively (Singh et al., 2003). Soil and microorganisms are considered as sources and sinks as well, and since they are the object of the entire manuscript, a detailed description of the VOCs emissions from those sources will be given in chapter I.V.

I.4.2 Feedbacks effects of bVOC on climate

Lately, bVOCs gained attention because of their pivotal role in climate changes. In the past, bVOCs were precluded from having a significant role on climate change because of their short lifetime in the atmosphere. It is now clear that the increase of bVOCs due to the warming and global change have direct and indirect effects on the greenhouse gas budgets (Peñuelas and Staudt, 2010). Indeed, stresses linked to climate changes (i.e., heat and drought) or other type of environmental stresses (i.e., insect infestations) affect the composition and quality of the VOCs emissions. For instance, Zhao et al., 2017, studied the type of VOCs emissions emitted from a boral forest under unstressed and stressed conditions. VOCs emissions from unstressed condition were dominated by isoprene and monoterpenes. Isoprene and monoterpenes were found to affect the size of the SOA particles (Figure I-4). Under stressed condition, VOCs emissions were dominated by sesquiterpenes. Sesquiterpenes were found to influence the higroscopicity of the SOA particles. The size and the higroscopicity of the SOA particles are crucial factors on the cloud condensation nuclei (CCN) formation. This study concluded that the CCN formation by SOA due to changes in biogenic VOC emissions, will influence cloud formation, and ultimately impact climate (Figure I-5). (Zhao et al., 2017).



Figure I-6. Interactions between environmental conditions, biogenic volatile organic compounds emissions, secondary organic aerosol, cloud formation and climate as potential feedback loops. VOC constitutive (unstressed conditions) = higher emission rate of isoprene and monoterpenes, VOC induced (stressed conditions) = higher emission rate of sesquiterpenes, k=hygroscopicity (Zhao et al., 2017).

Biogenic VOCs are also involved in the reactions explained in paragraph 3.2.2 leading to the ozone formation. Furthermore, bVOCs react with the ozone thus contributing to its consumption as well. Nevertheless, the net formation of O_3 depends mainly on the VOC/NOx ratio (see paragraph 3.2.2 for more details). Besides, bVOCs emissions are likely to indirectly increase other greenhouse gasses (i.e., methane) impoverishing OH radical concentration in the atmosphere. OH radicals are very reactive oxidants and act as the primary cleansing agent for the atmosphere (Lelieveld et al., 2008).

Several studies have been focused on bVOCs emissions by plants and forests. These sources of VOCs are considered the most important because of the higher VOCs flux emitted (Kesselmeier and Staudt, 1999). Recently, other bVOCs sources gained attention because of their possible underestimated contribution on the total bVOCs emissions (Bachy et al., 2016). Those sources are soil and microorganisms, and they will be detailed in the following paragraphs.

I.5 Soil as source and sink of VOCs

Soil emits VOCs between 1-2 orders of magnitude less than vegetation. BVOCs from soil are the result of multiple abiotic and biotic processes. Biotic processes leading to the bVOCs emissions in soils are the decomposition of litter and dead organic material, and the metabolism of microorganisms living in soil and on roots. Furthermore, VOCs mediate the interactions between organisms living in the soil. An overview of the interactions mediated by VOCs in soil is represented in **Figure I-7**. Since VOCs are often the result of complex interactions, understanding the processes leading to their emissions is challenging. For example, root exudate and litter increase the microbial activity in soil; this can lead to a shift in the consumption or production of VOCs in soil (Rinnan et al., 2013). Moreover, it was shown that other abiotic factors affect the VOCs emissions from soil such as water content, nutrient availability and temperature (Asensio et al., 2007b).



Figure I-7. Illustration of the complexity of VOCs exchanges in soil. rVOCs = emissions from plants roots, mVOCs = emissions from microorganisms, fVOCs = fungi VOCs emissions. Red lines indicate negative effects (like inhibition of growth, toxicity), while positive effects are indicated by the green arrows (i.e. growth promotion). Blue lines represent the flux of VOCs emitted by soil (reproduced from Peñuelas et al., 2014).

I.5.1 Abiotic factors affecting soil-atmosphere VOCs exchange

VOC fluxes, even if biotic sources produce them, are dependent on abiotic factors affecting the concentration gradient between the soil and the atmosphere. VOC transfer in the soil indeed follows molecular diffusion mechanisms driven by the concentration gradient, which leads to a flux directed from high concentrated zone to a less concentrated one. However, VOCs transfer in soils is also affected by adsorption mechanisms onto water, and the mineral and organic fraction of soil. The VOCs equilibrium between liquid and gaseous phase in soil follows the Henry's law: in thermodynamical equilibrium, the gas quantity dissolved in the liquid phase is proportional to the pressure of the VOCs on the liquid surface. The concentration gradient depends on soil porosity, pore water content, organic carbon fraction, pH, soil particles distribution depending on the type of soil (Uteau et al., 2013) and is finally affected by the chemical properties of the VOC (Williams et al., 1996). Another

important factor is the soil organic matter, which, changing the soil porosity, affects the gas diffusivity (Hamamoto et al., 2012). Besides, high water content in pores boosts the emissions of non-soluble VOCs, while, in dry conditions, soluble VOCs will mainly be retained (Provoost et al., 2011). The percolation threshold which represents the air content in soil at which diffusive gas transport decrease due to water pore blockage, is known to be higher in organic soils than in mineral⁶ ones (Freijer, 1994). Soil properties and soil composition also affect the VOCs emissions and absorption in soil. Ruiz et al. (1998) studied the absorbing VOCs potential of clay, sand, and limestone. This study demonstrated that for the 8 VOCs analyzed (n-hexane, n-heptane, n-octane, toluene, p-xylene, m-xylene, ethylbenzene, and methyl ethyl ketone) clay absorbed a higher rate of VOCs. Approximatively, clay absorbs an order of magnitude more than sand and two orders of magnitude more than limestone (Ruiz et al., 1998).

Furthermore, Serrano and Gallego, (2006) studied the absorption of 25 VOCs compounds in alkaline and acid agricultural soils. They reported a higher absorption in alkaline soils than acid ones and a positive correlation between the content of organic carbon in soil and the absorption of VOCs for alkaline soil while for the acid ones VOCs absorption decreases with organic carbon content.

I.6 Microorganisms living in soil: structure and functions

The soil is a highly complex, heterogeneous, and nutrient-limited habitat consisting of a mixed organic-mineral matrix with liquid and gaseous pores, owning the highest microbial diversity on earth (Jangid et al., 2010). The range of microbial biomass in soil goes from 1 T ha⁻¹ to 10 T ha⁻¹ representing a considerable fraction of the biomass on Earth (Fierer et al., 2007). The diversity of terrestrial microbial communities is complex and variable at different levels of biological organization. It includes genetic variability within taxa (species), number (richness) and relative abundance of taxa (evenness) as well as functional groups within the community (Torsvik and Øvreås, 2002). Microorganisms colonizing soil are bacteria, archaea, nematodes, protozoa, and fungi. Besides, it has been estimated that only a very small fraction

⁶ Soils with organic content < 3 % are defined *mineral soils*, soils with organic content >3% and <15%, are classified as *mineral soils with organics*; when the organic content exceeds 15% but < 30% they are called *organic soils*. (Huang et al., 2009)

of the microorganisms colonizing soil is known. For bacteria and fungi we know 13000 and between 18000-35000 species respectively which correspond to 1-2 % of the total estimated species (Barrios, 2007).

Microorganisms are especially abundant in the narrow region of soil attached to plant roots, the rhizosphere. They are essential in soil ecosystem due to their essential role in all ecosystem functions and biogeochemical processes in soil (Baumann et al., 2013; Falkowski et al., 2008). The ecosystem services related to soil functioning driven by microorganisms are listed in **Table** I-2.

Microbial group	Process	Ecosystem service	Ecosystem service category
HETEROTROPHIC BACTERIA/ARCHAEA	Organic matter breakdown, mineralization	Decomposition, nutrient recycling, climate regulation, water purification	Supporting and regulating
PHOTOAUTOTROPHIC BACTERIA	Photosynthesis	Primary production, carbon sequestration	Supporting and regulating
CHEMO(LITHO)AUTOTROP HIC	Specific elemental transformations (e.g., NH+4, S−2, Fe+2,CH₄oxidati on)	Nutrient recycling, climate regulation, water purification	Supporting and regulating
UNICELLULAR PHYTOPLANKTON	Photosynthesis	Primary production, carbon sequestration	Supporting and regulating
ARCHAEA	Specific elemental transformation (e.g., metals, CH ₄ formation, NH ₄ * oxidation), often in extreme habitats.	Nutrient recycling, climate regulation, carbon sequestration	Supporting and regulating
PROTOZOA	Mineralization of other microbes	Decomposition, nutrient recycling, soil formation	Supporting
FUNGI	Organic matter breakdown and mineralization	Decomposition, nutrient recycling, soil formation, primary production (i.e., mycorrhizal fungi)	Supporting
VIRUSES	Lysis of hosts	Nutrient recycling	Supporting
ALL	Production of metabolites (e.g., antibiotics, polymers), degradation of xenobiotics, genetic transformation, and rearrangement	Production of precursors to industrial and pharmaceutical products	Provisional
ALL	Enormous diversity, versatility, environmental and biotechnological applications	Educational purposes, getting students interested in science	Cultural

Table I-2. Ecosystem services and major functions of the microorganisms living in soil (Bodelier,2011).

As **Table** I-2 shows, different microorganisms in soil can accomplish the same task. Species colonizing an ecosystem and contributing equally to an ecosystem function can be substituted by another. This characteristic is named functional redundancy. At first, functional redundancy gave less importance to the preservation of microbial diversity in soils since the soil functions remained constant even if erosion of microbial diversity due to environmental stresses were noticed (Chapin et al., 1997).

However, it has been reported that microbial species, surviving a perturbation, and which supposed to have the same ability to perform a function, might not have the same competitive ability or growth rate as the original community members (Riah-Anglet et al., 2015). Furthermore, the new community shaped after a perturbation might not be able to perform the functions with the same efficiency, or finally, they might perturb directly or indirectly the activity of other population in the community (Riah-Anglet et al., 2015).

I.6.1 Factors affecting microbial diversity in soil

Generally, high-input agricultural practices decrease microbial biodiversity while the low-input practices enhance microbial diversity in soil (Girvan et al., 2003; Munyanziza et al., 1997). Lupwayi et al., (2001) has given an example recording higher microbial diversity on soils under conventional tillage than zero tillage. Furthermore, Wolińska et al., (2017), compared cultivate soils with non-cultivated soil reporting a decrease of the microbial diversity in soil up to 30% in the cultivated soil. Monoculture agricultural practice also reduced bacteria biodiversity in soil, while fungi biodiversity seems to not be affected (Liu et al., 2014). Soil microbial diversity decrease with increasing latitude and correlate positively with measures of atmospheric temperature and pH (Staddon et al., 1998). Soil microbial diversity may be lower in northern sites due to decreased productivity, nutrient limitation, and higher acidity. The diversity and abundance of soil bacteria and fungi are also reduced in the arid lands (Maestre et al., 2015).

I.6.2 Microorganisms and biogeochemical cycles in soil

The survival of plants and animal life depend on the microorganisms' activity because of their implication on the biogeochemical cycles of carbon, nitrogen, sulfur, and other elements. In soil, microorganisms are involved on the humus formation, decomposition of plant litter and thus, energy production. An example of the roles played by microorganisms in soil is given by the description of the nitrogen cycle (**Figure I-8**). The nitrogen cycle is mostly driven by microorganisms: nitrogen-fixing bacteria can transform atmospheric nitrogen (N_2) in ammonium by fixation and ammonification processes. Secondly, nitrifying bacteria transform ammonia in nitrites and nitrates, which are assimilated by the plant in order to build

proteins, amino acids, RNA and DNA, and may lead to NO emissions. Finally, denitrifying bacteria use nitrate for the oxidation process releasing N_2 in the atmosphere, but also leading to N_2O emissions.

Moreover, the mineralization of organic matter, a central process of the carbon cycle, is mostly carried out by heterotrophic soil microorganisms which reduce complex molecules into smaller molecules, easily assimilated by plants. Due to their metabolic plasticity, soil microorganisms are also involved in the degradation and immobilization of pollutants (i.e., pesticides) brought from the agricultural or industrial environment. Some microorganisms have also got a significant impact on plant health and growth by creating symbioses (Barrios, 2007) or by inducing diseases. Other microorganisms are involved in the formation and stability of soil aggregates through the production of binding agents or the establishment of physical bonds made by the hyphae of fungi (Six et al., 2002).

All the metabolic processes leading to the transformation of the elements in soil, such as the nitrogen and carbon cycles just described, can release VOCs (Schmidt et al., 2015). Moreover, in a complex system such as the soil, VOCs are also used to communicate or more in general for intraspecific and interspecific interactions (Tyc et al., 2017). In the following paragraphs, the description of possible metabolic processes leading to the production of VOCs is given.



Figure I-8. Nitrogen cycle (source: EarthLabs:Climate and the Carbon Cycle)

I.6.3 Biosynthesis of VOCs from microorganisms

Microbial VOCs can be released from primary metabolism in order to produce energy or in the secondary metabolism implicated in the communication and defense mechanisms. In this paragraph, we will describe the primary metabolisms leading to VOCs production.

VOCs production from **sugar degradation** is mainly released from these three pathways: Embden-Meyerhof, Entner-Doudoroff and heterolactic/homolactic pathway. During sugar degradation, several precursor compounds of the VOCs production are formed: glyceraldehyde-3-phosphate, pyruvate, lactate, acetate, and CO₂ are among those. From the compounds listed above (except CO₂) *Saccharomyces* and a few more bacteria can synthesize ethanol (Sniegowski et al., 2002). *Bacillus* species can produce 2,3-Butanediol and acetoin (Ryu et al., 2003) while *Clostridium* species produce butanol and acetone from the fermentation process (Smith, 1975). Microorganisms in soil need to degrade the organic matter in order to produce energy. When the **biodegradation of the soil organic matter** is incomplete, several VOCs, as intermediate products of the degradation, are released (Leff and Fierer, 2008). For example, pyruvate is an intermediate product of the **hemicellulose and cellulose degradation** and if the degradation is incomplete higher quantities of ethanol are released (Lamsen and Atsumi, 2012). An essential factor affecting degradation processes is oxygen availability. When anaerobic conditions are present, the alcoholic fermentation becomes the most likely metabolic process.

Another important pathway generating microbial VOCs is the **shikimate pathway** which releases aromatic compounds (Schulz and Dickschat, 2007). The shikimate pathway (Shikimic acid pathway) is a seven-step metabolic route used by bacteria, archaea, fungi, algae, and plants for the biosynthesis of folates and aromatic amino acids (phenylalanine, tyrosine, and tryptophan). Gosset, (2009) reported that the most emitted VOCs releases from the shikimate pathway are 2-phenylethanol, p-hydroxycinnamic acid, p-hydroxystyrene, and p-hydroxybenzoate.

Bacteria can synthesize sulphur-containing VOCs. An example is given by the lactic bacteria which generate a degradation product, the l-methionine, that leads to the formation of H_2S , dimethyl sulphide (DMS), dimethyl disulphide (DMDS), and methanethiol (Schulz and Dickschat, 2007).

Compounds such as alkenes, aliphatic alcohols, hydrocarbon compounds, and ketones are the result of fatty acid metabolism (Peñuelas et al., 2014). During the **fatty acid biosynthesis**, several transformations occur such as the decarboxylation that leads to alkenes or methyl ketones or the reduction of carboxyl group which generates aldehydes and 1-alkanols (Schulz and Dickschat, 2007).

Microorganisms are also responsible for the production of the VOCs giving the typical earthy odour: Geosmin (terpene) and 2-methyisoborneol. Those compounds are emitted by *actinomycetes myxobacteria* and *cyanobacteria* (Citron et al., 2012). Finally, another class of compounds that is likely to be emitted from microorganisms is monoterpenes. More details concerning the different pathways leading to the VOCs formation are represented in **Figure** I-9.



Figure I-9. Main metabolic pathways for the production of microbial volatiles. VOCs are represented in colored dashed rectangles indicating different chemical classes. Representative examples are given per class: alcohols (ethanol), aldehydes (benzaldehyde), alkanes (undecane), alkenes (1-undecene), aromatic compounds (2-phenylethanol), esters (2-phenylethyl ester), fatty acids (butyric acid), isoprene, lactic acid, lactones (gamma-butyrolactone), methylketones (acetone), monoterpenes (farnesol), nitrogen compounds (benzonitrile), sesquiterpenes (pinene) and sulphur compounds (dimethyl disulphide) (Schmidt et al., 2015).
I.6.4 Interactions mediated by VOCs

Culture-based studies have revealed that even a single bacteria or fungi strain can produce a vast array of secondary metabolites which are not directly involved in the growth, development or reproduction of the microorganism (Tyc et al., 2017). Within those secondary metabolites, we can find VOCs. VOCs, thanks to their physiochemical properties (i.e., small molecules <300 Da, easy diffusion and volatility), are the perfect candidates to mediate the cooperation and the competition between soil microorganisms; even when they are not adjacent to each other. VOCs by microorganisms mediate several functions such as (Kai et al., 2009a; Ryan and Dow, 2008):

- Communication between inter and intra-specific organisms;
- Cell-to-cell signals;
- Growth promoting and inhibiting agents.

In the following paragraph, we will be focused on the VOCs emitted from bacteria and fungi which inhibit or promote growth.

I.6.4.1 VOCs inhibiting and promoting fungi and bacteria growth

For fungi, the exposure to bacterial VOCs can change fungal morphology, enzyme activity and gene expression (Kai et al., 2009a; Vespermann et al., 2007; Wheatley, 2002). Some VOCs have antifungal properties even at low concentrations: trimethylamine, benzaldehyde, and N,N-demethyloctylamine (Chuankun et al., 2004a). Fungi emit several VOCs implied on antibiotic activity. For instance, the emissions profiles of the basidiomycetes *Hypholoma Fasciculare* and *Resinicium Bicolor* change when they are sharing the same space (Hynes et al., 2007). The VOCs emitted while those two species are in contact inhibit mutually the growth of the fungi. Moreover, Chakraborty and Chatterjee, (2008), studied the *Trichoderma* spp. inhibiting activity mediates by antibiotic VOCs versus pathogenic fungi (i.e., *Fusarium solani*) on plants. The antibiotic VOCs released by *Trichoderma* spp. can reduce the pathogenic fungi colonization on plants up to 75% (Chakraborty and Chatterjee, 2008).

VOCs which mediate bacteria-to-bacteria interactions can inhibit the growth of other bacteria species. For instance, *Veillonella* and *Bacteroides fragilis* inhibit the growth of

enteropathogenic bacteria such as *Escherichia coli*, *Salmonella*, *Shigella* and *Yersinia* (Wrigley, 2004). A widespread VOCs produced among bacteria with a strong growth inhibitor power is Indole. Indole is generated through the degradation of tryptophan by tryptophanase enzyme and inhibits biofilm formation in *E. coli*, *P. fluorescens*, and *P. aeruginosa*. Other studies as Kai et al., 2009a reported that the typical red colony coloration of the *B. cepacia* species diminishes on exposure to volatiles from *S. odorifera* and *Serratia plymuthica*, showing that volatiles of bacteria can influence the metabolism of other bacteria. Another example is the release of volatile short chain fatty acids from *Veilonella* species and *Bacteroides fragilis*, which thus control the growth of the enteropathogens *Salmonella typhimurium*, *Salmonella enteritidis*, *E. coli*, and *Pseudomonas aeroginosa* (Hinton and Hume, 1995). Finally, positive interactions between bacteria and fungi have been reported. Wheatley, (2002) reported that bacteria VOCs emissions are able to stimulate fungi growth by up to 35 %.

I.7 Organic Waste Products (OWPs)

Organic waste products are defined as the exogenous organic matter applied in soils with the aim of improving fertilization and soil structure. OWPs include all the organic wastes deriving from agricultural (i.e. crops residues), urban and industrial activities (Van-Camp et al., 2004). In Europe every year about 1.6 billions tons of OWPs are produced: 61% comes from animal residues, 25% comes from crops residues, 7% comes from municipal wastes and finally 7 % comes from industrial wastes. In France, the majority of the annual production of OWPs is derived from manure (300 Mt, ADEME, 2006), and the 50% of this production is returned to the soil. Regarding municipal and industrial OWPs France produces about 32 Mt (ADEME, 2006). In this case, about 40% are recycled as OWP amendment to soil (ADEME, 2006). In this study four different organic waste products were taken into account:

- **Municipal solid waste compost** (MSW) made from residual municipal waste after the selective collection of dry and clean packaging;
- **Biowaste compost** (BIOW) made from selectively collected fermentable fraction of municipal wasted co-composted with green wastes;

- Green waste and sludge (GWS) a compost resulting from the co-composting of sewage sludge, green wastes, and wood chips;
- Farmyard manure (FYM) obtained from a dairy farm.

All those OWPs are the result of a composting process leading to a monitored transformation of the organic waste products driven by the microbial community. The final products are rich in stabilized organic products, humic compounds, and nutrients (ITAB, 2002).

I.7.1 Effects of organic waste products amendment on soil organic matter and microbial communities

The intensive agricultural practices widely used in agricultural land contribute to a decrease in soil organic matter content with negative consequences on soil fertility. To contrast, this trend the recycling of organic waste products (OWPs) have been widely encouraged in Europe (European Commission, 2010) especially for cropped soils. In fact, OWPs improve soil fertility and contribute to carbon storage in soil by improving soil porosity, aggregation, bulk density and structure stability (Abiven et al., 2018). Furthermore, OWPs represent a potential source of nutrients (N, P, K...) for crops that can partially substitute the use of mineral fertilizers (Chalhoub et al., 2013). In addition to positive effects on soil fertility, OWPs application improves soil biodiversity and biological activities (García-Gil et al., 2007). OWPs also impact on chemical fertility through the pH regulation, as well as increase CEC (Diacono and Montemurro, 2010). The effects of the OWP in soil have been investigated on long-term and short-term experiments. In the following paragraphs, an elucidation of the effects on microbial community and on the organic matter of both type of experiment will be given.

I.7.1.1 Long-term effects

Repeated fertilizations over several years are a commonly used practice under field conditions to maintain soil fertility and thus crop yields. It has been also observed that compared to punctual organic fertilization, long-term OWP applications had more persistent impacts on soil characteristics (Obriot et al., 2016), plant growth (Clark et al., 2007), and microbial diversity and activity (Francioli et al., 2016; Giacometti et al., 2014). Studies focusing on the impact on soil microbial community demonstrated an increase of soil

microbial biomass with recurrent OWP amendment (Sadet-Bourgeteau et al., 2018). However, the response of other soil microbial community parameters such as diversity, composition, and structure seemed to depend on the time between the OWP application and soil sampling and the number of years of application. In fact, for applications shorter than 6 years no changes in soil microbial community structure and composition were observed (Poulsen et al., 2013). On the other hand, an increase of soil bacterial and fungal diversity and stimulation of some microbial groups such as *Firmicutes*, *Proteobacteria*, and *Zygomycota* were observed after more than 20 years of OWP application (Francioli et al., 2016). Others parameters, such as the quantity and the type of OWPs applied, seemed to modify the impact of the microbial communities in soil (Diacono and Montemurro, 2010; Sadet-Bourgeteau et al., 2018).

Long term application of OWPs in soil have also negatives effects. OWPs may contain contaminants such as pathogens, organic contaminants or trace elements including metals (Belon et al., 2012). Those contaminants are accumulated in soil leading to a possible transfer to plant and water (Cambier et al., 2014). Additional negative impacts are the decrease of the pH that leads to less O_2 availability in soil, or an excessive input of nutrients due to simultaneous N and P addition (Obriot et al., 2016). These impacts on soil are strictly related to the quality of the organic amendment, the applied doses, the frequency of the application and the cropping system.

I.7.1.2 Short-term effects

Short-term effects of OWP amendments to soils were investigated by Leroy et al., (2008) who studied aggregate stability, hydraulic conductivity, and on the OC in soil organic matter (SOM) fractions. Their study reported an increase of the aggregate stability and hydraulic conductivity after less than 1 year from the first OWP application. The OWP applications also improved the amount of OC in all the fractions of the SOM (Leroy et al., 2008). Federici et al., (2017a) reported the same results as Leroy et al., (2008) after only 120 days from the OWP application. These results showed that OWPs have pronounced effects on soil physical properties starting from the first year of application. Concerning the microbial community, after three days only, all OWPs induced rapid modifications of both fungal and bacterial communities (Federici et al., 2017a). However, Federici et al., (2017a) also reported that while the bacterial community restored to its initial state after 120 days, suggesting their high resilience capacity, the fungal community changes remained modified after this period.

I.7.2 Effects of the organic waste products amendment on the VOCs emissions from soil

Organic waste products affect soil biological and chemical parameters. Changes in soil characteristics also affect the VOCs emissions. It has been reported for instance that moisture content, pH and temperature leads to variation of VOCs flux (Asensio et al., 2007a; Raza et al., 2017a). Due to the complexity of the soil ecosystem, literature does not provide exhaustive information about the dynamics of the VOCs emitted by OWP, despite recent studies have started to fill this lack. For example, Seewald et al. (2010) studied VOCs production by adding glucose to soils regularly amended by different composts and mineral fertilizers. They also reduced the O_2 availability in order to impair the degradation of the VOCs produced by microorganisms. Results reported by this study showed that organic waste composts and mineral fertilization showed the opposite effect. Moreover, Potard et al. (2017) showed that different soil organic fertilizers such as pig slurry and methanized pig slurry impacted on a different way soil VOCs emissions: pig slurry released double quantities of VOCs while methanized pig slurry emitted even less than the unamended samples. This is as expected since methanization tends to drastically reduce the carbon content of the slurry.

Woodbury et al., (2016) reported an emission of volatile sulfurs compounds after the application of beef cattle manure. Contrasting type of VOCs emissions from soil have been reported from several studies. For instance, in Mediterranean soils the most emitted compound was methanol (Asensio et al., 2007a) while in soil amended with straw it was acetone (Zhao et al., 2016). In fact, emissions of VOCs are strongly related to the substrate quality and it is important to note that even small variations in nutrient composition may change the type and the amount of individual VOCs produced (Wheatley, 2002). It is also important to bear in mind that a significant proportion of the VOCs are produced by microorganisms (Isidorov and Jdanova, 2002). Hence, since the microbial communities, as explained in previous paragraphs, are widely altered by the OWP amendment (Obriot et al., 2016; Sadet-Bourgeteau et al., 2018) VOCs are also altered.

Objectives

During these three years, my work has focused on the characterization of VOCs emissions from soil. In particular, the aim was to characterize, in terms of composition and quantities, the VOCs emitted from soils amended with different type of organic waste products and to determine the role of the microbial diversity in these emissions. We also looked into VOCs emissions responses to OWPs application in the short term compared to long term by measuring VOCs emissions after 49 h, one and two years following OWPs applications.

This Ph.D. objectives were hence constructed around three main research questions:

- a. Do recurrent amendments of differentiated types of OWPs affect the VOCs emissions by soil in magnitude and composition? Since OWPs amendments affect soil characteristics and microbial composition, we hypothesized that physical and chemical parameters modulated by the application of OWPs (i.e. pH, soil organic matter, total organic carbon...) would induce VOCs emissions changes.
- b. What is the effect of microbial diversity on VOCs emissions composition and magnitude? We further questioned if these changes would affect the OWPs amended soils in the same manner.
- c. How do OWPs amendments affect VOCs emissions and microbial community by soils in the first two days following application? We further tested whether the structure of a microbial community adapted to OWPs amendment would respond differently compared to a microbial community that never received OWPs amendment in the last ~20 years, and whether this changes VOCs emissions.

Chapter II gives an overview of the methods used in this work, while chapters III, IV and V are the core of the work presenting results from three experiments designed to answer the three research questions listed above. Chapter VI is a general discussion that makes the link between these three questions and Chapter VII gives conclusions and perspectives to this work.

Chapter III was dedicated to question *a*. For this purpose, we measured the composition and quantity of VOCs emitted by soils regularly amended with OWPs. Soil samples were taken from the QualiAgro site where soils have received OWPs since 1998. We measured VOCs emissions from soil amended with 4 different OWPs and a control sample that never received organic amendment. Soils were sampled two years after the last application in order to avoid any short term emission effects. In this chapter, we discuss the differences in magnitude and composition of the VOCs emitted and we discuss the links with soil characteristics (pH, organic matter, I_{roc} indicator, cation exchange capacity, carbon/nitrogen ratio, total nitrogen, and organic carbon). Chapter III was published in Science of the Total Environment journal.

The aim of **chapter IV** was to give an answer to question *b*. Soil samples were collected at the same site as the first experiment, one year after the last OWPs application. Soil samples were sterilized and inoculated with three different microbial diversity dilution levels (high, medium and low) to prepare microcosms that were incubated for 6 weeks. VOCs emissions were then measured and the microbial composition characterized. The links between the microbial community structure, as affected by diversity manipulation, and VOCs emissions composition and magnitude were explored.

Chapter V is dedicated to question c. In this experiment, we measured the dynamics of VOC emissions and microbial structure during two days following the fresh application of green waste and sludge (GWS). We further compared the response of a soil recurrently receiving OWPs to soil that had never received OWPs for ~20 years. Addition of GWS to these soils was performed 1h before the detection of VOCs emissions. The VOCs emissions from the microcosms were detected 10 times during 49h after the application of the GWS amendment. We characterized the microbial community structure before and at the end of the 49h. We then discussed the short term VOC emissions and the links with changes in the microbial community.

For all the performed experiments the detection of VOCs was made using the PTR-Qi-TOF-MS technique; while the evolution of the microbial diversity was characterized after the VOCs detection by a high throughput sequencing approach targeting 16S and 18S ribosomal genes. The experimental setups are presented in Chapter II in details. **Table I- 3**, however, summarizes the main items of the three experimental setups underlining this work.

CHAPTERS TITLE	SITE	EXPERIMENTAL CONDITIONS	SOIL MANIPULATION	NUMBER OF SAMPLES (N) AND REPLICATES (R)	MICROBIAL DIVERSITY ANALYSIS	VOCs EMISSIONS ANALYSIS	TYPES OF OWPs	DELAY AFTER THE LAST OWPs APPLICATION	MEASUREMENTS
Chapter III - Profiles of volatile organic compound emissions from soils amended with organic waste products			 Dried; 2mm sieved; 60% WHC 	N=15, R=3		 Total VOCs emissions Most emitted compounds ANOVA Shannon index BCA 	GWS MSW FYM BIOW <i>Vs</i> CN	2 years	VOCs: PTR-QiTOF-MS
Chapter IV - Volatile organic compounds emissions from soils are linked to the loss of microbial diversity	QualiAgro	Dynamic chambers under laboratory conditions	 Dried; 2mm sieved; 60% WHC; 	N=45, R=3	 Microbial Biomass; Ratio F/B; Shannon index; Bacterial and fungi relative abundance 	 Total VOCs emissions Most emitted compounds; ANOVA Shannon index; PCA; 	GWS MSW FYM BIOW <i>Vs</i> CN	l year	VOCs: PTR-QiTOF-MS MICROBIAL COMMUNITY: quantitative PCR and High throughput sequencing
Chapter V - Microbial VOCs dynamics after green waste and sludge amendment	-		 Sterilized; re-inoculated with a soil suspension of the same non-sterilized soil; 6 weeks incubation 	N=18, R=3	 Microbial Biomass; Shannon index; Bacterial abundance. 	 Total VOCs emissions; Most and less emitted compounds; Shannon index; VOCs emissions dynamic along time. 	GWS Vs CN	49h	VOCs: PTR-QiTOF-MS MICROBIAL COMMUNITY: quantitative PCR and High throughput sequencing

 Table I- 3. Summary of the performed experiments. GWS= green waste and sludge, MSW=municipal soil waste, FYM=farmyard and manure, BIOW=biowaste, CN=control without organic input, ratio F/B= ratio Fungi/Bacteria.

Chapter II Materials and methods

II. Materials and methods

II.1 Site description

The experimental site was situated in the Plain of Versailles, at Feucherolles. The site name is QualiAgro and it takes part of the SOERE-PRO-network⁷. SOERE- PRO is a network of experimental field sites for long duration experiments. The aim of the SOERE-PRO network was to observe the effects of organic waste products (OWPs) amendment on different crops. QualiAgro site was subdivided on plots allowing the study of the evolution in a long-term scale of soil, plants, air, and OWPs of the amended ecosystem.

Feucherolles is located in the northwest of France 35km from Paris (48°52'N, 1°57'E, alt 150 m), in the Yvelines department (**Figure II-1**).



Figure II- 1. Localization of the QualiAgro site.

The soil has a loamy-clayey texture and is classified as a hortic glossic Luvisol (IUSS Working Group WRB, 2014), representative of the Parisian Basin. The characteristics of these soils are loam texture of a depth greater than 1.2 m, lack of clay (<15%) initial pH of 6.9 in the surface (0-30 cm), good drainage and it is based on loessic carbonate loam which appears at around 1.60 m. At the beginning of the experiment, in 1998, the site had an organic

⁷ https://www6.inra.fr/qualiagro_eng/Nos-partenaires/The-SOERE-PRO-network

matter content lower than the critical threshold⁸ (1.1 %) (Meersmans et al., 2012), which is not the case anymore, in fact, organic matter content is now around the 2%. The increase of organic matter content is due to the application of OWPs since 1998 at a rate of ~4 tC ha⁻¹ every two years on the wheat stubbles in September, after harvesting. The crop rotation was wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.). The wheat crop residues were exported, whereas the maize residues were returned to the soil. Finally, the site weather station reports an annual average precipitation is 583 mm with 11°C of annual average temperature. Mineral fertilization occurs twice per year for wheat (early March and early April) and once for maize (early May).

II.2 Site structure and sample collection

The QualiAgro site is a 6 ha experiment divided in 4 blocks of 10 plots (40 plots of 10x45 m each). The distance between plots in the same block was 6 m while the distance between plots in different blocks was 25 m, separated by a wide buffer strip (Erreur ! Source du renvoi introuvable.). The experiment was a randomized block design with 4 replicates comparing 4 organic waste products: BIOW (bio-waste compost derived from the co-composting of green wastes and source-separated organic fractions of municipal solid wastes), GWS (compost derived from the co-composting of green wastes and public gardens), FYM (farmyard manure) and MSW (municipal solid waste compost derived from the composting of residual solid wastes after removing dry and clean packaging); plus a control without organic input (CN). **Table II-1** shows the composition of the different organic waste products regarding the dry matter content, the organic carbon, the total N, the organic carbon/total N ratio and the pH.

Furthermore, the OWPs application was crossed with 2 mineral N treatments. 20 field plots were additionally amended with mineral N in order to reach the N optimal quantity in soil. Application of N optimal was reached adding: 140 (\pm 37) kg N ha⁻¹ for wheat and 82 (\pm 34) kg N ha⁻¹ for maize.

Sampling was performed in the ploughed layer (0-30 cm). For each plot, 10 pooled subsamples were collected. The collection for the first experiment was made in September 2015 in the 20 plot receiving the N optimal amendment, 2 years after the application of OWPs.

⁸ Critical threshold of organic content 1.5%

		GWS	BIOW	MWS	FYM
Dry matter (DM)	%	63.3± 8.2	70.1 ± 8.5	67.8 ± 12	39.6 ±9.1
Organic carbon	g kg ⁻¹ DM	265 ±44	208 ± 47	308 ± 45	320 ± 67
Total N	g kg ⁻¹ DM	$23.5{\pm}2.7$	17.4 ± 4.5	17.6 ± 2	$21.9{\pm}~3.1$
Organic carbon/total N		11.4±2.1	12.1±8.5	17.8±4	14.7 ± 2.8
рН		7.5±0.6	8.1±0.5	7.5 ± 0.5	9.1 ± 0.3

Table II-1. Mean characteristics of the OWPs (Obriot et al., 2016).

For the second and the third experiment samples were collected in September 2016, 1 year after the application of OWPs and we only collected samples in the first block receiving the optimal N amendment.



Figure II- 2. Design of the QualiAgro field experiment.

II.3 Samples preparation

After the samples collection for the first experiment, samples of the same plot were mixed, homogenized and dried at room temperature during 2 weeks. The homogenization of the samples has been obtained by passing samples through a 2 mm sieve and removing aboveground plant materials, roots and stones.

For the second and the third experiment, samples were prepared as the first experiment except for the dry up temperature which was 40 °C for three days. Secondly, samples were sent to the sterilization process in order to proceed with the inoculation and thus the creation of the microcosms (see paragraph Microcosms preparation pg. 40).

II.4 Flux chambers

All the experiments described in this work were performed in a laboratory environment. In order to detect the VOCs emissions dynamics chambers were used. The two Pyrex chambers used in the first experiment soil samples had a surface of 106 cm². Pirex chambers allow better detection of the VOCs emitted by soil because of their low interaction with VOCs molecules. Furthermore, the connection between the chambers and the PTR-QiTOF-MS was performed using PEEK tubes which are made of inert material. For the first experiment, 60 g of soil were homogeneously spread in the chamber surface. One chamber was used as a blank (no samples were inserted) in order to detect the VOCs emitted by the empty chamber.

For the second and the third experiment, 30 g of inoculated soil were transferred in a flask (**Figure II- 3**). The flask had a volume of 150 cm³ and a surface of 15 cm². The flask had a caoutchouc plug with two PEEK tubes, one connected with the PTR-QiTOF-MS and the other connected to the synthetic air (**Figure II- 3**). In **Figure II- 4** the laboratory system used for the detection of VOCs is shown.



Figure II- 3. Picture of the flasks used during the second and the third experiment.



Figure II- 4. Laboratory system used for the detection of the VOCs from the microcosms.

II.5 Microcosms preparation

Humidity and water holding capacity (WHC) of the sterilized soils were measured for all samples. The samples collection was performed one month before the sterilization. Once soils were sterilized a period of 8 weeks passed before the inoculation. In fact, sterilized soils need a stabilization period in order to verify that the sterilization worked and no microbial contaminations were still colonizing the samples.

The day after the inoculation, on sterilized conditions, 30 g of soil for each sample was transferred in a flask; 5 mL of MilliQ water were added in order to reach the 50% of the 60% of the WHC (**Figure II- 5**).



Figure II- 5. Sample humidification in sterilized conditions

The day of the inoculation samples were homogeneously hydrated. The preparation performed is explained in **Figure II-6**. Soil suspension was prepared by mixing 30 g of soil and 90 mL of sterilized water. Three levels of microbial dilution were created: one pure, the second one had a water ration of $1:10^3$ and the third one with $1:10^5$ water rates.



Conditions stériles

Figure II- 6. Preparation of the soil suspensions.

After obtaining the different dilution levels, 5 mL of the different soil suspensions were added to the corresponding microcosms. The addition of the soil suspension allowed reaching the 60% of the WHC within the microcosms. Before incubation microcosms were hermetically sealed; the incubation was 6 weeks long at 20°C. Every week, on Monday, microcosms were aerated in order to avoid the accumulation of CO_2 . If a weight loss higher than 0.1g were observed by weighing the microcosms, the addition of pure water was carried out.

II.6 Biomolecular analysis

II.6.1 DNA extraction

The DNA extraction has been performed for all microcosms following the protocol developed by GenoSol platform (INRA, Dijon, France, www.dijon.inra.fr/plateforme_genosol) (Terrat et al., 2012) for application in large-scale soil survey (Terrat et al., n.d.). The protocol consists of mixing 1 g of each soil sample with 2 g of 100 mm diameter silica beads, 2.5 g of 1.4 mm diameter ceramic beads and 4 glass bead of 4 mm diameter and 5 mL of a solution containing 100 mMTris (pH 8.0), 100 mMEDTA (pH 8.0), 100 mM NaCl, and 2% (wt/vol) sodium dodecyl sulfate on mixing in a 15 mL Falcon tube. Then, we proceed homogenizing the samples in a FastPrep-24 (MP-Biomedicals, Santa Ana, CA, USA) during 90 s and incubated for 30 min at 70 °C before centrifugation at 7000 g for 5 min at 20 °C. The deproteination was achieved by collecting 1 mL of the supernatant and incubating for 10 min on ice with 1/10 volume of 3M potassium acetate (pH 5.5) and centrifuged at 14.000 g during 5 min. The precipitation of the proteins was performed with one volume of ice-cold isopropanol. The last step of the extraction consisted of washing the nucleic acid with 70% ethanol. DNA concentrations of crude extracts were determined by electrophoresis in 1% agarose gel stained with ethidium bromide using a calf thymus DNA standard curve, and used as estimates of microbial biomass (Dequiedt et al., 2011). After quantification, nucleic acids were separated from the residual impurities, particularly humic substances, by centrifuging through two types of minicolumn. Aliquots (100 µl) of crude DNA extract were first loaded onto polyvinyl polypyrrolidone minicolumns (BIORAD, Marne-la-Coquette, France) and centrifuged at $1000 \times g$ for 4 min at 10 °C. The eluates were then purified using the Geneclean turbo kit (Mp Biomedicals, Illkirch, France) (Ranjard et al., 2003). DNA concentration in each sample was fluorometrically quantified with the Quant-iT

PicoGreen dsDNA Assay Kit (Invitrogen, CergyPontoise, France) following the manufacturer's instructions.

II.6.2 Quantitative PCR (qPCR)

The polymerase chain reaction (PCR) is an in vitro molecular biology method for DNA or RNA amplification. The quantitative real-time PCR is a technique used to monitor the progress of a PCR and quantify the amount of PCR product in real-time (DNA or RNA). The real-time PCR is based on the detection of the fluorescent molecules that bind to the double-strained DNA. In this study, we used the SYBR © Green detection system, which consists in the most common fluorescent dye that binds by intercalating between the DNA bases. The fluorescence can be measured at the end of each amplification cycle determining the quantify 16S (bacterial quantification) and 18S (fungi quantification) ribosomal DNA (rDNA) sequences. It was thus possible to estimate the fungal bacterial ratio (F:B). Bacterial and fungal quantitative PCR assays were performed using a StepONE (Applied Biosystems, Courtaboeuf, France) with a SYBRGreen® detection system. The primers used for the bacterial and fungi quantification are reported in **Table II-2**.

	Primer code	Primers	References	
Bacteria	341F	5' - CCT ACG GGA GGC AGC AG - 3'	(López-Gutiérrez et al. 2004)	
	515R	5' - ATT ACC GCG GCT GCT GGC A - 3'		
Fungi	FR1	5'-AIC CAT TCA ATC GGT AIT-3',	(Chamidlin Prévost Douré et al. 201	
	FF390	5'-CGA TAA CGA ACG AGA CCT-3'	(Chemidini Pievost-Boure et al. 2011)	

Table II-2. Primers used for the quantification of fungi and bacterial DNA

II.6.3 The high throughput sequencing and the bioinformatics analysis

The high throughput sequencing refers to a method used for determining the order of the nucleotides bases in a DNA molecule. In this study, we amplified 440-base 16S rRNA from and 350-base 18S rRNA each DNA sample in order to obtain the bacterial and the fungi diversity respectively. The primers used are detailed in **Table II-3**. The pool was sequenced with a MiSeq Illumina instrument (Illumina Inc, San Diego, CA) operating with V3 chemistry and producing 250 bp paired-reads. After the sequencing, we performed the Bioinformatic analysis using the GnS-PIPE developed by the Genosol platform (INRA, Dijon, France)

(Terrat et al., 2012). At first, all the 16S and 18S raw reads were organized according to the multiplex identifier sequences. All raw sequences were checked and discarded if: (i) they contained any ambiguous base (Ns), (ii) if their length was less than 350 nucleotides for 16S reads or 300 nucleotides for 18S reads, (iii) if the exact primer sequences were not found (for the distal primer, the sequence can be shorter than the complete primer sequence, but without ambiguities). A PERL program was then applied for rigorous dereplication (i.e. clustering of strictly identical sequences). The dereplicated reads were then aligned using Infernal alignment (Cole et al., 2009), and clustered into operational taxonomic units (OTU) using a PERL program that groups rare reads to abundant ones, and does not count differences in homopolymer lengths. A filtering step was then carried out to check all single-singletons (reads detected only once and not clustered, which might be artifacts, such as PCR chimeras) based on the quality of their taxonomic assignments. Finally, in order to compare the datasets efficiently and avoid biased community comparisons, the reads retained were homogenized by random selection. The details of the random selection are given in Chapter IV and V since the number of reads was different. The retained high-quality reads were used for taxonomyindependent analyses and determining the Shannon index.

	Primer code	Primers	References	
Bacteria	F479	5'-CAG CMG CYG CNG TAA NAC-3'	(Tardy et al., 2014).	
	R888	5'-CCG YCA ATT CMT TTR AGT-3'		
Fungi	FF390	5'-CGA TAA CGA ACG AGA CCT-3'	(Prévost-Bouré et al., 2011)	
	FR1	5'-ANC CAT TCA ATC GGT ANT-3'		

Table II-3. Primers used for sequencing fungi (18S rRNA) and bacteria (16S rRNA).

II.7 Techniques for the detection of VOCs

In order to detect VOCs emissions in a laboratory system, several techniques and instruments can be used. Several studies analysed the emissions in the field using detection chambers while other studies used soil samples in the field and analysing them in a laboratory. The most common instruments used for the detection of VOCs emissions are the gas chromatography mass spectrometer (GC-MS) and the Proton Transfer Reaction Mass Spectrometry (PTR-MS). The gas chromatography can be coupled with: a flame ionization detector (GC-FID), a mass spectrometer (GC-MS) and a flame photometric detector (GC-

FPD). For all those VOCs detection systems a method for the extraction of VOCs is needed. This means that they are off lines techniques requiring a pre-concentration of VOCs in absorption traps (eg. Solid phase micro-extraction - SPME). Only after the collection of these VOCs they can be subjected to the detection of a GC system. On the other hand, PTR-MS allows the online detection of the VOCs released from samples with a very high mass resolution, especially if coupled with a time of flight (TOF) detector.

In the present study, VOCs emissions were detected with a PTR-QiTOF-MS (Proton Transfer Reaction-Quadrupole Ion guide Time of Flight- Mass Spectrometry) technique.

II.7.1 PTR-QiTOF-MS

The advantages given by this technique are the online detection of the VOCs and the low limit of detection (10 pptv). Furthermore, a PTR-MS coupled with a time of flight detector has two more advantages: the rapidity of the detection of the VOCs and the high resolution. A scheme summarizing the PTR-QiTOF-MS technique is shown in **Figure II- 7**.



Figure II- 7. Scheme of the PTR-QiTOF-MS technique.

The VOCs released from the flux chamber connected to the PTR-QiTOF-MS passed through the drift tube where the collision with the ions produced within the ion source happens. The ion source is divided in two chambers.



Figure II- 8. First chamber of the ion source.

In the first chamber a potential difference between a cathode and an anode is produced (**Figure II-8**), while in the second one the ions are created as follows:

$$H_2^+ + H_2 0 \rightarrow H_2 0^+ + H_2$$
 Equation 5

or

$$H^+ + H_2 O \rightarrow H_2 O^+ + H$$
 Equation 6

or

$$0^+ + H_2 0 \rightarrow H_2 0^+ + 0$$
 Equation 7

Leading to the final equation:

 $H_2O^+ + H_2O \rightarrow H_2O - H^+ + OH$ Equation 8

The final molecule colliding with the VOCs is the ion H_3O^+ . Within the drift tube, the ionization of the VOCs is performed. The ionization happens due to the transfer of one proton of the H_3O^+ to the emitted molecule (M) from the sample. The proton transfer was possible only for molecules having a proton affinity higher than the proton affinity of the water (691.7 kJ mol⁻¹). The molecule resulting from the interaction is a protonated molecule (MH⁺). Furthermore, the ionization of the molecule can be possible thanks to the charge transfer reaction $(A++B \rightarrow A-B^+)$ Charge transfer reaction Equation 10).

$$AH^+ + B \rightarrow A - BH^+$$
Proton transferEquation 9 $A^+ + B \rightarrow A - B^+$ Charge transfer reactionEquation 10 $H_3O^+ + R \xrightarrow{K} RH^+ + H_2O$ Chemical ionization reactionEquation 11

Once the molecule is protonated it passes through the ion-guide where only the range of molecule of the select m/z range pass to the time of flight. Finally, the molecule is detected with a Multiple Channel Plate (MCP), an electron multiplier. From the signal of the H_3O^+ ions, the signal of the protoned mass (MH⁺) we can calculate the concentration of the molecule [M] using the following equation (Lindinger et al., 1998):

$$[MH^+] = [H_3O^+]_0 0(1 - e^{-k[M]t}) \approx [H_3O^+]_0 [M]kt$$
 Equation 12

Where *t* is the reaction time of H_3O^+ in the drift tube, and where *k* is the reaction rate coefficient between [M] an H_3O^+ according to the tabulation provided by Cappellin et al., (2012). If *k* is not available a standard value can be used (2*10⁻⁹ cm³ s⁻¹).

Despite all the advantages of the PTR-QiTOF-MS listed before, some disadvantages are reported. In fact H_3O^+ can form a cluster leading to the formation of a molecule with a +19 m/z $(M - H_3O^+)$ instead of +1 m/z (MH^+) . Furthermore, events like fragmentation can happen caused by the collision of the molecules within the drift tube. Those events lead to a complicated identification of the detected molecules. Finally, the PTR-QiTOF-MS is not able to separate isomers, for this reason, it is often coupled with a GC.

II.7.2 Peaks analyses and mass table

The detection of the peaks was performed using the software PTR viewer 3.1.019 (Ionicon, Analytik GmbH). The integration of the peaks has been done using the software and selecting the peaks one by one in order to include the largest number of peaks. An example of VOCs spectrum is reported in **Figure II- 9**. For more details concerning the peak, selection see paragraph

Peak detection of the mass spectra and mass calibration pg. 59.



Figure II- 9. Example of a spectrum detected from soil sample.

Not all peaks detected have been inserted in the mass table. In fact, m/z smaller than 40 m/z have not been considered in this analysis (except for 31 m/z, the formaldehyde and 33 m/z, methanol). Furthermore, masses derived from the water cluster (37.03 m/z, 38.03 m/z, 39.03 m/z, and 55.03 m/z) have been deleted from the final mass table. Once the selection of all peaks have been completed, the same mass table has been applied to all samples.

Once the mass table was ready calculations of the concentration of the compounds were performed, and the calculation of the average spectra was carried out. In order to do that, software using LabVIEW has been created (**Figure II- 10**). The aim of this software is to cut the spectrum when the signal was stable. For all samples, we took a minimum of 60 spectra to calculate an average spectrum used on the further analysis.



Figure II- 10. Image of the LabVIEW software developed for the selection of the stable signals used to calculate the average spectrum.

Chapter III

Profiles of volatile organic compound emissions from soils amended with organic waste products

Profiles of volatile organic compound emissions from soils amended with organic waste products

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Graphical Abstract



Abstract

Volatile Organic Compounds (VOCs) are reactive compounds essential to atmospheric chemistry. They are mainly emitted by living organisms, and mostly by plants. Soil microbes also contribute to emissions of VOCs. However, these emissions have not yet been characterized in terms of quality and quantity. Furthermore, long-term organic matter amendments are known to affect the microbial content of soils, and hence the quantity and quality of VOC emissions. This study investigates which and how much of these VOCs are emitted from soil amended with organic waste products (OWPs). Four OWPs were investigated: municipal solid waste compost (MSW), green waste and sludge co-compost (GWS), bio-waste compost (BIOW) and farmyard manure (FYM). These OWPs have been amended every two years since 1998 until now at a rate of ~4 tC ha⁻¹. A soil receiving no organic inputs was used as a reference (CN). VOCs emissions were measured under laboratory conditions using a Proton Transfer Reaction-Quadrupole ion guide Time of Flight-Mass Spectrometry (PTR-QiToF-MS). A laboratory system was set up made of two Pyrex chambers, one for samples and the second empty, to be used as a blank. Our results showed that total VOC emissions were higher in BIOW than in MSW. Further findings outlined that the most emitted compounds were acetone, butanone, and acetaldehyde in all treatments, suggesting a common production mechanism for these compounds, meaning they were not affected by the OWP amendment. We isolated 21 VOCs that had statistically different emissions between the treatments and could, therefore, be considered as good markers of soil biological functioning. Our results suggest that organic matter and pH jointly influenced total VOC emissions. In conclusion, OWPs in soil affect the type of VOC emissions and the total flux also depends on the pH of the soil and the quantity of organic matter.

Keywords: VOC, soil, organic waste products, PTR-QiTOF-MS, VOCs fluxes.

III.1 Introduction

The family of volatile organic compounds (VOCs) is composed of a large range of molecules with different functional groups and different chemical and physical properties. The common traits between VOCs are the high vapor pressure under ambient conditions and low boiling points which explain their presence in the atmosphere. Volatile organic compounds sources have been extensively studied because of their contribution to global warming and pollution (Kesselmeier and Staudt, 1999). It has been demonstrated that VOCs are involved in many chemical reactions in the atmosphere among which the production cycle of tropospheric ozone (O_3) in combination with the NO_x cycle (Seinfeld and Pandis, 2016). Another important chemical processes in the atmosphere that involves VOCs are the photolysis of formaldehyde and other carbonyls in presence of NO which are responsible of hydroxyl (OH) formation (Atkinson, 2000). Additionally, VOCs are precursors of secondary organic aerosol (SOA) (Singh et al., 1995) and are involved in the production of nitrates in the troposphere (Monson and Holland, 2001). For these reasons VOCs alter the air quality and thus they have indirectly consequences on human and ecosystems health.

Several studies demonstrate that biogenic VOCs contribute to 90% of the total VOCs emissions (Atkinson, 2000), while anthropogenic sources such as industries, solvents, transportations etc. contribute only for the 10% of the total VOCs emissions. Among the most important biogenic sources of VOCs are living plants, with a global emission of 1Pg C y⁻¹, mainly monoterpenes from broadleaves forests (Harrison et al., 2013). Furthermore, it has been shown that the soil and litter can release around 12 to 136 % of monoterpenes compared to canopy emissions in spring and fall (Faiola et al., 2014).

Another source of biogenic VOCs is the interaction between soil and microorganisms. Soils VOCs emissions are up to three times lower than the VOCs release from the canopy. Nevertheless, it has been demonstrated that under certain temperatures and soil water contents, and for specific ecosystems, VOCs emissions from soil could reach the same magnitude as canopy emissions (Peñuelas et al., 2014). Additionally, little is known about the emissions from agricultural fields, although they are recognised as large contributors of methanol and acetone to the atmosphere (Bachy et al., 2018). It however appears from recent measurements that soils may contribute to a large extent to the overall balance of VOC fluxes in crop rotations (Bachy et al., 2016).

From another perspective, intensive agriculture may lead to the decrease of soil organic matter contents (Chan et al., 2002) and Europe is encouraging the use of organic waste

products (OWPs) in crops (European Commission, 2010) to favour carbon storage in soils (Peltre et al., 2012). The recycling of OWPs in soils also represents an alternative to waste management by landfilling or incineration. The application of OWPs also provides nutrients such as nitrogen (N) and phosphorous (P) which make possible the substitution of mineral fertilizer, improves soil biodiversity and has positive impacts on soil physical properties such as aggregate stability (Diacono and Montemurro, 2010). The negative impacts are not negligible though; OWPs release contaminants that can be transferred to plants, water or to the atmosphere (Belon et al., 2012). OWPs change the pH in soil. As Brinton (1998) reported, the pH is one of the chemical properties that impact on VOCs emissions. Positive and negative impacts in soil vary with the applied organic waste product (Obriot et al., 2016). For example, Seewald et al. (2010) studied VOCs production by adding glucose to soils regularly amended by different composts and mineral fertilizers. They also reduced the O₂ availability in order to impair the degradation of the produced VOCs by microorganisms. Results reported by this study shown that organic waste compost did not alter the VOC emissions compared to the untreated control, while sewage sludge composts and mineral fertilization showed distinct effects. Moreover, Potard et al. (2017) showed that different soil organic fertilizers such as pig slurry and methanized pig slurry impact in a different way soil VOCs emissions: pig slurry releases double quantities of VOCs while methanized pig slurry emits even less than the unamended samples. Contrasting type of VOCs emissions from soil have been reported from several studies. For instance, in Mediterranean soils the most emitted compound was methanol (Asensio et al., 2007a) while in soil amended with straw it was acetone (Zhao et al., 2016). In fact, emissions of VOCs are strongly related to the substrate quality and it is important to note that even small variations in nutrient composition may change the type and the amount of individual VOCs produced (Wheatley, 2002).

Significant emissions of the VOCs released by soil are produced by microorganisms (Isidorov and Jdanova, 2002). It has been reported that the composition and amount of VOCs emitted are linked to the microbial community living in the soil (Seewald et al., 2010). Microorganisms produce VOCs as a result of the degradation of sugar, alcoholic fermentation, amino acid and fatty acid degradation, terpenoid biosynthesis and sulphur reduction. For instance, acetaldehyde, which is one of the compounds most emitted from soil, is formed during the alcoholic fermentation that produces ethanol (Castaldelli et al., 2003). Previous studies have reported that microorganisms degrade sugar following three major pathways: (1) Embden-Meyerof pathway, (2) Heterolactic/homolactic pathway and (3) Entner-Douoroff pathway. In these processes, intermediate compounds are produced such as

pyruvate, glyceraldehyde-3-phosphate, lactate and acetate which are precursors for the biosynthesis of several VOCs (Peñuelas et al., 2014). VOCs are also released as molecules resulting from the secondary metabolisms of micro-organisms (Werner et al., 2016). Secondary metabolites might be produced under certain circumstances or development stages; hence they might be used as indicator of the cellular state (Patti et al., 2012). Furthermore, the nature of the substrate can affect microorganisms' community and thus change VOCs emissions (Peñuelas et al., 2014). Moreover, the application of OWPs in soil stimulate its diversity, the abundance and the activities of microorganisms in soil compared to soils that only received mineral fertilizers. This is a consequence of the addition of microorganisms previously present in the OWP (Ros et al., 2006).

The aim of this study was to characterise VOCs emissions from soil amended with OWPs in order to quantify and qualify emissions from amended soil to the atmosphere. Furthermore, soils regularly amended with different organic waste products for a long period can have a differentiated volatile organic compound signature and different total VOCs fluxes. In order to reach this aim, four soils amended for 20 years with organic wastes products were considered: bio-waste compost (BIOW), green waste and sludge compost (GWS), municipal solid waste compost (MSW) and farmyard manure (FYM). All these soils were compared with a control sample that had never received any OWP. We measured VOCs fluxes under standardised laboratory conditions with a Proton Transfer Reaction - Quadrupole Ion guide Time of Flight - Mass Spectrometer (PTR-QiTOF-MS). The VOCs released were identified and quantified and the relationships between VOCs emissions and soil chemical characteristics (pH, organic matter, I_{roc}, cation exchange capacity, carbon/nitrogen ratio, total nitrogen, organic carbon) were studied.

III.2 Methods

III.2.1 Site description

Samples were collected in the QualiAgro site, a field station taking part of the SOERE-PRO-network (https://www6.inra.fr/qualiagro_eng/Nos-partenaires/The-SOERE-PROnetwork). The QualiAgro agronomic set up which is described in this study started in September 1998 and lasted until September 2015. The QualiAgro site is located at Feucherolles in northwestern France (35 km west of Paris; 48°52'N, 1°57'E, alt 150 m), on a silt loam textured soil. The soil of QualiAgro site of Feucherolles is classified as a hortic glossic Luvisol (IUSS Working Group WRB, 2014), representative of the Parisian Basin. The main characteristics of these soils are represented by the lack of clay, a silt-loam texture (15.0% clay, 78.3% slit) and an initial pH of 6.9 in the surface horizon (0-30 cm) and good drainage. Moreover, the QualiAgro field experiment is in a cropland dominated region, which lead to a low organic carbon and low organic matter concentrations (initial content of 1.1%) (Meersmans et al., 2012). On the other hand, observing QualiAgro site on a smaller scale, differences in the organic carbon content, total nitrogen or pH between samples are due to the different applications of OWPs.

The experiment was a randomized block design with 4 replicates comparing 4 organic waste products: BIOW (bio-waste compost derived from the co-composting of green wastes and source-separated organic fractions of municipal solid wastes), GWS (compost derived from the co-composting of green wastes with sewage sludge), FYM (farmyard manure) and MSW (municipal solid waste compost derived from the composting of residual solid wastes after removing dry and clean packaging); plus a control without organic input (CN). Moreover, the 20 field blocks studied were additionally amended with mineral N to reach optimal N application. The field was cropped with a winter wheat – maize rotation. The wheat crop residues were exported, whereas the maize residues were returned to the soil. Since 1998, the organic waste products (OWPs) have been applied at a rate of ~4 tC ha⁻¹ every two years on the wheat stubbles in September, after harvesting. In each plot, 5 soil cores were randomly sampled at 0-30 cm depth using a core drill and stored in a cold chamber at 4°C prior to analysis. The sampling was performed in early September 2015, two years after the last amendment of OWPs. Each type of amended soil was sampled as described by Noirot-Cosson et al. (2016).

III.2.2 Experimental setup

III.2.2.1 Soil analysis

All soil analysis was realized once on the dried and 2mm-seived composite sample, further 250 μ m-ground when indicated. Soil pH was determined following the standard NF ISO 10390 protocol, where a glass electrode was used in a 1:5 (volume ratio) suspension of soil in MilliQ water. Total carbon in soil was measured by dry combustion of 0.05 g of 250 μ m-ground soil and organic carbon calculated after correction of the carbonate content (ISO 10694). Total N was determined simultaneously, also by dry combustion (ISO 13878).

Finally, organic matter in soil was calculated by multiplying C organic x 1.72, while organic matter for composts was determined by loss on ignition of 1 g of ground composts at 550°C. The soil cation exchange capacity was determined with cobaltihexamine following NF X 31-130 (AFNOR 1999). Briefly, 2.5 g of dried and 2mm-seived soil were shaken for 2 hours with 50 ml of 50 mmol/L of cobaltihexamine chloride. The soil cation exchange capacity was determined based on the difference of cobaltihexamine chloride before and after soil contact.

III.2.2.2 Soil sample preparation for PTR-QiTOF-MS analysis

Samples of the same plot were mixed and homogenized by passing through a 2 mm sieve to remove aboveground plant materials, roots and stones, then dried up at room temperature (~23°C) for 2 weeks.

III.2.2.3 Laboratory flux chambers

In order to measure VOC emissions, soil samples were first moisturised to the field capacity by adding ultrapure water (resistivity 18.2 M Ω). The amount of water necessary was determined on a sub-sample and used for all samples (15 mL of water for each sample). Soil samples were then inserted, one at a time, in a Pyrex chamber having a volume of 106 cm³. The quantity of soil inserted in the chamber $(m_{drv soil})$ was 60 g (dry mass) for each measurement. Soil was homogeneously spread on the chamber surface (132 cm²). An empty chamber was used as a reference for zero emissions. Figure III- 1 represents the design of the measurement scheme. An air flow (Q_{air}) of 0.2 L min⁻¹ (equivalent volumetric flow at 0°C and 1 atm) of dry synthetic air (Alphagaz 1 Air: 80% nitrogen, 20% oxygen, 99.9999%, Air Liquide®) was passed through a hydrocarbons and humidity filter (Filter for fuel gas, final purity=99.999%, Restek®) and a Hydrocarbon Trap (Supelco, Supelpure® HC) prior to injection in the chambers. A mass flowmeter (Bronkhorst® model F-201CV, accuracy: standard 0.5% Rd plus 0.1% FS, range: 0.2 L min⁻¹ to 5 L min⁻¹ air) was used to control the synthetic air flow rate. Air was sampled at the chamber outlet into a PTR-QiToF-MS with a 0.05 L min⁻¹ flow rate with a 2 m long, 1 mm internal diameter PEEK tube, heated at 80 °C. A measurement cycle consisted in measuring the VOC mixing ratio at the outlet of the chamber containing the soil ($x_{VOC \ soil}$ in ppb) for 180 s. Then air was sampled for 120 s on the empty chamber to determine $x_{VOC \ empty}$ (ppb). We sampled the empty chamber less than the chamber with soil because the signal was stable after only ~10 s. Only the last 60 s of each measurement were kept to calculate averaged mixing ratios in order to ensure a stable VOCs mixing ratio. The VOC emission (E_{VOC} in nmol g⁻¹ s⁻¹ dry soil) was calculated as:

$$E_{VOC} = \frac{Q_{air} \times (\chi_{VOC \ soil} - \chi_{VOC \ empty})}{V_{mol}^{air} \times m_{dry \ soil}}$$
(1)

Where V_{mol}^{air} is the air molar volume at standard temperature and pressure (22.4 L mol⁻¹ at 0°C and 1 atm). In total 15 samples were measured: 3 replicates for each of the 5 treatments. Each measurement was further repeated 3 times to compute the mean and standard deviation of the measurement. This led to 45 measurements in total. After each measurement, the chamber was cleaned with diluted sodium hydroxide in order to destroy any organic compound that could affect the next measurements, and then rinsed with ultrapure water (resistivity 18.2 M\Omega).



Figure III- 1. Design of the experimental set-up. The chamber on the left is the empty chamber used as a zero, the one to the right is the chamber used to detect the emissions released from the samples.

III.2.3 VOCs analysis with the proton transfer reaction time of flight mass spectrometer (PTR-QiTOF-MS)

The VOCs were analysed with a PTR-QiTOF-MS (PTR-Quadrupole Ion guide-TOF, Ionicon, Analytik GmbH, AU). The analyser is described in details by Sulzer et al., 2014. It consists in a drift tube where VOCs are ionised and accelerated prior to be injected into a quadrupole ion guide and electromagnetic lenses. The electromagnetic lenses focus the ions in a time of flight mass spectrometer detector (TOF, TofWerk, Switzerland) which separates the ions by inertia prior to detection. The detection is made with a multi-channel-plate (MCP) detector and a time-to-digital converter (Burle Industries Inc., Lancaster, PA, USA). In this study, ionisation was carried out with H_3O^+ as proton donor. The transfer reaction was effective for VOCs with a proton affinity higher than 691.7 kJ mol⁻¹, which is the proton affinity of the water. In the drift tube, the pressure was tuned to 4 mbar, the temperature to 80°C, and the drift voltage to 1000 V, while the extraction voltage at the end of the tube (UDx) was 44 V. The E/N ratio (Electric field/density of natural particles) was 132 Td where 1 Td is 10 V m². These parameters were controlled in order to maintain constant ionization conditions within the drift tube. The setup of the time of flight timing was: TOF extraction period 40000 ns, pulse width 2000 ns, trigger delay 100 ns. The number of channels was 240.000. This gave a mass spectrum measurement up to 510 m/z. The measurement period was set to 1 s, which means that each sample corresponded to 60 acquisitions of 25000 individual spectra. Raw PTR-QiToF-MS data were recorded by TofDaq software (Tofwerk AG, Switzerland).

III.2.3.1 <u>Peak detection of the mass spectra and mass calibration</u>

We used the PTR viewer 3.1.0.29 software (Ionicon, Analytik GmbH) to detect mass peaks in each spectrum. The mass calibration was performed using the oxygen isotope of the ion source $H_3^{18}O^+$ (21.022 m/z) and acetone, $C_3H_7O^+$, (59.0449 m/z). These two were chosen as they were present in all samples. Mass calibration was made over the entire dataset allowing for a change of 0.1 m/z around the peak maximum between consecutive samples and using a moving average of 50 successive samples for better stability. Overall the instrument mass resolution, which is defined as the peak m/z divided by the peak width at mid-height was around 4000 (no unit). In order to create a peak table including the largest number of peaks, we used the automatic research feature of the software selecting as search mode "multiple peaks" with a threshold of 5 counts per second. Following this step, a manual check of each peak was performed in order to correct for any software miss-fits. Each peak was manually readjusted. The modes used to auto-select and manually adjust the peak areas that are used for mixing ratio calculation were the Gaussian or the multi-peak modes. The Gaussian peak mode was used for the analysis of isolated peaks; while for a better fit of multiple peaks we used the multi-peak mode. The range of the mass detection was limited between 31 and 400 m/z. Finally, masses deriving from the water cluster such as 37.03 m/z,
38.03 m/z, 39.03 m/z, and 55.03 m/z were not taken into account during the analysis of the dataset.

III.2.3.2 Isotopes and fragments identification

In order to make sure that a compound was not twice counted, we identified likely isotopes and fragments by performing a correlation analysis on the mass spectra. The Pearson's correlation coefficient was computed between each m/z based on all measured mixing ratios during the experiment (N=45). The m/z having a correlation coefficient larger than 0.99 were considered either fragments or isotopes depending on the m/z difference. In total 16 isotopes and 2 likely fragments were identified (**Table S1**).

III.2.3.3 <u>PTR-QiTOF-MS mixing ratio calibration</u>

The MCP provides the counts per seconds of detected ions after the TOF. In order to calculate the mixing ratio of gases in ppb that enter the PTR, a series of calculations were performed. The mixing ratio was calculated using the PTR-viewer software, which uses the following equation:

$$\chi_{\text{voc standard transmission}} = 1.657 e^{-11} \frac{U_{\text{drift}} T_{\text{act}}^2[K]}{k p_{\text{act}}^2[\text{mbar}]} \frac{R^+}{H_3 O^+} \frac{TR_{H_3 O^+}}{TR_{R^+}}$$

Where $\chi_{i \ standard \ transmission}$ is the mixing ratio of the compound in ppb, U_{drift} is the voltage of the drift (V), T_{act} is the drift-tube temperature in Kelvin, R⁺ is the signal of the product ion in count per second (cps), k is the protonation reaction rate constant (2 × 10⁻⁹ cm³ s⁻¹) assumed equal for all compounds, $TR_{H_3O^+}$ is the transmission factor for mass of primary ion, TR_{R^+} is the transmission factor for mass of product, p_{act} is the pressure of the drift, ion H_3O^+ is the primary ions signal in cps. The standard transmission curve from supplier was used to compute $TR_{H_3O^+}/TR_{R^+}$ (see Table S2 in supplementary material).

We used the following primary ions mass: 21.02 m/z (H₃¹⁸O⁺, multiplier factor 487.56), 38.03 m/z (water cluster H₂¹⁸O•H₃¹⁸O⁺ with two ¹⁸O isotopes, multiplier factor 819.51) and mass 55.03 m/z (H₂O•H₃O⁺ •H₃O⁺ multiplier factor 1.0008).

In order to account for the normal loss of sensitivity of the PTR-QiTOF-MS, due to the MCP degradation over time, the change of $H_3^{18}O^+$ produced in the source, or other factors, we used a calibration procedure to account for the actual sensitivity of the analyser. We used benzene, toluene and ethylbenzene as reference compounds for this calibration. The mixing ratio of

(2)

these compounds was modulated using a dilution system with a standard cylinder containing 102 ppb of benzene, 104 ppb of toluene, 130 ppb of ethylbenzene and 336 ppb of xylene (Btex, Messer®). We used the same synthetic air for the dilution system as the one described for the chamber set up. The sensitivity S^{norm} was calculated as the slope of the regression between measured mixing ratio with the standard transmission provided by Ionicon and mixing ratio calculated with equation (2), and forcing a zero intercept. The calibrated mixing ratio of all compounds was then calculated as:

$$\chi_{voc} = \left[\chi_{voc \ standard \ transmission} - \chi_{voc}_{(0)}\right] \times S^{norm}$$
(3)

Where χ_{voc} is the mixing ratio of the compound in ppb, $\chi_{voc \ standard \ transmission}$ is the mixing ratio calculated using the standard transmission as in equation (2), $\chi_{voc}_{(0)}$ is the mixing ratio of the compound measured for dry synthetic air.

The procedure was applied for the three compounds cited above for the calibration. We found that the sensitivity was 3.11 ± 0.15 . A single sensitivity value of 3 was applied for all masses, since the protonation reaction constant was assumed equal for all masses. In total we selected 754 peaks in the mass spectra used for these analyses.

III.2.3.4 VOC identification

We first identified the molecular formula with the Spectra Analyser within the PTR viewer 3.1.0.29 software (Ionicon, Analytik GmbH). The spectra analyser is a tool that allows the manual selection of the peaks chosen for the identification. The identification of the compounds corresponding to each mass peak was performed by searching the possible combinations of elements that led to the closest molecular weight. The following elements were selected for all peaks: C, N, O, and H. Other compounds were selected on purpose for masses higher than 70 m/z like S, Si, Cl, Ca and K, when C, N, O and H did not lead to a good agreement with the peak mass. The maximum number of atoms per molecule was set to 50, while the error on the molecular weight was fixed to ± 0.01 m/z. Subsequently, a list of possible compounds was produced. The compounds listed represent the best matches for the selected exact m/z. Finally, a match of the isotopes of each compound was evaluated. Once the molecular formula was determined, we tried to identify the potential compounds with the use of the literature reviews. We have to stress that this method does not provide an undoubtable identification of the compounds as several compounds could have the same molecular mass but not the same chemical structure. Moreover, even with this procedure, some compounds could not be identified at all.

III.2.4 Data analysis

Several statistics test were performed on the VOCs emissions calculated for each treatment in order to identify the VOCs that mark the differences between the OWP treatments. All statistical analysis were performed using R software (Version 1.0.153 – © 2009-2017 RStudio). First, a normality test (Shapiro-Wilk test, W> 0.9) has been applied to verify that the mean mixing ratios were normally distributed for each VOC. Secondly, the homogeneity of the variances was verified for each treatment using the Levene test. Once the normality and the homogeneity of de variances were validated, the ANOVA test followed by Tukey post hoc test were performed. Finally, the Between Class Analysis (BCA, Package *Ade4* Version 1.0.153 – © 2009-2017 R Studio) was performed. The ANOVA and BCA were specifically used to identify the VOCs that differentiate between each OWP treatment. The Between-Class analysis is a particular case of a Principal Component analysis, it calculates the axes maximizing the covariance between the different treatments analysed. The Shannon index of diversity (**Figure 3b**) was calculated with the diversity function of the vegan package (version 2.4-3) in the R software (version 3.2.3). The diversity index is calculated as $H = \sum_{VOC} E_{VOC} \log(E_{VOC})$, where the sum is over all VOCs recorded in the mass table.

III.3 Results

III.3.1 Soil characteristics of each treatment

The **Figure 2** summarizes soils characteristics as a function of OWP. The carbon content is in the range 10-18 g kg⁻¹ DM which spans from typical to high carbon content for a silt-loam. Organic matter was mainly found in the clay fraction of the soil (60%) and in the smaller fraction of the particulate organic matter, mainly in the form of O-alkyls, Alkyls and Aryls (Paetsch et al., 2016).

The CN samples showed the lowest cation exchange capacity, organic matter content, total nitrogen content, pH and organic carbon while its C/N ratio was not different from BIOW and GWS treatments (**Figure III- 2**). The treatment with the highest cation exchange capacity was BIOW while we found the highest C/N ratio in the FYM. Furthermore, the total N, organic carbon and organic matter were higher for the BIOW and the GWS treatments. Concerning the pH, BIOW and MSW had the highest values, while CN and GWS had the lowest values.

These results show that the repeated application of OWP has significantly increased soil organic carbon content (OC in Figure 2) in all organic treatments compared to the CN, but not in the same amount. Although all OWP are applied based on the same input of organic C, their different characteristics can explain their varying efficiencies at increasing soil organic carbon (Peltre et al., 2012). Indeed, the index of residual organic carbon (Iroc) that represents the portion of organic carbon potentially incorporated into soil organic carbon after the organic waste application (Lashermes et al., 2009) was similar to OC: Iroc was the lowest for the MSW samples (I_{roc} =49% Org C), and was the highest for BIOW and GWS (I_{roc} =77% and 75 % Org C, respectively) (Obriot et al., 2016). Total organic nitrogen (Ntot) was also greater in the organic treatments compared to CN was ordered similarly as soil organic carbon. The C/N ratio is in the same order of magnitude for all the samples. The soil CEC was increased in treatments receiving OWP, which is in line with previous finding, that CEC increased with organic matter in soils (Kaiser et al., 2008). Finally, the BIOW and MSW treatments increased soil pH mainly because of their contents in carbonates. FYM only slightly increased pH compared to control while GWS had no effect on soil pH. Paetsch et al., (2016) further showed that the C/N ratio of the fine fraction of particulate organic matter rather represented the crops residues than organic waste inputs. They deduced lower degradability of organic matter in BIOW and GWS although they show the highest OM content.



Soil characteristics

Figure III- 2. Average soil characteristics analysis for the different treatments. DM= dry matter, CEC= cation exchange capacity, CN ratio= carbon/nitrogen ratio, Ntot= total Nitrogen, OC= organic carbon, M= organic matter. MSW: Municipal solid waste compost, GWS: Green waste and sludge compost, BIOW: Bio-waste compost, FYM: farmyard manure, CN: control without organic inputs. Point= median, boxes= interquartile, whiskers= minimum and maximum.

III.3.2 VOCs Emissions

In order to verify whether the treatments affected the bulk production of VOCs, the fluxes of all compounds detected with the PTR-QiTOF-MS were summed up. They ranged between $36 \pm 8 \ 10^{-4} \text{ nmol s}^{-1} \text{ g}^{-1}$ and $52 \pm 12 \ 10^{-4} \text{ nmol s}^{-1} \text{ g}^{-1}$ (**Figure III- 3a** and Supplementary material **Table S3**). The treatment with the highest total flux rate was the BIOW and the treatment with the lowest total flux rate MSW. The other three treatments had non-significantly different total VOC emissions (ANOVA and a post hoc Tukey test with p-value < 0.05). Moreover, the Shannon diversity indexes (Figure III- 3b) were similar in all treatments, though slightly smaller in BIOW indicating that BIOW less compounds contribute to the overall VOC flux.



Figure III- 3. (a) Total VOCs emission rates per soil treatment. Bold line=median, boxes= interquartile, whiskers= minimum and maximum. Letters indicate significant differences according to the Tukey test with p.value>0.05. (b) Shannon diversity index of the VOC signature indicating the diversity of VOCs emitted. BIOW= bio-waste compost, MSW= municipal solid waste compost, CN= control without organic input, FYM= farmyard manure, GWS= green waste and sludge compost.

III.3.2.1 Differentiating VOCs for each treatment

In order to determine if OWP amended soils affect the profile of VOCs emissions, we performed a Between Component Analysis (BCA). The first two components of the BCA explained 40.2% and 33.8% of the total dataset variability. In **Figure III- 4** we observe that GWS and BIOW treatments are isolated from the other OWP (FYM, MSW, and CN), which means that they have a VOC profile which is different from the other OWP. The VOCs that most explained the variance in the two first components of the BCA were selected as those having a coordinate larger than 0.9 on the first component and 0.5 on the second one (in absolute value). The m/z and a tentative identification of these VOCs are shown in **Figure III- 4**.

Furthermore, an ANOVA analysis was performed in order to determine the VOCs that significantly differentiated the treatments. A Tukey test was performed on those compounds to detect significance in means differences. The emission rates of the 21 compounds statistically different are plotted in **Figure S1**. **Table III- 1**summarises the most emitted VOCs and those highlighted by the BCA and the ANOVA analysis. These 49 compounds listed in **Table III-1** contribute between 70% and 91% of the total emission rate. For all treatments includes the control we found that acetone (contributing for the 30% of the total emissions), butanone (contributing for the 25% of the total emissions) and acetaldehyde (contributing for the 8.1% of the total emissions) were the most emitted compounds always as first, second and third. In **Table III-1** we added a tentative identification of the compounds and the corresponding chemical class. BIOW generally showed larger emissions than other treatments for small weighed compounds while GWS showed clearly higher emissions than other treatments for compounds in the mass range 80-130 m/z.



Figure III- 4. Effect of OWP on the VOCs emissions. The m/z of the compounds that are most explaining the variance in the two first components are shown on the graph. The eigenvalues show the percentage of the variance explained by the 4 first components (the percentage is also shown on each ax).

m/z	Most likely formula	Tentative identification	Class of chemical compounds	Detected in BCA or ANOVA		E1 (nm	mission rates ± ol s ⁻¹ g ⁻¹ (DW))	sd x 10 ⁴		$\begin{array}{c} Average \\ \pm sd \\ emission \\ rates^{1} \\ (\mu g \ m^{-2} \\ h^{-1}) \end{array}$	Averaged percentage contribution
					CN	BIOW	FYM	GWS	MSW		
59.04	C ₃ H ₆ O	Acetone	Carbonyl/ketone	Х	9.4 ± 2.4	15.0 ± 4.9	11.0 ± 2.7	10.0 ± 2.7	8.8 ± 1.1	1000 ± 240	30.0%
73.06	C_4H_8O	2-Butanone, MEK	Carbonyl/ketone	Х	6.8 ± 1.9	11.0 ± 3.1	6.7 ± 1.9	6.4 ± 1.4	5.5 ± 0.78	$\begin{array}{c} 870 \pm \\ 260 \end{array}$	25.1%
45.03	C_2H_4O	Acetaldehyde	Carbonyl/aldehyde		3.5 ± 0.85	4.9 ± 1.4	3.7 ± 0.97	3.9 ± 1.2	3.1 ± 0.32	280 ± 51	8.1%
57.06	C_4H_8	Butene	Alkene		1.7 ± 0.29	2.3 ± 0.54	1.9 ± 0.6	2.3 ± 0.96	1.6 ± 0.23	180 ± 29	5.3%
47.04	C ₂ H ₆ O	Ethanol	Alcohol	Х	0.76 ± 0.12	0.85 ± 0.11	0.76 ± 0.24	0.9 ± 0.44	0.69 ± 0.079	150 ± 16	4.3%
33.03	CH ₄ O	Methanol	Alcohol		0.50 ± 0.14	0.43 ± 0.085	0.53 ± 0.12	0.64 ± 0.19	0.47 ± 0.14	110 ± 17	3.1%
115.10	$C_7H_{14}O$	Heptanal/heptanone	Carbonyl /ketone		1.2 ± 0.16	2.0 ± 0.66	1.2 ± 0.27	1.2 ± 0.55	1.1 ± 0.6	100 ± 27	3.0%
43.05	C_3H_6	Propene	Alkene		0.54 ± 0.14	0.56 ± 0.088	0.44 ± 0.09	0.55 ± 0.22	0.4 ± 0.042	71 ± 10	2.0%
129.10	$C_8H_{16}O$	Octanal/Octanone	Carbonyl /ketone		0.92 ± 0.24	1.3 ± 0.51	1.5 ± 0.5	1.3 ± 0.53	1.0 ± 0.54	66 ± 13	1.9%
87.07	$C_5H_{10}O$	Pentanal, MBO	Carbonyl /aldehyde		0.45 ± 0.24	0.33 ± 0.12	0.39 ± 0.16	0.46 ± 0.12	0.31 ± 0.12	59 ± 10	1.7%
41.03	C_3H_4	Propyne	Alkyne		0.42 ± 0.11	0.38 ± 0.1	0.41 ± 0.13	0.47 ± 0.23	0.36 ± 0.065	46 ± 4.6	1.3%
69.06	C ₅ H ₈	Isoprene	Terpene		0.21 ± 0.023	0.29 ± 0.091	0.41 ± 0.1	0.26 ± 0.056	0.41 ± 0.31	42 ± 12	1.2%
93.06	C ₇ H ₈	Toluene	Aromatic		0.55 ± 0.08	0.65 ± 0.12	0.55 ± 0.13	0.62 ± 0.17	0.47 ± 0.13	40 ± 4.9	1.2%
81.07	C ₆ H ₈	Fragment monoterpenes/Hexenal	terpene		0.13 ± 0.011	0.21 ± 0.036	0.15 ± 0.03	0.59 ± 0.12	0.25 ± 0.03	39 ± 28	1.1%
65.05	$C_2H_2F_2$				0.26 ± 0.04	0.3 ± 0.05	0.31 ± 0.049	0.32 ± 0.087	0.27 ± 0.086	34 ± 2.9	1.0%
71.08	C5H10	Pentene	Alkene		0.26 ± 0.045	0.39 ± 0.095	0.26 ± 0.06	0.25 ± 0.031	0.23 ± 0.046	34 ± 8	1.0%
74.06				Х	0.27 ± 0.088	0.43 ± 0.14	0.29 ± 0.067	0.29 ± 0.14	0.25 ± 0.15	33 ± 8	0.94%
60.04				Х	0.54 ± 0.29	0.53 ± 0.16	0.41 ± 0.18	0.5 ± 0.26	0.41 ± 0.12	32 ± 4	0.9%
43.01	C_2H_2O	Ethenone	Carbonyl /ketone		0.14 ± 0.041	0.068 ± 0.04	0.13 ± 0.022	0.12 ± 0.046	0.12 ± 0.036	30 ± 7	0.85%
91.06	$C_4H_{10}S$	Diethyl sulphide	Organosulfur		0.1 ± 0.021	0.1 ± 0.055	0.12 ± 0.016	0.39 ± 0.057	0.16 ± 0.018	27 ± 19	0.77%
93.03	C ₆ H ₄ O			Х	0.24 ± 0.044	0.35 ± 0.087	0.27 ± 0.054	0.25 ± 0.045	0.24 ± 0.029	27 ± 4.7	0.77%
63.04	$C_2H_6O_2$	1,2 Ethanediol	Diol	Х	0.3 ± 0.15	0.36 ± 0.14	0.23 ± 0.069	0.23 ± 0.087	0.23 ± 0.039	19 ± 4.3	0.55%
157.15				Х	0.14 ± 0.036	0.2 ± 0.048	0.18 ± 0.02	0.18 ± 0.034	0.14 ± 0.049	17 ± 2.9	0.50%

77.05	C.H.O.			x	0.082 ± 0.011	0.13 ± 0.031	0.1 ± 0.02	0.098 ± 0.02	0.08 ± 0.02	12 ± 2.6	0.36%
111.08	CaHuoO			x	0.056 ± 0.011	0.061 ± 0.007	0.069 ± 0.014	0.083 ± 0.011	0.066 ± 0.022	12 ± 1.9	0.35%
63.02	C ₂ H ₁₀ O	Dimethysulfide	Organosulfur	X	0.049 ± 0.018	0.024 ± 0.014	0.043 ± 0.009	0.039 ± 0.01	0.039 ± 0.014	9.9 ± 2.4	0.29%
111.07	021100	Diniculysunde	organosunur	x	0.044 ± 0.008	0.048 ± 0.005	0.05 ± 0.011	0.071 ± 0.011	0.05 ± 0.012	9.5 ± 1.9	0.27%
111.04	C/H/O			X	0.044 ± 0.025	0.032 ± 0.018	0.046 ± 0.017	0.032 ± 0.012	0.042 ± 0.026	8.7 ± 1.5	0.25%
135.10	C10H14	n-cymene	Aromatic		0.023 ± 0.007	0.032 ± 0.021	0.047 ± 0.016	0.027 ± 0.006	0.05 ± 0.04	8.0 ± 2.7	0.23%
157.11	010114	p cymene	Thomate	x	0.028 ± 0.003	0.038 ± 0.016	0.033 ± 0.01	0.079 ± 0.012	0.038 ± 0.002	7.9 ± 3.7	0.23%
137.10	C10H16	Monoterpenes		21	0.032 ± 0.02	0.025 ± 0.017	0.042 ± 0.021	0.027 ± 0.014	0.03 ± 0.019	6.2 ± 1.3	0.18%
75.06	C ₃ H ₉ NO		N containing compound	X	0.049 ± 0.009	0.06 ± 0.012	0.064 ± 0.012	0.064 ± 0.011	0.048 ± 0.014	5.9 ± 0.83	0.17%
121.10	C ₉ H ₁₂	Propylbenzene/cumene/mesytilene	aromatic		0.023 ± 0.011	0.02 ± 0.008	0.025 ± 0.007	0.021 ± 0.004	0.029 ± 0.016	4.9 ± 0.74	0.14%
92.05	C ₇ H ₈			Х	0.02 ± 0.005	0.011 ± 0.005	0.012 ± 0.004	0.011 ± 0.005	0.011 ± 0.003	4.8 ± 1.4	0.852%
127.10	C ₈ H ₁₄ O	Octenal/octenone	Carbonyl/ketone		0.017 ± 0.004	0.022 ± 0.004	0.019 ± 0.005	0.055 ± 0.009	0.026 ± 0.005	4.2 ± 2.3	0.12%
95.02	$C_2H_6S_2$	Dimethydisulfide	Organosulfur	Х	0.026 ± 0.005	0.034 ± 0.008	0.03 ± 0.007	0.046 ± 0.008	0.03 ± 0.01	4.1 ± 0.93	0.12%
113.05	$\mathrm{C_6H_{12}N_2}$		N containing compounds	Х	0.015 ± 0.003	0.016 ± 0.002	0.017 ± 0.004	0.021 ± 0.003	0.015 ± 0.005	3.1 ± 0.45	0.089%
80.03	C ₅ H ₅ N	Pyridine	Heterocyclic organo-compound (N containing compound)	X	0.018 ± 0.007	0.01 ± 0.006	0.018 ± 0.004	0.033 ± 0.008	0.019 ± 0.009	3.0 ± 1.3	0.088%
94.03	C ₆ H ₅ O		-	Х	0.009 ± 0.002	0.008 ± 0.004	0.01 ± 0.001	0.028 ± 0.005	0.012 ± 0.002	2.1 ± 1.3	0.060%
221.15				Х	0.021 ± 0.002	0.011 ± 0.003	0.02 ± 0.005	0.013 ± 0.002	0.013 ± 0.005	2.1 ± 0.56	0.059%
63.00				Х	0.008 ± 0.001	0.009 ± 0.003	0.009 ± 0.002	0.013 ± 0.002	0.007 ± 0.001	1.7 ± 0.4	0.050%
113.01	$C_6H_{12}N_2$		N containing compounds	Х	0.007 ± 0.002	0.004 ± 0.001	0.005 ± 0.001	0.004 ± 0.002	0.004 ± 0.001	1.0 ± 0.27	0.030%
101.00					0.006 ± 0.003	0.004 ± 0.001	0.008 ± 0.004	0.007 ± 0.002	0.006 ± 0.004	1.0 ± 0.24	0.029%
136.02				Х	0.011 ± 0.001	0.006 ± 0.004	0.011 ± 0.002	0.012 ± 0.002	0.008 ± 0.002	0.99 ± 0.25	0.029%
110.06	C ₆ H ₇ NO	Benzamide	Amide (N containing compound)	X	0.003 ± 0.001	0.003 ± 0.0	0.004 ± 0.001	0.008 ± 0.001	0.005 ± 0.001	0.83 ± 0.39	0.024%
76.03	$C_2H_5NO_2$		N containing compound		0.005 ± 0.0	0.001 ± 0.001	0.004 ± 0.003	0.007 ± 0.002	0.003 ± 0.002	0.48 ± 0.29	0.014%

42.00	C ₂ OH	Hydroxymethyl	Х	0.001 ± 0.00	$1 0.002 \pm 0.002$	NA	NA	NA	$\begin{array}{c} 0.041 \pm \\ 0.064 \end{array}$	0.001%
1	_									

¹average and standard deviations over all treatments.

Table III- 1. VOCs identified as most emitted, most contributing to the BCA and most explaining the variance in the ANOVA test. The compounds are sorted in descending order of average emission rates in mass for all treatments.

III.4 Discussion

II.4.1 Identification and quantification of VOCs emitted

III.4.1.1 <u>Most emitted VOCs: acetone, butanone, acetaldehyde, methanol, butene, and</u> <u>ethanol</u>

This study was focused on VOCs emissions from soils regularly amended with 4 different organic waste products compared to unamended soil. However, the soils were sampled two years after the last OWPs application. Thus the differences in VOCs emitted could be associated to long-lasting effects of the OWP applications and to changes in soil organic matter characteristics due to these repeated OWP applications. We first notice that in all treatments including the control, the 3 most emitted compounds were acetone, 2-butanone and acetaldehyde regardless of amendments.

This study showed acetone emissions of $1000 \pm 240 \ \mu g \ m^{-2} \ h^{-1}$. Smaller acetone emissions are usually reported from laboratory experiments such as those by Asensio et al. (2007a) for a Mediterranean soil who found 10 times smaller fluxes than what we measured here. On the contrary, Schade and Goldstein (2001) reported acetone emissions in the same range as our study using a field chamber and a dual GC-FID system on a ponderosa pine plantation; they indeed found peak emissions of 800 μ g m⁻² h⁻¹ following rain but smaller emissions 260 μ g m⁻² h⁻¹ on average. Peñuelas et al. (2014), in their review, reported emissions ranging from 4 to $800 \ \mu g \ m^{-2} \ h^{-1}$. Acetone is often observed in rural areas (Lamanna and Goldstein, 1999). Acetone emissions seem to be mainly driven by biogenic sources such as litter decomposition (Gray et al., 2010), microorganisms in soils (Peñuelas et al., 2014) but also functioning leaves (Mozaffar et al., 2018). However, acetone was also found to be produced from abiotic degradation of plant material in the soil at a rate of 100 µg g⁻¹DM by Warneke et al., (1999). Emissions of acetone from soils depend on moisture, temperature and on the technique used to detect the fluxes which could explain the differences between the fluxes reported in the studies cited previously (Peñuelas et al., 2014). Organic matter seem to enhance acetone emissions as reported by Zhao et al. (2016) who found that acetone was the most emitted compound from soil amended with straw contributing to $50 \pm 9\%$ of the total VOCs emissions. For comparison, in our study acetone contributed to around 30% of the total mass of VOC emissions (Table III-1).

Butanone was the second most emitted compound from our samples contributing up to 25.1% of the total VOCs emissions. Zhao et al. (2016) also reported butanone emissions with a contribution of $9 \pm 2\%$ to the total VOCs emissions. McNeal and Herbert (2009) also detected butanone production by soil microcosm, while Larsen and Frisvad (1995a; 1995b) showed that butanone was one of the VOCs emitted from fungi. Apart from these references butanone was not detected in other studies, at least not as one of the major compound emitted such as we found here.

The third most emitted compound from soil was acetaldehyde which was detected by Asensio et al. (2007a) as the second most emitted compound from Mediterranean soil samples. Schade and Goldstein, (2001)detected a flux of acetaldehyde of 100 μ g m⁻² h⁻¹ at 24°C quite similar to what we found ~280 μ g m⁻² h⁻¹. Similar to this study, though smaller, Peñuelas et al., (2014) reported between 2 and 100 μ g m⁻² h⁻¹ emissions of acetaldehyde for different type of soil, such as: forest soil, ponderosa pine plantation and shrubland soil. Gray et al., (2014) reported much smaller emissions (0.2 μ g m⁻² h⁻¹), but they demonstrated that acetaldehyde emissions were largely coming from the roots system which was partly removed from our measurements by sieving at 2 mm. Mancuso et al. (2015) also detected acetaldehyde as the most emitted compound. It is noticeable that acetaldehyde with PTR-MS (not TOF ones) may catch some CO₂.H⁺ and hence include some soil respiration. Indeed, although CO₂ has a proton-affinity lower than the one of water, we found that CO₂ was protonated in our TOF at m/z = 44.997 (Loubet, personal communication).

Methanol, isoprene and ethanol were also detected with a significant emission rate in this study (**Table III- 1**). Methanol has been reported by Mancuso et al. (2015), Seewald et al. (2010) and was the compound most emitted as reported by Asensio et al. (2007) and Ramirez et al. (2010). Peñuelas et al., (2014) reported emissions in field conditions ranging from 4 to 530 μ g m⁻² h⁻¹, while Bachy et al., (2018) found a range from -50 to 200 μ g m⁻² h⁻¹, which agreed with Schade and Custer, (2004) measuring the emissions from a bare soil. Our results (~ 66 μ g m⁻² h⁻¹) are in good agreement with the cited studies. Ramirez et al. (2010) showed that methanol contributed to 89% of the total VOCs emissions by a forest soil while in our study methanol emissions contributed around 3.1% of the total mass VOCs emissions only. Methanol was also reported by Potard et al. (2017) as one of the most emitted compounds by soil. In fact it contributed between 20% to 40% of the total VOCs emissions in the control treatment and between 20% and 90% in soil amended with methanized pig slurry (Potard et al., 2017). Several studies showed different methanol sources from soil, i.e. abiotic production

through SOM degradation (Schade and Custer, 2004), biotic production from the litter (Ramirez et al., 2010, Bachy et al., 2018). Methanol might be produced via the microbial breakdown of plant pectin (Jayani et al., 2005). The small amount of plant residues in our samples may explain the lower contribution of methanol to VOCs emissions compared to Potard et al., (2017) and Ramirez et al., (2010). It is also important to stress that methanol, as a soluble compound is adsorbed and desorbed from water films (Laffineur et al., 2012). This mechanism may have boosted emissions due to water evaporation in the chamber exposed to dry air.

Butene contributed for the 5.3% of the total VOCs emissions. Emissions smaller than 2 μ g m⁻² h⁻¹ were detected from forest floor (Hellén et al., 2006) and from agricultural soils amended with straw (Wang et al., 2015) with a contribution between 3-7 % of the total emissions. Wang et al., (2015) also reported a positive correlation between butene and microbial biomass.

Ethanol (4.3% of the total emissions in our study, see **Table III-1**) is one of the compounds emitted during the sugar degradation as showed by Seewald et al. (2010). Schade and Goldstein (2001) reported emission of ethanol from bare soil between 300 and 500 μ g m⁻² h⁻¹ while we found lower emissions around 100 μ g m⁻² h⁻¹).

III.4.1.2 Other compounds detected

Several aromatic compounds have been observed as emitted from our soil samples. Microorganisms and plants release aromatic compounds through the shikimate pathway or by the degradation of phenylalanine or tyrosine. Aromatic compounds are known to be emitted by plants under stress conditions (Misztal et al., 2015) and absorbed by humic substances in soil (Lu and Pignatello, 2004). Toluene is one of the aromatic compounds most emitted by our samples (at a rate of 1.2%), but in a similar amount between treatments which was also reported by Asensio et al. (2007b). Toluene is rather a compound linked to oil, either natural (crude oil) or from derivatives, such as solvents, gasoline or other fuels as well as in the production process of coke from coal. The flux of this compound from soil is always very low but due to the several impacts that this compound might have on the health is important to quantify and characterize the emissions. It is interesting to notice that we also detected monoterpenes (Gratien et al., 2011). We found around 0.018 nmol m⁻² s⁻¹ which is higher than the range of monoterpenes emissions detected by Asensio et al. (2007a) (between -0.004 nmol m⁻² s⁻¹). Hayward et al. (2001) reported that emissions of

monoterpenes from soil of Sitka spruce forest were 0.026 nmol $m^{-2} s^{-1}$, and were strongly related to soil temperature (Raza et al., 2017b). It is also known that monoterpene emissions from forests exceed those from agriculture (Kesselmeier and Staudt, 1999). Assuming soil emissions are linked to plant residues degradation in the soil this might explain the higher emissions from forest's soils compared to what we find here.

Other noticeable compounds detected are the organosulfur compounds such as dimethylsulfide, dimethethydisulfide and diethylsulfide. Other studies have reported emissions of these compounds from soils. For instance, Potard et al. (2017), Veres et al. (2014), Zhao et al. (2016), Mayrhofer et al. (2006), and (Raza et al., 2017b)) reported emissions of dimethylsulfide from soils. Dimethethydisulfide is a fungistatic produced by *Bacillus, Pseudomonas* and *Actinomycetes* (Schöller et al., 1997; Wilkins, 1996). It represents also a precursor of atmospheric aerosols which hence has impacts on climate and health (Ayers and Cainey, 2007).

Propene is also in our list of characteristic compounds and was reported by Zhao et al. (2016) from amended agricultural soil as one of the most emitted compounds with a significantly positive correlation between this compound and bacterial communities in soil. In Zhao et al. (2016), propene contributed to 1.1% of the total emissions similar to what we found in this study (2.0 %).

Isoprene was reported as a compound emitted by agricultural soils amended with straw at only 0.1% of the total emissions by Zhao et al. (2016). Our results showed that around 6% of the total VOCs emissions were represented by isoprene emissions. Mancuso et al. (2015) measured isoprene emissions from agricultural and forest soil samples. Isoprene is a metabolite directly related to the presence of microorganisms in soil even if the role of this compound in microorganisms cell has not been clarified yet (Hess et al., 2013).

The VOCs emitted from the soil samples may be derivated from the microbial activity in soil. In fact, different VOCs derive from several metabolic pathways. Fermentative pathways such as ethanol fermentation, with acetaldehyde as an intermediate product, and butyric acid, acetone and methanol fermentation take part of the microorganisms' primary metabolism (Raza et al., 2017b). On the other hand, more complex molecules with a higher molecular weight may derive from secondary metabolism pathways, such as production of signaling compounds or antibiotics (Mathivanan et al., 2008). Some VOCs produced by these pathways are 2,4-diacetylphloroglucinol, 3-4-dihydroxycarotane and trichodermin.

It is important to bear in mind that in this study measurement of VOCs emissions is not bi-directional since the inlet was synthetic air which was free of VOCs. This may have led to consistent differences when compared to other in-situ measurements that captured the bidirectionality of some fluxes (e.i. Mozaffar et al., 2018). Moreover, we should also stress that evaluations of the percentage of total VOC emissions are affected by the number of peaks identified. Indeed, we could expect higher percentages by studies that use PTR-MS which detect a smaller number of peaks than those based on PTR-TOF that screens all peaks.

III.4.2 Effects of organic waste product applications on VOCs emission rates by soils

Total VOCs emissions were statistically higher in BIOW than in MSW (**Figure III- 3a**). Despite the fact that the amount of carbon imported in these two treatments was the same over 17 years, the I_{roc} indicator which is the fraction of carbon stored in the soil to that applied was found to be lower in MSW (49% Org C) than in BIOW (77% Org C) (Lashermes et al., 2009). As a consequence, the organic matter (OM) content was higher in BIOW compared to MSW (**Figure III- 2**). This suggests that total VOCs production is linked to OM content in soil. However, the OM content cannot be the only factor affecting total VOCs emissions. Otherwise, GWS, which has the highest OM content, should have had also the largest total VOCs emissions and it was not the case.

BCA and ANOVA analysis showed that emitted compounds from BIOW and GWS were the most differentiated. BIOW and GWS were also differentiated from CN, MSW and FYM emissions, which were grouped. It is noticeable that the same classification was also found in the C/N ratio of the small particle organic matter (POM) fraction reported by Paetsch et al., (2016) on the same site. Indeed, they found higher C/N ratios of these fractions in CN, MSW and FYM. Higher C/N ratios indicate higher organic matter degradability. This would mean that the VOC compounds that are mostly associated with CN, MSW and FYM treatments may be more representative of the degradation of the small fraction of POM than those measured in the BIOW and GWS treatments.

Overall, BIOW emitted more alcohols and alkenes, N compounds and terpenes than the other treatments, while GWS mainly emitted more aromatic compound (**Figure S2**). Peñuelas et al., (2014) reported that bacterial VOCs emissions contain mostly alkenes, ketones, terpenes and pyrazines while fungi emit more aldehydes, benzenoids, nitriles and alkynes. We could hence argue that BIOW promotes bacterial-mediated VOCs emissions as shown by the dominance of alkenes and terpenes, and also by the larger total VOC emissions (**Figure III**-

3a). Since BIOW has also the lowest VOC diversity index (**Figure III- 3b**); this suggests that BIOW may promote more specific VOC emission processes than other treatments.

A notable difference in the measured soil characteristics between BIOW and GWS treatments is the soil pH. Since soil pH affects nutrient availability and the physiological state of microorganisms, we could also expect that pH may affect VOCs emission profiles. Insam and Seewald, (2010) reported that VOCs are largely absorbed by the mineral and organic fraction of soil. This adsorption depends on soil pH, with higher adsorption in alkaline soils and lowers in acid soils. We tend to observe a complex dependency to pH which also interacts with organic matter. The total emission of VOC tends indeed to increase with the product of pH with organic matter (**Figure S3**).

Furthermore, it has been shown by Seewald et al. (2010) that anaerobic conditions lead to increased amount and diversity of VOCs emitted as opposed to aerobic conditions that leads to CO₂ as the end product of microbial decomposition. The increase of soil organic matter in treatments receiving OWP is known to improve soil structure and aggregate stability (Diacono and Montemurro, 2010). Gupta and Larson, (1979) have shown that OM content increases water soil retention which may promote anaerobic conditions in microsites and hence modify VOC emissions diversity and amounts. OM content may also modify the porosity and hence gas diffusivity which can affect the emission and storage processes of VOCs in soil system (Peñuelas et al., 2014). One should however keep in mind that the soils used in our study were sieved before VOCs analysis, which would suppress any effects of OM on soil porosity above 2 mm.

According to the BCA and ANOVA analysis, GWS and BIOW have different VOC emission spectra: 21 compounds were found to have statistically different emissions between the treatments. BIOW released more acetone, more 74.06 m/z (unidentified compound), and $C_3H_8O_2$ (77.05 m/z). At this stage we are not able to say why the BIOW samples released these three compounds in higher quantities than the other samples. What we can conclude is that the compounds cited above can be used as marker compounds for the bio-waste treatment. The GWS treatment has other marker compounds such as: 94.03 m/z, 95.02 m/z, 110.06 m/z (benzamide), 111.07 m/z and 113.01 m/z. Benzamide is an intermediate compound produced by microorganisms during the degradation of 2,6-dichlorobenzonitrile which is a persistent herbicide in soil (Holtze et al., 2006). The last herbicide application was two years before the sampling period. The content of benzamide could be related also to the herbicide present in the green wastes used for the GWS compost. Similarly, CN can be

differentiated from the other treatments with high emissions of 136.02 m/z and 221.15 m/z. For the FYM and MSW no distinctive compound was found.

III.5 Conclusions

VOCs emissions by soils are involved in atmospheric chemistry and climate change. This study underlines the importance of the VOCs emissions characterization from soil in order to fill the lack of information in this field. Our results showed that VOCs emissions were affected by the type of OWP amended to soils. The BIOW compost emitted the highest total flux rate, while MSW compost the lowest. Our results further suggested that organic matter content and pH jointly influenced total VOC emissions, with larger emissions for higher joint pH and OM content.

The most emitted compounds were acetone, butanone and acetaldehyde whatever the OWP amended, suggesting a universal production mechanism for these compounds little affected by the OWP amendment. On the contrary, we found that 21 compounds had statistically different emissions between the treatments, and could be hence considered as good markers of soil biological functioning. Our results also suggest that the VOC emissions profiles are linked to the C/N ratio of the small fraction of particulate organic matter which is an indicator of OM degradability. It is however difficult to draw conclusions concerning the biological pathway (i.e. degradation of sugar, mineralization of the organic matter ;...) leading to VOCs emissions observed. Other analysis of microorganisms' activity and diversity are necessary to unveil these aspects.

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Chapter IV

Volatile organic compounds emissions from soils are linked to the loss of microbial diversity

Volatile organic compounds emissions from soils are linked to microbial diversity

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Abstract

Biogenic volatile organic compounds (bVOCs) emissions are essential players of atmospheric chemistry. Within the sources of bVOCs microorganisms and soils have been less characterized in term of impact on the ecosystems and on atmospheric chemistry than plants, which are greater VOCs releasers. The role played by microbial VOCs on soil ecosystem has been increasingly demonstrated in recent years. Even though, little is known about the influence of the microbial community structure and diversity on VOCs emissions. In this study, we manipulated the microbial diversity level by diluting and measured the effects on VOCs emissions using a highly sensitive PTR-QiTOF-MS. Soil microcosms were prepared with three microbial dilution levels on soils amended with organic waste products. VOCs emissions and microbial RNA were measured after six weeks incubation. We found that microbial diversity in soil reduced VOCs emissions and increased VOCs diversity. Furthermore, we found that *Proteobacteria* and *Bacteroidetes* phyla were VOCs emitters while other bacteria were both absorbing and emitting VOCs.

IV.1 Introduction

Volatile organic compounds (VOCs) have a large influence on atmospheric chemistry. In particular, VOCs play a role in atmospheric photochemistry by reducing hydroxyl (OH) concentration, increasing tropospheric ozone (O_3) and stimulating the secondary organic aerosol (SOA) in the atmosphere (Atkinson, 2000). VOCs are released from biogenic (i.e., plant, forests, soils) and anthropogenic (i.e., industries, solvents, transportation) sources. Biogenic VOCs contribute up to 90% of the total VOCs emissions (Guenther et al., 1995), with isoprene as the most emitted compound and broadleaves forest dominating the budget (Guenther et al., 2006). Recent studies have been focused on biogenic VOCs released by aboveground biomass because of their contribution to atmospheric chemistry (Karl et al., 2009; Lindfors and Laurila, 2000; Zemankova and Brechler, 2010). Often, VOCs fluxes by soil, and in particular by microorganisms, are not directly considered. This is illustrated for example by the most used Guenther global emission model which does not include soil emissions, although it is based on Volatile organic compounds (VOCs) have a large influence on atmospheric chemistry. In particular, VOCs play a role in atmospheric photochemistry by reducing hydroxyl (OH) concentration, increasing tropospheric ozone (O₃) and stimulating the secondary organic aerosol (SOA) in the atmosphere (Atkinson, 2000). VOCs are released from biogenic (i.e., plant, forests, soils) and anthropogenic (i.e., industries, solvents, transportation) sources. Biogenic VOCs contribute up to 90% of the total VOCs emissions (Guenther et al., 1995), with isoprene as the most emitted compound and broadleaves forest dominating the budget (Guenther et al., 2006). Recent studies have been focused on biogenic VOCs released by aboveground biomass because of their contribution to atmospheric chemistry (Karl et al., 2009; Lindfors and Laurila, 2000; Zemankova and Brechler, 2010). Often, VOCs fluxes by soil, and in particular by microorganisms, are not directly considered. This is illustrated for example by the most used Guenther global emission model which does not include soil emissions, although it is based on whole canopy flux measurements (Guenther et al., 1995). Bachy et al., (2016) underlined that VOCs from vegetation could be overestimated compared to soil emissions; reporting comparable fluxes of methanol from bare soil and canopy. Moreover, VOCs emissions by soils are affected by agricultural practices. Woodbury et al., (2016) reported higher fluxes of volatile sulfur compounds under no-tillage condition than tilled one; also underlining the important role played by the organic amendment on the VOCs emissions. Furthermore, the role of the organic amendments on

VOCs emissions by soil have been reported by several recent studies (Abis et al., 2018; Potard et al., 2017; Raza et al., 2017; Woodbury et al., 2016). VOCs emissions may be boosted by organic amendment, due to soil organic matter (SOM) content and soil microbial biomass increase compared to soil receiving mineral amendments (Girvan et al., 2004). Among the different organic amendment, organic waste products (OWPs) resulting from human activities (i.e., sewage sludge, municipal solid waste composts, farmyard manure) are being increasingly used since they facilitate the recycling of nutrients and improve soil fertility. OWPs are also responsible for increasing soil microbial diversity in soil (Sadet-Bourgeteau et al., 2018) due to the incorporation of exogenous microorganisms colonizing the organic amendment (García-Gil et al., 2007). Microbial diversity in soil ensures several soil ecosystems functions such as (i) carbon (C) balance between organic C sequestration and C mineralization (Orgill et al., 2017), (ii) organic matter break down (Baumann et al., 2013), and more generally, (iii) nutrient recycling. Initially, no importance was given to preserve the microbial diversity since the functional redundancy ensured ecosystem functions. In other words, Species colonizing an ecosystem and contributing equally to an ecosystem function can be substituted by another (Chapin et al., 1997). Recently, it has been demonstrated that independently of their redundancy, functions of soil organic matter decomposition are affected by a reduction in microbial diversity (Baumann et al., 2013; Philippot et al., 2013). Additionally, microorganisms are directly involved in the production of VOCs emitted by soil (Stahl and Parkin, 1996) since they are involved in several processes as the communication between organisms (Wenke et al., 2010), alterations of microbial nutrient cycles (Bending and Lincoln, 2000) and growth rate of microorganisms colonizing the ecosystem (Stotzky and Schenck, 1976). For instance, it has been reported that VOCs production by bacteria living in soil suppresses the growth of specific fungal phytopathogens (Kai et al., 2007). Other studies suggested that microbial VOCs may also be able to indirectly influence plant growth rate by inducing systemic tolerance to abiotic stresses (i.e., drought, heavy metals) in plants (Farag et al., 2013). Microbial community is not only a source of VOCs but also a sink which means that microorganisms use VOCs as a nutrient source. For example, Paavolainen et al., (1998) reported an increase in respiration rate and microbial biomass by introducing a high concentration of VOCs in an in vitro soil incubation experiment. Another important information about microbial VOCs was reported by Isidorov and Jdanova, (2002) and Misztal et al., (2018) who demonstrated that the microbial VOCs emissions could also depend on the taxa. For instance, it has been shown that VOCs emissions from P. syringae were dominated by methanol while other microbial emissions were often dominated by compounds with higher carbon number (Misztal et al., 2018)

Up to our knowledge, no studies reported VOCs emissions measurements in response to microbial diversity and organic waste amendment in the soil. Hence, the effect of microbial diversity dilution on VOCs emissions is still an unexplored land. Since microorganisms play a central role in soil VOCs emissions, we hypothesized that the manipulation of the microbial community in soil might lead to some variation in the quantity and quality of the VOCs emissions.

The manipulation of the microbial diversity was performed by inoculating 30 g of sterile soils with three dilution levels of a soil microbial suspension taken from the same soil. After six weeks incubation at water holding capacity and 20°C in the dark, VOCs emissions were measured with a dynamic chamber method using a highly sensitive proton transfer, quadrupole injection, time of flight mass spectrometer (PTR-Qi-TOF-MS, Ionicon). The VOCs concentration was measured every second during 3 min for each sample (n=45, replicates= 3). The mass spectra were analyzed using PTR-MS viewer-3 software in order to integrate each ion peak. The microbial biodiversity was measured after VOC emission measurements by a high throughput sequencing approach targeting 16S and 18S ribosomal genes. Four soils were selected that had 20 years of differentiated OWPs amendments together with a control soil that received no organic inputs during the same period were selected. Three replicates of each combination of soil and dilution were used for providing enough data for statistical analysis. All statistical analyses were performed with R (R Version 1.0.153 – © 2009-2017 R-Studio). In particular, ANOVA followed by a post hoc Tukey test were performed as well as principal component analysis (PCA, Package Ade4).

IV.2 Results

IV.2.1 Soil microbial biomass

After six weeks of incubation, microbial biomass was not significantly different between microbial dilution levels for each type of OWPs except for FYM (farmyard manure), where the higher dilution level had the higher microbial biomass (**Table IV-1**). A normalization of the VOCs emissions by the biomass levels showed no significant difference in the variation of VOCs emissions with dilution compared to non-normalized data. This indicates that these small differences in microbial biomass did not affect conclusions on VOC emissions. Whatever the dilution levels considered, the microbial biomass was statistically higher in MSW (municipal solid waste compost derived from the composting of residual solid wastes after removing dry and clean packaging), FYM and GWS (compost derived from the co-composting of green wastes with sewage sludge) than CN (control without organic input) and BIOW (bio-waste compost derived from the co-composting of green wastes and source-separated organic fractions of municipal solid wastes).

Organic Waste Product	Dilution level	Biomass μg DNA g sol ⁻¹ ± sd	Tukey test
	D0	13.52 ± 2.41	а
Control without organic input	D1	12.48 ± 2.09	а
	D2	12.69 ± 2.30	а
	D0	11.24±1.07	а
Biowaste	D1	15.83±1.33	а
	D2	17.38±2.77	а
	D0	16.25 ±4.35	а
Farmyard manure	D1	21.22 ± 2.20	ab
	D2	28.52 ± 3.48	b
	D0	18.30 ± 1.18	а
Green waste and sludge	D1	20.26 ± 4.78	а
	D2	21.99 ±3.77	а
	D0	22.31 ±2.12	а
Municipal solid waste	D1	21.52 ± 1.19	а
	D2	23.97 ± 1.39	а

Table IV-1. Microbial biomass in soil samples. D0: microbial dilution equal to 1. D1: microbial dilution equal to 10^{-5} .

IV.2.2 Microbial diversity

The bacterial Shannon diversity index is a measure of bacterial diversity. It shows a significantly decreasing of bacterial diversity with increasing dilution (p.value < 0.05), confirming the efficiency of the diversity manipulation (**Figure IV-1**). The dilution of diversity was also efficient for the FYM samples, even though a higher biomass level was found for the highest dilution (D2, **Table IV-1**). For fungi, the Shannon index revealed a larger number of fungi phyla in the higher dilution level, which was unexpected (**Figure IV-S1**). In our microcosms the number fungi:bacteria ratio was, however, smaller compared to the number of fungi to bacteria ratio which was lower than previously observed in similar studies which was always higher than 0.35 (Maron et al., 2018) (**Figure IV-S2**).



Figure IV-1. Shannon index for bacteria in the soil for each dilution level. D0: microbial dilution equal to 1. D1: microbial dilution equal to 10^{-3} . D2: microbial dilution equal to 10^{-5} . Bold line=median, boxes= interquartile, whiskers = minimum and maximum. Point = Shannon Index of each sample. Letters indicate significant differences according to the Tukey test with p-value > 0.05.

As expected, the diversity manipulation tended to strengthen the presence of the most abundant bacteria species and reduce the presence of the less abundant ones. This was indeed observed with phyla *Proteobacteria* and *Bacteroides* which were the most abundant in the lowest dilution samples and showed an increased abundance in the highest dilution samples. *Firmicutes, Crenarchaeota, Actinobacteria, Thaumarchaeota, Planctomycetes, Gemmatimonadetes,* and *Acidobacteria* showed the opposite behavior **Figure IV-** 2**a**. Concerning fungi, this was not the case: Basidiomycota, *Chytridiomycota, Glomeromycota,* and *Neoclimastigomycota* were more abundant in the highest dilution samples than in the lowest ones, although they were not the most abundant fungi in D0 (**Figure IV-** 2**b**). The contrary was observed for *Ascomycota* and *Blastoclamidiomycota* which were the most present in D0.



Figure IV- 2. Relative abundances of the phylum. (a) Relative abundances of the bacterial phyla. (b) Relative abundances of the fungal phyla. D0: microbial dilution equal to 1. D1: microbial dilution equal to 10^{-3} . D2: microbial dilution equal to 10^{-5} .

IV.2.3 VOCs emissions from manipulated soils

Total VOCs emissions increased with dilution rates and hence decreased with microbial diversity in soil (**Figure IV-** 2). The higher dilution level reported a total VOCs flux between 0.5 and 3 times higher than the lower dilution levels. This was true for all the considered soils (**Figure VI-S3**, Tukey test on OWPs). This meant that the effect of microbial dilution in driving soil VOC emissions overpasses the effect of OWPs amendment. The VOCs diversity (Shannon index on VOCs emissions) was lower in the highest dilution, meaning that the larger total emission rates were combined with a lower number of VOCs emitted (**Figure VI-S4**).



Figure IV-3. Total VOCs emissions rate per microbial dilution in soil. Bold line=median, boxes= interquartile, whiskers = minimum and maximum, empty points (white) = outliers, points (grey) = value of the total VOCs emissions for each sample. Letters indicate significant differences according to the Tukey test with p.value >0.05. D0: microbial dilution equal to 1. D1: microbial dilution equal to 10^{-3} . D2: microbial dilution equal to 10^{-5} .

The diversity of the VOCs emitted analyzed by PCA (**Figure IV-4**) also showed that the highest dilution level (D2) was isolated from the other microbial dilution levels while microbial dilution levels D0 and D1 had similar VOCs profiles. Several compounds are responsible for the differences in microbial dilution. Tentative identification of the 15 compounds explaining the variance in Figure 4 was reported in **Table IV-S1**, together with the 50 most emitted compounds in all dilution levels. These compounds contribute to almost 99% of the total emissions rate. The most emitted compounds were m/z 121.097 (tentatively identified as Propylbenzene, isopropylbenzene or 1,3,5-trimethylbenzene), m/z 93.066 (Toluene), m/z 107.081 (tentatively identified as xylenes, ethylbenzene or benzaldehyde) and m/z 135.113 (p-cymene) and represented 70% of the total VOCs emission rate (**Table IV-S1**). Significant results underlined by **Table IV-S1** concerned the emissions of acetaldehyde, butanone, and acetoin that were emitted in double quantities in samples with lower dilution diversity levels compared to samples with higher dilution levels.



Figure IV-4. Effect of the different microbial dilutions on VOCs emissions by soil. The m/z of the 20 compounds that are explaining the variance in the two first components are shown on the graph. The percentage of the variance explained by the 2 first components is shown on each axis. The ellipses represent the similarity between samples. Samples in the same ellipsis are more similar than samples displayed in two different ellipses. D0: microbial dilution equal to 1. D1: microbial dilution equal to 10^{-3} . D2: microbial dilution equal to 10^{-5} .

By correlating the VOCs emissions with the bacterial and fungi phyla abundance, we found that in general VOC emission rates were negatively correlated with bacterial phyla except for *Bacteroides* and *Proteobacteria* which were positively correlated with almost all compounds (**Figure IV-5**). An opposite behavior was observed for the fungus phyla with positive correlations except for *Cryptomicota*. This result suggests that most bacteria were VOCs consumers while fungus were mostly VOCs emitters, although we cannot tell in which proportions. VOCs profiles of bacteria phyla showed higher consumption of VOCs than the fungal phyla.



(To be continued in the next page)





IV.3 Discussion

IV.3.1 Microbial diversity

It was found that, after six weeks of incubation, the microbial biomass was similar in all dilutions levels except for FYM samples. As previously reported by Bevivino et al. (2014), pioneer species can colonize the microcosms faster than others, especially in reduced competition conditions, such as we have generated in D2. The larger biomass observed in the highest dilution level in FYM microcosms could hence result from a faster colonizing process promoted by reduced competition conditions and specific to that soil. Nevertheless, Tukey tests confirmed that the decrease of bacteria phyla diversity was effective in all D2 trials compared to D0. This decrease was explained by a lower abundance of phyla reported to be mainly composed of slow-growing organisms such as *Acidobacteria, Actinobacteria, Planctomycetes*, and *Gemmatimonadetes* (Fierer et al., 2007), and an increased dominance of phyla described as fast-growing such as *Proteobacteria* and *Bacteroidetes* and *Firmicutes* (Fierer et al., 2007).

IV.3.2 Interactions between bacteria and fungi diversity

The increase of biomass for some fungi phyla and the larger fungi diversity observed in the highest dilution level D2 might also be the consequence of interactions between fungi and bacteria. Indeed, Mille-Lindblom et al., (2006) reported that fungi, grown up in a substrate with a reduced competition with bacteria, had higher biomass than in substrates with higher competition with bacteria. Moreover, de Boer et al. (2003) and Garbeva et al., (2011) reported that bacterial secondary metabolisms produced fungistatic VOCs limiting the colonization of the microcosms by fungi. More specifically, Chuankun et al., (2004) found that some VOCs were only found in strongly fungistatic soils and others VOCs had higher concentrations in fungistatic soils (Table S2). The bacteria emitting fungistatic volatiles span a wide phylogenetic spectrum (Chuankun et al., 2004). Campos et al. (2010) further reported that several bacteria from the *Firmicutes* phylum inhibited the growth of common fungal species. These findings have been supported by Zou et al., (2007) who observed that the benzaldehyde produced by *Bacillus* sp. (*Firmicutes* phylum) was one of the inhibitors of the fungi mycelia growth. Our results reported a higher relative abundance of *Firmicutes* in the lowest dilution level D0 compared to D2 (**Figure 2**). Higher emission rate of the mass tentatively identified as benzaldehyde in the higher dilution level $(32.69 \pm 6.16 \text{ nmol s}^{-1} \text{ g}^{-1} \text{ DW} \times 10^4)$ than in the lower one $(5.66 \pm 8.2 \text{ nmol s}^{-1} \text{ g}^{-1} \text{ DW} \times 10^4)$ were reported. At the same time, the higher dilution level reported the lowest fungi-bacteria ratio, which is perfectly in line with Campos et al., (2010) and Zou et al., (2007) results. We hence hypothesize that the higher production of the benzaldehyde probably produced by *Firmicutes* phylum might be responsible for a lower quantity of fungi in the higher dilution. Another compound that was probably responsible for the lower F/B ratio in the higher dilution level was dimethyl disulfide which was emitted 10 times more in the higher dilution level than in the lower one. Kai et al., (2009) have reported that dimethyl disulfide was an inhibitor of the mycelium growth.

IV.3.3 Microbial diversity affects VOCs total emission rates

Although Abis et al. (2018) reported a significantly higher VOCs emission rate for BIOW treatment, in this study, we noticed that the effect of the substrate on VOC emissions was negligible compared to the effect of the bacteria diversity (Erreur ! Source du renvoi introuvable.Erreur ! Source du renvoi introuvable. and S3-S5). Furthermore, in the previous study of Abis et al., (2018), using the same soil samples without performing the microbial diversity manipulation, it was found that the most emitted compounds were acetone, butanone, and acetaldehyde. In this study, we found the masses m/z 121.097 (tentatively identified as propylbenzene, isopropylbenzene or 1,3,5-trimethylbenzene), m/z 93.066 (Toluene), m/z 107.081 (tentatively identified as xylenes, ethylbenzene or benzaldehyde) and m/z 135.113 (p-cymene). Acetone was not emitted, or at least not between the 50 most compounds emitted which meant that its contribution to the total VOCs emissions was lower than 0.01%. Acetaldehyde and butanone were emitted 100 and 10 times less respectively, compared to Abis et al., (2018). Even for the most emitted compound, the influence of the microbial diversity in soil was more important than the OWPs amendment effect on VOCs emissions. It is important to note that differences on the VOCs emissions might also be due to a different samples treatment.

The three most emitted compounds reported in this study are aromatic compounds. It has been reported by Gosset, (2009) and Schmidt et al., (2015) that the emissions of aromatic compounds are released from the shikimate pathway leading to the production of aromatic aminoacids. In particular, this pathway is used by microorganisms in order to produce amino acids like phenylalanine, tyrosine, and tryptophan used to build proteins.

It has been shown by several studies that microorganisms are a source and a sink of VOCs (Müller et al., 2013; Schulz-Bohm et al., 2015). Furthermore, Paavolainen et al., 1998, in an *in vitro* soil incubation, reported that when VOCs were increased the respiration rate was higher, suggesting that VOCs were used as a nutrient source for some bacteria. In our study we found more than 250 VOC having a detectable flux. We found that VOC emissions rates increased with decreasing bacterial diversity. We further found that VOC emissions were positively correlated with only two bacterial phyla (*Proteobacteria* and *Bacteriodetes*), which also those were increased emission from these two phyla together with decreased absorption by other bacteria. VOCs are released either by the process of the primary metabolisms or by secondary metabolisms sources (Citron et al., 2012). Since *Proteobacteria* and *Bacteriodetes* are the most abundant phyla, the emissions of VOCs might be correlated to the secondary metabolism which is related to the colonization of the space within the microcosms.

IV.3.4 Bacterial and fungi VOCs emissions profiles

Bacteria and fungi are both capable of producing and consuming VOCs. The heatmap (Erreur ! Source du renvoi introuvable.) shows that some phyla are mostly positively correlated with VOC compounds emissions while others are mostly negatively correlated with VOC compounds emissions. Previous studies reported that the attribution of a unique VOCs emission pattern to a phylum was not possible (Bäck et al., 2010). For this reason, the aim of this study was not to find common intraspecific VOCs patterns. However, it is interesting to underline that the correlation between VOCs emissions and microorganisms have a lower correlation rate (Spearman coefficient <0.50) while the negative correlation was stronger (Spearman coefficient between -0.50 and -0.75). This meant that VOCs compounds that showed decreased emissions with increasing phyla abundance had a steeper decrease than those increasing. Mayrhofer et al., (2006) found, as in the present study, positive and negative correlation between microorganisms and specific VOCs, and hypothesized two possible VOC-bacteria interaction, for both positive and negative correlation. A positive correlation with a VOC can occur due to the emissions from a microorganism or due to a stimulated growth of the microorganisms where the VOCs is present, suggesting that microorganisms are using VOCs as a carbon source. A negative correlation may either result from the gradual exhausting of a VOC used by microorganism as a carbon source or the presence of a specific VOC might inhibit the presence of a microorganism.

IV.3.5 VOCs mediating interaction between phyla

VOCs were shown to be essential intermediates in the interactions between bacteria, playing a role together with the competition for nutrients (Schulz-Bohm et al., 2015). In particular, some VOC produced by bacteria having access to nutrients have shown to stimulate the activity, but suppress the growth of starved bacteria (Schulz-Bohm et al., 2015). Compounds tentatively identified in this study as 1,3-butadiene are known to be used as biocontrol agents against other bacteria. In particular, *Pseudomonas* sp. which form part of the *Proteobacteria* phylum can inhibit the growth of *Bacillus* sp, which form part of the *Firmicutes* phylum (Tahir et al., 2017). In this study, for the higher level of dilution, the higher flux rate of this compound was found.

Furthermore, in the same dilution level, the bacteria *Proteobacteria* phylum abundance increased while *Firmicutes* abundance decreased, giving indirect evidence of the effect of 1,3-butadiene in the interaction between these two phyla. Another interesting compound detected was acetoin. Acetoin was detected in the three dilution levels, with a contribution in the total emissions increasing from 0.1 - 0.8% in the lower dilution level to about 2% in the higher dilution level. The acetoin (3-hydroxy-2-butanone) is derived from the pyruvate fermentation under anaerobic conditions (Ryu et al., 2003), and was shown to increase the virulence factors of *Proteobacteria* in the colonization process (Audrain et al., 2015). Bunge et al., (2008) also found a positive correlation between the bacteria cell numbers of *Salmonella enterica* (*Proteobacteria* phylum) with the signal of an unidentified signal from the mass 89, which can be identified as the ionized acetoin. Furthermore, acetoin is a promoter of plants growth inducing systemic resistance in plants (Chuankun et al., 2004; Farag et al., 2013; Ryu et al., 2003). We could hence hypothesize that acetoin could have been a vector promoting *Proteobacteria*, in reduced microbial diversity conditions, which might have a positive effect on plants growth.

Our results also showed a double emission rate of acetaldehyde and butanone emissions in the higher dilution level compared to the lower ones. Bunge et al., (2008) reported a positive correlation between the number count of *Shigella flexneri* and *Salmonella enterica* (*Proteobacteria* phylum) cells and the emissions of acetaldehyde and butanone. Acetaldehyde and butanone emissions might also be correlated to the *Proteobacteria* phylum VOCs emissions.

IV.4 Conclusions and perspectives

The major important conclusion of this work is that decreases of the microbial dilution levels in soil increased VOCs total emissions rate while decreased the VOCs diversity. It is important to keep in mind that the experiment was under controlled laboratory conditions and studies on the field are needed in order to confirm our results. Furthermore, several microbial processes involved in the production of VOCs within the microcosms have been hypothesized, underlining the possibility of both VOCs production pathways: primary and secondary metabolisms production. Finally, *Proteobacteria* and *Bacteroidetes* might be phyla correlated with the emissions on VOCs from soils while other phyla seemed to be sources and sinks of VOCs at the same time.

IV.5 Methods

IV.5.1 Sampling and site description

Samples were collected in the QualiAgro site, a field station taking part of the SOERE-PRO-network (https://www6.inra.fr/qualiagro_eng/Nos-partenaires/The-SOERE-PROnetwork). The QualiAgro agronomic set up which is described in this study started in September 1998 and lasted until September 2015. The QualiAgro site is located at Feucherolles in northwestern France (35 km west of Paris; 48°52'N, 1°57'E, alt 150 m), on a silt loam textured soil. The soil is classified as a hortic glossic Luvisol (IUSS Working Group WRB, 2014), representative of the Parisian Basin. The main characteristics of these soils are represented by the lack of clay, a silt-loam texture (15.0% clay, 78.3% slit) and an initial pH of 6.9 in the surface horizon (0-30 cm) and good drainage. Moreover, the QualiAgro field experiment is in a cropland dominated region, which lead to a low organic carbon and low organic matter concentrations (initial content of 1.1%) (Meersmans et al., 2012).

The experiment was a randomized block design with 4 replicates comparing 4 organic waste products: BIOW (bio-waste compost derived from the co-composting of green wastes and source-separated organic fractions of municipal solid wastes), GWS (compost derived from the co-composting of green wastes with sewage sludge), FYM (farmyard manure) and MSW (municipal solid waste compost derived from the composting of residual solid wastes after removing dry and clean packaging); plus a control without organic input (CN). Samples were collected in 5 blocks of the site amended with mineral N in order to reach optimal N application. Since 1998, the organic waste products (OWPs) have been applied at a rate of ~4
$tC ha^{-1}$ every two years on the wheat stubbles in September, after harvesting. In each plot, 5 soil cores were randomly sampled at 0-30 cm depth using a core drill and stored in a cold chamber at 4°C prior to analysis. The sampling was performed in early September 2016, one year after the last amendment of OWPs.

IV.5.2 Microcosms and Experimental setup

Soil samples were sieved at 4 mm and homogenized before gamma-ray sterilization (35 kGy; Conservatome, Dagneux, France). The sterility of the irradiated soil was verified by spreading serial dilutions of the soil onto nutrient agar plates. After the sterilization process soils were inoculated with a diluted soil suspension prepared with the same soil before sterilization (Wertz et al., 2006). The soil suspension was created by mixing 30 g of soil with 90 mL of sterilized water. From this soil suspension that was used pure (10^{0}) two levels of dilution were prepared with a water ratio of $1:10^3$ for the first one and $1:10^5$ for the second one. The second step was the re-inoculation of the sterilized soil with the three different soil suspensions (pure or 10^{0} , 10^{-3} and 10^{-5}). In order to create the microcosms, 30 g of every sample were transferred in a flask, and we added 50% of the water necessary to reach the 60% of the water holding capacity (WHC). We completed the microcosms adding one of the three soil suspension until 60% of the WHC. Soil samples consisting of soils amended with 4 different OWPs and a control without organic input. Finally, three replicates of every combination of soil and microbial diversity level were created obtaining thus 45 microcosms (n=45, replicates=3). Microcosms were sealed hermetically and pre-incubated at 20°C in the dark. Once a week during six weeks the microcosms have been aerated keeping constant the soil moisture at 60% of the WHC.

One week before the detection of the emissions with the PTR-TOF-MS caoutchouc the flask plugs were substituted with Teflon plugs. This was necessary in order to reduce the influence of the emissions of VOCs released from the caoutchouc. In fact, Teflon is an inert material reducing the VOCs emissions that would have been released from the caoutchouc plug. After the six week incubation, the microcosms were connected to the PTR-QiTOF-MS in order to detect the VOCs emissions.

IV.5.3 PTR-TOF-MS detection system

Every flask plug was equipped with two PEEK tubes, one allowing the connection with the PTR-QiTOF-MS and the other one was connected with a bottle of dry synthetic air (Alphagaz 1 Air: 80% nitrogen, 20% oxygen, 99.9999%, Air Liquide®). The flasks had a volume of 88 cm³. The detection of the VOCs emissions from the microcosms was performed during 180 s for every sample. An empty flask was used as a reference for zero emissions. In order to characterize the emissions released from the Teflon plug, after the detection of the emissions of the flask containing the microcosms the plug was moved to an empty flask and the emissions of the plug and the empty flask were detected during 180 s. An air flow (Q_{air}) of 0.3 L min⁻¹ (equivalent volumetric flow at 0°C and 1 atm) of dry synthetic air was passed through a hydrocarbons and humidity filter (Filter for fuel gas, final purity=99.999%, Restek®) and a Hydrocarbon Trap (Supelco, Supelpure® HC) prior to injection in the flask. A mass flowmeter (Bronkhorst® model F-201CV, accuracy: standard 0.5% Rd plus 0.1% FS, range: 0.2 L min⁻¹ to 5 L min⁻¹ air) was used to control the synthetic air flow rate. Air was sampled at the chamber outlet into a PTR-QiToF-MS with a 0.05 L min⁻¹ flow rate with a 2 m long, 1 mm internal diameter PEEK tube, heated at 80 °C. A measurement cycle consisted in measuring the VOC mixing ratio at the outlet of the flask containing the microcosms $(x_{VOC mic} \text{ in ppb})$ for 180 s. Then air was sampled for 180 s on the empty chamber with the Teflon plug sealing the microcosms just measured to determine $x_{VOC empty}$ (ppb). Only the last 60 s of each measurement were kept to calculate averaged mixing ratios in order to ensure a stable VOCs mixing ratio. The VOC emission (E_{VOC} in nmol g⁻¹ s⁻¹ dry soil) was calculated as:

$$E_{VOC} = \frac{Q_{air} \times (\chi_{VOC\ micro} - \chi_{VOC\ empty})}{V_{mol}^{air} \times m_{dry\ soil}}$$

(1)

Where V_{mol}^{air} is the air molar volume at standard temperature and pressure (22.4 L mol⁻¹ at 0°C and 1 atm). After each measurement, the flask was cleaned and the soil transferred in a -40 °C chamber before the DNA extraction.

IV.5.3.1 VOCs data analysis

The analysis of the peaks of VOCs detected, the mass calibration, and the creation of the mass table with all the compounds detected were performed using Spectra Analyser within the PTR viewer 3.1.0.29 software (Ionicon, Analytik GmbH) following the protocol published in Abis et al., (2018). Likely isotopes and fragments were identified using a correlation coefficient of 0.99. Hence, the ions having a correlation coefficient higher than 0.99 were considered as isotope or fragment depending on the m/z difference. Furthermore, correlated

masses that are closer than the resolution of the used PTR-QiTOF-MS were removed in order to not count twice the same peak.

IV.5.4 Microbial analysis

IV.5.4.1 DNA extraction

The DNA extraction has been performed for all microcosms following the protocol developed GenoSol platform (INRA, Dijon, by France, www.dijon.inra.fr/plateforme_genosol) (Terrat et al., 2012) for application in large-scale soil survey (Terrat et al., n.d.). The protocol consist of mixing in a 15 mL Falcon tube 1 g of each soil sample with 2 g of 100 mm diameter silica beads, 2.5 g of 1.4 mm diameter ceramic beads and 4 glass bead of 4 mm diameter and 5 mL of a solution containing 100 mMTris (pH 8.0), 100 mMEDTA (pH 8.0), 100 mM NaCl, and 2% (wt/vol) sodium dodecyl sulfate. Then, we proceeded homogenizing the samples in a FastPrep-24 (MP-Biomedicals, Santa Ana, CA, USA) during 90 s and incubated for 30 min at 70 °C before centrifugation at 7000 g for 5 min at 20 °C. The deproteination was performed by collecting 1 mL of the supernatant and incubating for 10 min on ice with 1/10 volume of 3M potassium acetate (pH 5.5) and centrifuged at 14.000 g during 5 min. The precipitation of the proteins was performed with one volume of ice-cold isopropanol. The last step of the extraction consisted of washing the nucleic acid with 70% ethanol. DNA concentrations of crude extracts were determined by electrophoresis in 1% agarose gel stained with ethidium bromide using a calf thymus DNA standard curve, and used as estimates of microbial biomass (Dequiedt et al., 2011). After quantification, nucleic acids were separated from the residual impurities, particularly humic substances, by centrifuging through two types of minicolumn. Aliquots (100 µl) of crude DNA extract were first loaded onto polyvinyl polypyrrolidone minicolumns (BIORAD, Marne-la-Coquette, France) and centrifuged at $1000 \times g$ for 4 min at 10 °C. The eluates were then purified using the Geneclean turbo kit (Mp Biomedicals, Illkirch, France) (Ranjard et al., 2003). (Ranjard et al., 2003). Purified DNA concentrations were finally measured using the Quantifluor (Promega, Lyon, France) staining kit, according to the manufacturer's instructions.

IV.5.4.2 <u>Quantitative PCR (qPCR)</u>

Quantitative real-time PCR was performed on extracted DNA to quantify 16S and 18S rRNA gene sequences (Lienhard et al., 2012; Plassart et al., 2012), which led to the estimation of the

fungal to bacterial ratio (F:B). Bacterial and fungal quantitative PCR assays were performed using a StepONE (Applied Biosystems, Courtaboeuf, France) with a SYBRGreen® detection system. Purified DNA extract was amplified in a total reaction volume of 20 µl, containing 500 ng of T4 gene 32 protein (MP Biomedicals, France) and 10 µl of Power SYBR[®]Green PCR master mix (Applied Biosystems, France).

For bacterial quantification, the reaction mixtures contained 1 μ M of each primer (341F: 5' - CCT ACG GGA GGC AGC AG - 3' and 515R: 5' - ATT ACC GCG GCT GCT GGC A - 3') (López-Gutiérrez et al. 2004) and 2 ng of template DNA. The PCR conditions consisted of an initial step of 15 min at 95°C then 35 cycles of 15 s at 95°C, 30 s at 60°C, 30 s at 72°C and 20 s at 80°C. The 16S rDNA gene from a pure culture of *Pseudomonas aeruginosa* PAO was used as a standard for the bacterial quantitative PCR assay.

Soil fungi were quantified using 1.25 μ M of each primer (FR1: 5'-AIC CAT TCA ATC GGT AIT-3', and FF390: 5'-CGA TAA CGA ACG AGA CCT-3') (<u>Chemidlin Prévost-Bouré et al. 2011</u>), and 2 ng of template DNA. The PCR conditions were: an initial step of 10 min at 95°C for activation; followed by 35 cycles of 15 s at 95°C, 30 s at 50°C and 60 s at 70°C. Amplified DNA from a pure culture of *Fusarium oxysporum* 47 was used as a fungal standard. These measures were used to estimate the bacterial and fungal densities in the samples.

IV.5.4.3 High throughput sequencing of 16S and 18S rRNA gene sequences

Bacterial diversity was obtained by amplifying a 440-base 16S rRNA from each DNA samples. The corresponding primers were: F479 (5'-CAG CMG CYG CNG TAA NAC-3') and R888 (5'-CCG YCA ATT CMT TTR AGT-3') (Tardy et al., 2014). The amplification of the DNA was performed during a 25 μ L PCR (with 5 ng of DNA for each sample) under the following set up conditions: 94 °C for 2 min, 35 cycles of 30 s at 94 °C, 52 °C for 30 s and 72 °C for 1 min, followed by 7 min at 72 °C.

For fungal diversity, a 350-base 18S rRNA fragment was amplified from each DNA sample (5 ng) with the corresponding primers: FF390 (5'-CGA TAA CGA ACG AGA CCT-3') and FR1 (5'-ANC CAT TCA ATC GGT ANT-3') (Prévost-Bouré et al., 2011). For each sample, 5 ng of DNA were used for a 25 μ L PCR conducted under the following conditions: 94 °C for 3 min, 35 cycles of 30 s at 94 °C, 52 °C for 1 min and 72 °C for 1 min, followed by 5 min at 72 °C.

The purification of the PCR products was performed using the Agencourt® AMPure® XP kit (Beckman Coulter, Italy, Milano) and quantified with the Quantifluor (Promega, Lyon,

France) staining kit according to the manufacturer's instructions. Purified PCR products (7.5 ng of DNA for bacteria) were amplified twice in order to integrate to the 5' end of the primers a 10-bp multiplex identifiers allowing the specific identification of the samples and the prevention of PCR biases. For bacteria, the second PCR conditions were the same than previously described but with only seven cycles. For fungi, the second PCR conditions were optimized, with the number of cycles being reduced to seven and the denaturation step processed at 94 °C during 1 min. PCR products were purified with the MinElute gel extraction kit (Qiagen NV) and quantified with the Quantifluor (Promega, Lyon, France) staining kit according to the manufacturer's instructions. Equal amounts of each sample were pooled and then cleaned with the SPRI (Solid Phase Reverse Immobilization Method) using the Agencourt® AMPure® XP kit (Beckman Coulter, Italy, Milano). The pool was finally sequenced with a MiSeq Illumina instrument (Illumina Inc, San Diego, CA) operating with V3 chemistry and producing 250 bp paired-reads.

IV.5.4.4 Bioinformatic analysis of 16S and 18S rRNA gene sequences

The bioinformatics analyses were performed using the GnS-PIPE developed by the Genosol platform (INRA, Dijon, France) (Terrat et al., 2012). At first, all the 16S and 18S raw reads were organized according to the multiplex identifier sequences. All raw sequences were checked and discarded if: (i) they contained any ambiguous base (Ns), (ii) if their length was less than 350 nucleotides for 16S reads or 300 nucleotides for 18S reads, (iii) if the exact primer sequences were not found (for the distal primer, the sequence can be shorter than the complete primer sequence, but without ambiguities). A PERL program was then applied for rigorous dereplication (i.e. clustering of strictly identical sequences). The dereplicated reads were then aligned using Infernal alignment (Cole et al., 2009), and clustered into operational taxonomic units (OTU) using a PERL program that groups rare reads to abundant ones, and does not count differences in homopolymer lengths. A filtering step was then carried out to check all single-singletons (reads detected only once and not clustered, which might be artifacts, such as PCR chimeras) based on the quality of their taxonomic assignments. Finally, in order to compare the datasets efficiently and avoid biased community comparisons, the reads retained were homogenized by random selection (23 700 and 11900 reads for 16S and 18S rRNA gene sequences, respectively). The retained high-quality reads were used for: (i) taxonomy-independent analyses, determining the Shannon index (ii) taxonomy-based analysis using similarity approaches against dedicated reference databases from SILVA (Quast et al., 2013). The raw datasets are available in the EBI database system under project accession number PRJEB29286.

IV.5.5 Statistical analysis

Microbial biomass, microbial diversity Index (Shannon) and the relative abundance of bacterial, and fungal *phyla* in the microbial composition were processed by the ANOVA test. With the ANOVA test, we also analyzed the effect of the organic waste product on the microbial community for the different dilution level The dataset before the statistical analysis was made of 754 variables (number of peaks detected) and 45 samples comprising the 3 replicate for each sample. In order to select the most representing variables of the dataset, several statistical tests have been performed using the R software (Version 1.0.153 - © 2009-2017 RStudio). At first, the normality test (Shapiro-Wilk test, W> 0.9) has been applied to verify that the mean mixing ratios were normally distributed for each VOC. Secondly, the homogeneity of the variances was verified for each treatment using the Levene test. Once the normality and the homogeneity of the variances were validated, a t-test was performed in order to see if the VOC flux was significantly higher than 0. Moreover, correlated masses that are closer than the resolution of the used PTR-QiTOF-MS were removed in order to not count twice the same peak. Finally, an ANOVA test was performed. The final selected dataset comprised of 45 samples and 239 variables meaning that only 32% of the dataset was kept for further analysis. The ANOVA tests were followed by the Tukey post hoc test.

A principal component analysis (PCA, Package *Ade4* Version 1.0.153 – © 2009-2017 R Studio) was performed to see the different VOCs compounds differentiating the three dilution levels. The Shannon index of diversity (**Figure 3b**) was calculated with the diversity function of the vegan package (version 2.4-3) in the R software (version 3.2.3). The diversity index was calculated as $H = \sum_{VOC} E_{VOC} \log(E_{VOC})$, where the sum is over all VOCs recorded in the mass table. The correlation matrix between VOCs and microorganisms has been created selecting the VOC having a R² of the correlation microorganisms/VOC larger than 0.6, the final number of compounds displayed was 107.

Microbial biomass, microbial diversity Index (Shannon) and the relative abundance of bacterial, and fungal *phyla* in the microbial composition were processed by the ANOVA test. With the ANOVA test, we also analyzed the effect of the organic waste product on the microbial community for the different dilution levels. All significant effects were assessed by Tukey's Honestly Significant Difference (HSD) *post hoc* test (P < 0.05).

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Authors contributions

L.Abis wrote the article, carried out the experiment, and performed the statistical analysis with B. Loubet. B. Loubet and S. Bourgeteau-Sadet conceived the experiment and supervised the redaction and the statistical analysis of the manuscript. R. Ciuraru revised the manuscript and performed the tentative identification of the compounds. Pierre-Alain Maron conceived the experiment. F. Lafouge, V. Nowak and J. Tripied carried out the experiment. S. Dequiedt performed the bioinformatics analysis.

Chapter V

Short-term effect of green waste and sludge amendment on microbial diversity and VOCs emissions

Short-term effect of green waste and sludge amendment on microbial diversity and VOCs emissions

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Abstract

During the last decades, soil amendments with organic waste products (OWPs) have been widely encouraged in Europe in order to improve soil fertility. Short-term effect of the OWPs application causes wide changes on the microbial community structure and diversity. Those changes are known to affect the volatile organic compounds (VOCs) emissions by soil. This work aimed to characterize, in terms of quantity and composition, the effect of the green waste and sludge (GWS) application on soil VOCs emissions and microbial community over a short-term period (49 h after the last GWS application). Moreover, we used two soil types: one soil used to receive GWS input (GWS soil) and one that never received organic input (CN soil). This two were manipulated to generate a microbial dilution diversity gradient (low, medium and high) as explained in Abis et al., (2018-chapter IV). Results showed that Bacteroidetes phyla took advantage of the GWS application in all samples while Proteobacteria were penalized by the GWS amendment. Microbial structure differences between microbial diversity dilutions levels remained even after GWS application. GWS amendment induced an increase of total VOCs emissions for GWS soil only, while the VOCs Shannon diversity decreased among the incubation hours for both soils. We observed an increase in emissions of the most emitted compounds with GWS application: m/z 121.101

(tentatively identified as propylbenzene, isopropylbenzene or 1,3,5-trimethylbenzene), m/z 93.066 (Toluene), m/z 135.113 (p-cymene), m/z 79.049 (benzene), m/z 119.101 (Indane) and m/z 105.079 (Styrene). On the contrary, the less emitted compounds (m/z 98.100, m/z 84.084 and m/z 211.235) exponentially decreased among the incubation hours. Moreover, m/z 98.100, m/z 84.084 compounds were probably released by the GWS amendment itself and consumed by microorganisms during the incubation hours. Finally, we detected the presence of Indole compound, a great promoter of plant growth. The change in bacterial community structure and VOCs emissions are discussed.

V.1 Introduction

The recycling of the organic matter via amendment with organic waste products (OWPs) is an extensively used agronomic practice to increase soil fertility. Lately, this practice has been encouraged in Europe (European Commission, 2010) in order to contrast the decrease of soil organic matter content due to intensive agricultural practices (Chan et al., 2002). OWPs are potential sources of nutrients for crops, and they can partially substitute mineral fertilizers (Chalhoub et al., 2013). The use of OWPs affects several chemical and physical properties and thus the microbial structure and activity in soil (Blagodatskaya et al., 2007; Federici et al., 2017; Sadet-Bourgeteau et al., 2018). The effects of the OWPs application on soil chemical and physical characteristics and the microbial community depend on the duration of the OWPs application to soil. Repeated OWPs applications over several years maintain soil fertility and thus crop yields; they also have more persistent impacts on soil characteristics, plant growth (Clark et al., 2007) and microbial diversity (Francioli et al., 2016; Zhong et al., 2010) than punctual OWPs application. Some studies reported that repeated organic fertilization on soil microbial communities led to an increased soil microbial biomass (Francioli et al., 2016; Poulsen et al., 2013).

However, microbial communities parameters such as diversity, composition (i.e. number and relative abundance of present species in the community) and seemed to depend on the time between the OWP application and soil sampling, and the duration of the application (Francioli et al., 2016; Nasini et al., 2013; Poulsen et al., 2013; Proietti et al., 2015; Regni et al., 2017). Until now, it has been demonstrated that the most significant changes in the structure of the microbial community due to OWPs amendments occur in the short-term period (Pezzolla et al., 2015). Experiments on short-term periods following the OWPs application reported wide changes in the microbial community structure caused by a rapid turnover of microbial

biomass due to the r-strategists bacteria (Blagodatskaya et al., 2007). Federici et al., (2017) reported a rapid modification of both fungal and bacterial communities after three days from the OWPs spreading. Furthermore, in Federici et al., (2017) study, the bacterial community restored its initial state after 120 days demonstrating high resilience capacity, while the fungal population changes remained until the end of the trial (120 days). Other studies reported substantial changes in bacterial structure for a larger period after the application of OWPs. Calbrix et al., (2007) revealed that after the OWPs (~5 Mg ha⁻¹) application, the microbial community was strongly impacted on its functional and genetic structure during the first 3 months succeeding the amendment, followed by a period of resilience leading, after 6 months, to similar communities in both amended and control plots. Previous research thus suggests that a single OWP application has a transient impact on the abundance and diversity of soil microbial communities.

Changes on the microbial community are known to affect the volatile organic compounds (VOCs) emissions by soil. During the last decades, an increasing awareness concerning the VOCs emissions from a large number of microorganisms was observed. The production of VOCs from soil and microorganisms gained attention because of their contribution to biogenic VOCs emissions (Bachy et al., 2018, 2016; Mancuso et al., 2015). Abis et al., (2018-Chapter IV) showed that VOCs emissions from the microbial community are linked to the microbial diversity in soil. The dilution of the microbial diversity in soil led to an increase of the VOCs emissions from the soil up to 2-3 times more than soil with high diversity levels (Abis et al., to be submitted-Chapter IV). Furthermore, the effect of the OWPs applications on VOCs emissions by soil was less important compared to the influence of the microbial diversity dilution levels.

In this context, this study aims at characterizing the short-term effects of OWPs application to soils with varying microbial dilution levels on microbial structure and VOCs emissions. These parameters were measured after a 49 hours period following OWP application. We further compared the response of a microbial community used to receive OWPs applications (GWS soil) with a microbial community that never received organic waste input to an OWP application (CN soil). The measurements of VOCs emissions were performed, under controlled laboratory conditions using a dynamic chamber approach, from 18 microcosms with three different microbial dilution levels (high, medium and low). The VOCs emissions from the microcosms were detected with a PTR-QiTOF-MS, 1 hour before the application of the green waste and sludge (GWS) and during the following 49h. The

microbial community change due to the application of the GWS amendment was measured by a high throughput sequencing approach targeting 16S ribosomal genes.

V.2 Methods

V.2.1 Sampling and site description

The collection of the samples was performed in the QualiAgro site, located at Feucherolles, which is a station of the SOERE-PRO-network (https://www6.inra.fr/qualiagro_eng/Nospartenaires/The-SOERE-PRO-network). Feucherolles is in northwestern France (35 km west of Paris; 48°52'N, 1°57'E, alt 150 m), and the soil of the same zone are classified as glossic Luvisol (IUSS Working Group WRB, 2014). Important characteristics of these soils are: (i) low quantity of clay, (ii) slit-loam texture (15.0% clay, 78.3% slit), (iii) pH alkaline in the surface horizon (0-30 cm), and (vi) good drainage. QualiAgro site was used from September 1998 to September 2017 in order to study the long-term effect of the amendment with OWPs. The experiment consisted of amending the site every two years, in a randomized block design with 4 replicates with 4 different organic waste products: BIOW (bio-waste compost derived from the co-composting of green wastes and source-separated organic fractions of municipal solid wastes), GWS (compost derived from the co-composting of green wastes after removing dry and clean packaging); plus a control without organic input (CN).

The collection of the samples was performed in 2 plots of the site (GWS and CN). For each plot, we collected randomly five soil cores (0-30 cm depth) in early September 2016 one year following the last amendment (~4 tC ha⁻¹ of every OWP).

V.2.2 Microcosms preparation

Soil samples were sieved at 4 mm and homogenized before gamma-ray sterilization (35 kGy; Conservatome, Dagneux, France). Soil samples consisting of soils amended with GWS and control without organic input (CN). The sterility of the irradiated soil was verified by spreading serial dilutions of the soil onto nutrient agar plates. After the sterilization process soils were inoculated with a diluted soil suspension prepared with the same soil before sterilization (Wertz et al., 2006). The soil suspension, obtained by a mix of 30 g of soil with 90 mL of sterilized water, was used pure (D0=1), diluted with water 10^3 times (D1= 1: 10^3 water/soil suspension ratio) and finally, diluted 10^5 times (D2=1: 10^5 water/soil suspension

ratio). Microcosms were set up by placing 30 g of dry sterile soil in 150 ml plasma flask. The three levels of dilution of the soil suspension were used as inoculum to create a gradient of diversity as follow: low, for samples inoculated with D0 soil suspension, medium, for samples inoculated with D1 soil suspension, and high, for samples inoculated with D2 soil suspension. The soil moisture was fixed at 60% of the water holding capacity (WHC). Microcosms were at first sealed hermetically with a caoutchouc plug and pre-incubated at 20°C in the dark. Once a week during six weeks the microcosms have been aerated keeping constant the soil moisture at 60% of the WHC. One week before the measurement with the PTR-QiTOF-MS the caoutchouc plug was substitute with a Teflon plug in order to reduce the VOCs emissions released from the plug.

After six weeks incubation (20 °C), GWS were thoroughly mixed with soil samples CN+GWS and GWS+GWS, in its dry form (dose equivalent to 4 t C ha-1). The main characteristics of the GWS amendment are reported in **Table V-1**.

Treatment		GWS
Dry Matter (DM)	%	67.2 ± 1.3
Organic Carbon	$g kg^{-1} DM$	257 ± 2
Total N	$g kg^{-1} DM$	22.5 ± 0.5
P2O5 (Olsen)	$g kg^{-1} DM$	0.8 ± 0.1
C:N		13.2 ± 0.5
pH (water)		7.7 ± 0.1
Molecular Biomass	μg of DNA g^{-1} of soil	54.9 ± 19.1

Table V-1. Main characteristics of the organic waste product used. GWS= green waste and sludge. DM= dry matter.

CN and GWS samples were lyophilized and stored at -40 °C until use for molecular analyses based on soil DNA extraction. CN+GWS and GWS+GWS samples were then incubated for 49 hours in the dark at 20 °C and always 60 % water-holding capacity to ensure proper biomass activation. In total, there were 36 microcosms (2 treatments \times 3 levels of microbial diversity \times 2 dates of sacrifice \times 3 replicates). After 49h incubation, CN+GWS and GWS+GWS samples were sacrificed for microbial molecular analyses. In

Type of sample	Number of samples	Microbial analysis	VOCs detection
CN	9	Shannon index Microbial biomass Bacterial relative abundance	NA (sacrificed for microbial analysis)
CN+GWS	9		During 49 h
GWS	9		NA (sacrificed for microbial analysis)
GWS+GWS	9		During 49 h

Table V-2 we summarize the type samples and the performed analysis for each type of samples.

Type of sample	Number of samples	Microbial analysis	VOCs detection
CN	9	Shannon index Microbial biomass Bacterial relative abundance	NA (sacrificed for microbial analysis)
CN+GWS	9		During 49 h
GWS	9		NA (sacrificed for microbial analysis)
GWS+GWS	9		During 49 h

Table V-2. Type of samples and performed analysis. CN= control without organic inputs, CN+GWS= control without organic input with the addition of GWS amendment, GWS= green waste and sludge samples, GWS+GWS= green waste and sludge samples with the addition of GWS amendment. NA= not available.

V.2.2.1 <u>Timing of the VOCs measurements</u>

During the 49 hours incubation of the sample receiving GWS addition (CN+GWS and GWS+GWS), 10 VOCs measurements were carried out. The measurements have been performed 1h (T1), 3h (T2), 6h (T3), 9h (T4), 25h (T5), 27h (T6), 30h (T7), 33h (T8), 49h (T9) following the addition of GWS. Furthermore, we performed the VOCs detection just before the GWS addition (T0).

V.3 PTR-QiTOF-MS measurement set up

Two PEEK tubes were inserted in the plug of every flask, one connected to the PTR-QiTOF-MS and the other one connected to a bottle of dry synthetic air (Alphagaz 1 Air: 80% nitrogen, 20% oxygen, 99.9999%, Air Liquide®). VOCs detection lasted 180 s for every microcosm. The PTR-QiTOF-MS detection system and the equation used in order to calculate the VOCs fluxes were the same as in Abis et al. (2018), apart from the air flow which was set to 0.3 L min⁻¹ was used. Between each VOCs measurement, the microcosms were incubated in a 20°C chamber. After the last VOCs measurement, the flasks were cleaned and the soil transferred in a -40 °C chamber waiting for the DNA extraction.

V.2.4 Microbial Analyses

V.2.4.1 DNA extraction

The DNA extraction has been performed for all microcosms following the protocol developed GenoSol platform (INRA, Dijon, by France. www.dijon.inra.fr/plateforme_genosol) (Terrat et al., 2012) for application in large-scale soil survey (Terrat et al., n.d.). The protocol consist on mixing in a 15 mL Falcon tube 1 g of each soil sample with 2 g of 100 mm diameter silica beads, 2.5 g of 1.4 mm diameter ceramic beads and 4 glass bead of 4 mm diameter and 5 mL of a solution containing 100 mMTris (pH 8.0), 100 mMEDTA (pH 8.0), 100 mM NaCl, and 2% (wt/vol) sodium dodecyl sulfate. Then, we proceeded to homogenize the samples in a FastPrep-24 (MP-Biomedicals, Santa Ana, CA, USA) during 90 s and incubated for 30 min at 70 °C before centrifugation at 7000 g for 5 min at 20 °C. The deproteination was obtained by collecting 1 mL of the supernatant and incubating for 10 min on ice with 1/10 volume of 3M potassium acetate (pH 5.5) and centrifuged at 14.000 g during 5 min. The precipitation of the proteins was performed with one volume of ice-cold isopropanol. The last step of the extraction consisted of washing the nucleic acid with 70% ethanol. DNA concentrations of crude extracts were determined by electrophoresis in 1% agarose gel stained with ethidium bromide using a calf thymus DNA standard curve, and used as estimates of microbial biomass (Dequiedt et al., 2011). After quantification, nucleic acids were separated from the residual impurities, particularly humic substances, by centrifuging through two types of minicolumn. Aliquots (100 µl) of crude DNA extract were first loaded onto polyvinyl polypyrrolidone minicolumns (BIORAD,

Marne-la-Coquette, France) and centrifuged at $1000 \times g$ for 4 min at 10 °C. The eluates were then purified using the Geneclean turbo kit (Mp Biomedicals, Illkirch, France) (Ranjard et al., 2003). DNA concentration in each sample was fluorometrically quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, CergyPontoise, France) following the manufacturer's instructions.

V.2.4.2 High throughput sequencing of 16S rRNA gene sequences

Bacterial diversity was obtained by amplifying a 440-base 16S rRNA from each DNA samples. The corresponding primers were: F479 (5'-CAG CMG CYG CNG TAA NAC-3') and R888 (5'-CCG YCA ATT CMT TTR AGT-3') (Tardy et al., 2014). The amplification of the DNA was performed during a 25 μ L PCR (with 5 ng of DNA for each sample) under the following set up conditions: 94 °C for 2 min, 35 cycles of 30 s at 94 °C, 52 °C for 30 s and 72 °C for 1 min, followed by 7 min at 72 °C.

The purification of the PCR products was performed using the Agencourt® AMPure® XP kit (Beckman Coulter, Italy, Milano) and quantified with the Quantifluor (Promega, Lyon, France) staining kit according to the manufacturer's instructions. Purified PCR products (7.5 ng of DNA for bacteria) were amplified twice in order to integrate to the 5' end of the primers a 10-bp multiplex identifiers allowing the specific identification of the samples and the prevention of PCR biases. The second PCR conditions were the same than previously described but with only seven cycles. PCR products were purified with the MinElute gel extraction kit (Qiagen NV) and quantified with the Quantifluor (Promega, Lyon, France) staining kit according to the manufacturer's instructions. Equal amounts of each sample were pooled and then cleaned with the SPRI (Solid Phase Reverse Immobilization Method) using the Agencourt® AMPure® XP kit (Beckman Coulter, Italy, Milano). The pool was finally sequenced with a MiSeq Illumina instrument (Illumina Inc, San Diego, CA) operating with V3 chemistry and producing 250 bp paired-reads.

V.2.4.3 Bioinformatic analysis of 16S rRNA gene sequences

The bioinformatic analyses were performed using the GnS-PIPE developed by the Genosol platform (INRA, Dijon, France) (Terrat et al., 2012). At first, all the 16S raw reads were organized according to the multiplex identifier sequences. All raw sequences were checked and discarded if: (i) they contained any ambiguous base (Ns), (ii) if their length was less than 350 nucleotides for 16S reads, (iii) if the exact primer sequences were not found (for the distal

primer, the sequence can be shorter than the complete primer sequence, but without ambiguities). A PERL program was then applied for rigorous dereplication (i.e., clustering of strictly identical sequences). The dereplicated reads were then aligned using Infernal alignment (Cole et al., 2009), and clustered into operational taxonomic units (OTU) using a PERL program that groups rare reads to abundant ones, and does not count differences in homopolymer lengths. A filtering step was then carried out to check all single-singletons (reads detected only once and not clustered, which might be artifacts, such as PCR chimeras) based on the quality of their taxonomic assignments. Finally, in order to compare the datasets efficiently and avoid biased community comparisons, the reads retained were homogenized by random selection (15 000 reads for 16S rRNA gene sequences). The retained high-quality reads were used for taxonomy-independent analyses and determining the Shannon index.

V.2.5 Statistical Analysis

V.2.5.1 VOCs statistical Analyses

The dataset before the statistical analysis was made of 754 variables (number of peaks detected) and 18 samples comprising the 3 replicate for each sample. In order to select the most representing variables of the dataset, several statistical tests have been performed using the R software (Version 1.0.153 - © 2009-2017 RStudio). At first, the normality test (Shapiro-Wilk test, W> 0.9) has been applied to verify that the mean mixing ratios were normally distributed for each VOC. Secondly, the homogeneity of the variances was verified for each treatment using the Levene test. Once the normality and the homogeneity of de variances were validated, a t-test was performed in order to see if the VOC flux was significantly higher than 0.

Moreover, correlated masses that are closer than the resolution of the used PTR-QiTOF-MS were removed in order to not count twice the same peak. Finally, the ANOVA test was performed. The final selected dataset was made of 18 samples and 239 variables meaning that only 32% of the dataset was kept for further analysis. Then we performed the ANOVA test in order to analyze the statistical differences among the different timing. The performed ANOVAs were followed by the Tukey *post hoc* test.

The Shannon index of diversity (**Figure 3b**) was calculated with the diversity function of the vegan package (version 2.4-3) in the R software (version 3.2.3). The diversity index was calculated as $H = \sum_{VOC} E_{VOC} \log(E_{VOC})$, where the sum is over all VOCs recorded in the mass table.

V.2.5.2 <u>Microbial statistical analyses</u>

Microbial biomass, microbial diversity Index (Shannon) and the relative abundance of bacterial, and fungal *phyla* in the microbial composition were processed by the ANOVA test. With the ANOVA test, we also analyzed the effect of the organic waste product on the microbial community for the different dilution levels. All significant effects were assessed by Tukey's Honestly Significant Difference (HSD) *post hoc* test (P < 0.05).

V.3 Results

V.3.1 Microbial biomass and dilution diversity manipulation

After six weeks incubation, microbial biomass was not significantly different between the different microbial dilution levels (**Figure V-1**), while the Shannon index showed a significant decrease along the different microbial dilution levels (p.value < 0.05). The Shannon index with the microbial biomass results confirmed the efficiency of the diversity manipulation (**Figure V-2**).



Figure V-1. Microbial biomass for the different dilution levels. Bold line=median, boxes= interquartile, whiskers = minimum and maximum. Letters represent the significant differences according to the Tukey Test p-value > 0.05.



Figure V-2. Shannon index of the microbial diversity as a function of the dilution level for samples receiving GWS amendment (CN+GWS and GWS+GWS) and samples without any supplementary OWP addition (CN and GWS). Bold line=median, boxes= interquartile, whiskers = minimum and maximum, white point= outliers. Letters represent the significant differences according to the Tukey Test p-value > 0.05.

V.3.2 Bacterial relative abundance

The colonization from the different phyla within the microcosms showed a shift on the microbial community structure when the GWS was incorporated in both soil type (CN and GWS) (**Figure V-3** and **Figure V-4**). According to the Tukey test, this shift was statistically different for *Bacteroidetes* and *Proteobacteria* phyla. The relative abundance of the *Bacteroidetes* statistically increased while *Proteobacteria* statistically decreased among the dilution levels in CN+GWS and GWS+GWS samples compared to the relative abundance recorded in CN and GWS, respectively. Furthermore, a higher presence of *Bacteroidetes* was reported in CN+GWS and GWS+GWS than CN and GWS, respectively, for all the dilution levels. For the other phyla, no statistical shifts concerning the microbial relative abundance were reported after the addition of GWS amendment. The bacterial structure shift caused by the OWPs amendment was confirmed by the PCA (**Figure V-S1** and **Figure V-S2**).



Figure V-3. Relative phyla abundance from (a) CN= control without organic input and (b) CN+GWS=control without organic input and green waste and sludge amendment microcosms receiving a GWS amendment for the three dilution levels.



Figure V-4. Relative phyla abundance of microcosms (a) GWS=green waste and sludge and (b) GWS+GWS= green waste and sludge receiving a new GWS amendment for the three dilution levels.

V.3.3 VOCs emissions from the microcosms

According to an ANOVA and the post-hoc Tukey test, no difference on the VOCs total emissions was found between the three dilution levels for GWS+GWS samples. A statistical difference was however observed between the D0 and D2 total VOCs emissions for CN+GWS samples (**Figure V-** 5). We further measured a statistical increase in total VOCs

emissions during the two days following amendment for GWS+GWS (**Figure V-6**), (Tukey test, p.value <0.05) while it remained almost constant for CN+GWS (**Figure V-6**).



Figure V- 5. Total VOCs emission rates per soil treatment and dilution rate after the GWS amendment. D0= microbial diversity pure or 1, D1= microbial dilution diversity equal to 10⁻³, D2= microbial dilution diversity equal to 10⁻⁵. Black vertical bars= standard deviation, black points= outliers, Bold line =median, boxes= interquartile. Letters represent the statistical differences according to the Tukey test.

The Shannon index calculated for the VOCs emissions, which indicate how heterogeneous the emitted VOCs are, showed that VOCs are more differentiated when the emissions are lower. A decrease of the VOCs Shannon index was observed when the dilution rate and the incubation hours increased (**Figure V-7**). This is true for both CN+GWS and GWS+GWS samples. In contrast, the Shannon index of the VOCs emissions calculated only for the different microbial dilution rate showed no statistical differences (**Figure V-S3**).



Figure V-6. Total VOCs emission rates along the incubation hours after the fresh amendment with GWS. D0= microbial diversity equal to 1, D1= microbial dilution diversity equal to 10^{-3} , D2= microbial dilution diversity equal to 10^{-5} .



Figure V-7. Shannon index calculated along the incubation hours after the fresh amendment with GWS. D0= microbial diversity equal to 1, D1= microbial dilution diversity equal to 10^{-3} , D2= microbial dilution diversity equal to 10^{-5} .

The most emitted compounds were the m/z 121.101 (tentatively identified as propylbenzene, isopropylbenzene or 1,3,5-trimethylbenzene), m/z 93.066 (Toluene), m/z 135.113 (p-cymene), m/z 79.049 (benzene), m/z 119.101 (Indane) and m/z 105.079 (Styrene) which represented the 70% of the total emissions. **Figure V-8** shows an increase in the emissions of these compounds from T0 to T9. For the compounds less emitted such as m/z 98.100, m/z 84.084 and m/z 211.235, which represent less than 1% of the total VOCs emissions, we observed a decrease of the emissions from the T0 to the T9 (**Figure V-9**).



Figure V-8. Linear regression of the 6 most emitted compounds. m/z 121.101 (tentatively identified as propylbenzene, isopropylbenzene or 1,3,5-trimethylbenzene), m/z 93.066 (Toluene), m/z 135.113 (p-cymene), m/z 79.049 (benzene), m/z 119.101 (Indane) and m/z 105.079 (Styrene)



Figure V-9. Quadratic fit of the 3 less emitted compounds. as m/z 98.100, m/z 84.084 and m/z 211.235.

V.4 Discussion

V.4.1 Microbial diversity manipulation

The microbial biomass was constant among the three different dilution levels, indicating that after 44 days incubation, the microbial biomass was not affected by the dilution diversity manipulation performed. The Shannon index, calculated for CN, GWS and CN+GWS and GWS+GWS, indicated a decrease of the microbial diversity in the higher dilution level for all samples. Thus, the Shannon index confirmed successful microbial diversity dilution which was even increased following GWS amendment.

V.4.2 Effect of the GWS amendment on the bacterial community

Results showed that Bacteroidetes phylum takes advantage of the addition of the new GWS amendment whatever the history of the soil (CN and GWS soils). This result is in line with Simmons et al., (2014) study which reported an increase of the Bacteroidetes phylum in soil amended with organic amendment compared to soil that did not receive any organic amendment. Lupwayi et al., (2018) explained that the relative abundance of Bacteroidetes increased linearly with increasing N rate. Since GWS, is a source of N (see Table V-1), we can hypothesize that the increase of the *Bacteroidetes* relative abundance might be due to the increase of the N content in the microcosms. Proteobacteria was the most dominant phylum in all the microcosms and as suggested by Kuppusamy et al., (2016) this might be because functionally diverse groups of bacteria fall within this phylum. Nevertheless, Bastida et al., (2015) reported that the abundance of Proteobacteria was higher in compost-treated samples than in sludge-treated samples. Since our samples have been amended with GWS amendment that contains 30% of sludge, the development of the Proteobacteria might have been negatively influenced by the type of substrate added. Furthermore, in samples not receiving the new GWS amendment (CN and GWS) Proteobacteria, increased their relative abundance among the dilution levels may confirm that *Proteobacteria* are a great colonizer only when the conditions are favorable (Cleveland et al., 2007; Fierer et al., 2007). Fierer et al., (2007) reported that Bacteroidetes and Proteobacteria phyla were positively related with the C amendment level leading to the conclusion that these phyla can be classified as copiotrophic. Copiotrophics, are microorganisms requiring a high level of a nutrient, able to grow exponentially, colonize the habitat quickly with a relative short life due to the initial huge waste of energy involved on the exponential growth (Kjelleberg, 1985; Poindexter, 1981).

V.4.3 Dynamics of the total VOCs emissions

The detection of the VOCs emissions from the microcosms was performed 10 times during 49h. Total VOCs emissions of the GWS+GWS samples constantly increasing during the incubation hours. For the CN+GWS no statistical differences on the total VOCs emissions were observed along the incubation hours. This is possibly due to the faster degradation of the organic input from a microbial community used to the GWS application (GWS+GWS) than a microbial community not used to receive this organic input (CN+GWS). This efficiency in the organic matter degradation for the GWS+GWS microbial community might increase the VOCs emissions from soil. Even though, for CN and GWS higher VOCs emissions from the higher microbial dilution level were reported. Those results are in line with the previous study of Abis et al., (2018-chapter VI). Results concerning the Shannon index showed a lower diversity of the VOCs emitted while the total VOCs emissions increased (**Figure V**-10). Previous studies as Abis et al., (2018) and Abis et al., (to be submitted-chapter IV) reported the same trend, lower VOCs diversity for higher emissions.

V.4.4 Bacteria VOCs production dynamic of the most and less emitted

VOCs

In order to explain this phenome, we analyzed the emission dynamics of the 6 most emitted compounds, representing the 70% of the total VOCs emissions. Results showed an increase of the emissions with time. The opposite dynamic was observed for the compounds less emitted (representing less than 1% of the total VOCs emissions). In more details, the most emitted compound showed a constant emissions increase with time following GWS amendment, while the less emitted compounds showed an exponential decrease.

Compounds as 84.084 m/z and 98.100 m/z, which were less emitted, were detected from the GWS alone by Ciuraru et al., (personal communication). We can hence hypothesize that emission of these compounds, and by extension, all compounds showing an exponential decrease dynamics were due to emissions by GWS organic matter being processed rather than

an induced emission from soil. We can further hypothesize that the soil microbial community might have processed (consume) those VOCs as a carbon source. Indeed, the study of Mayrhofer et al., (2006) reported a negative correlation between the cells count of *Dysgonomonas caprocytophagoides* and the emissions of the ion mass m/z 84 underlining either a possible absorption by this bacterial species of the m/z 84 mass as a carbon source gradually exhausted (which is the likely cause in our case), or a suppression or inhibition of the emitting bacteria species.

Among the compounds which constantly increase following the GWS amendment, we found indole (m/z 119.101). Indole has been reported in several studies as a promoter of plant growth (Bhattacharyya et al., 2015; Park et al., 2014; Yu and Lee, 2013). Hence plants may benefit indirectly from GWS amendment via indole emissions. Other studies have reported indole emissions by *E. coli* culture (Bhattacharyya et al., 2015; Mayrhofer et al., 2006). In particular, Mayrhofer et al., (2006) reported a positive correlation between the increase of the *E. coli* cults count and the emissions of Indole.

V.5 Conclusions

This work aimed to characterize in terms of quantity and composition the effect of the GWS application on soil VOCs emissions and microbial community in the short-term period (49 h). We can conclude that GWS amendment boosted *Bacteroidetes* growth while penalized *Proteobacteria* colonization. Furthermore, the new addition of GWS in soil increased soil VOCs emissions in microcosms used to received GWS amendment and thus, more efficient on the degradation of the new organic input but not for soils unused to organic matter amendment. This work highlighted the possibility that the VOCs emissions released by the GWS amendment might be used as a carbon source by microorganisms. Finally, GWS might boost Indole emissions, promoter of plant growth.

Chapter VI

General discussions

VI. General discussions

In a global context were the emissions of VOCs gain more and more relevance due to the roles played on climate change and air pollution, with this work, we wanted to contribute by the addition of small tiles to the complex mosaic of the biogenic VOCs emissions. The present work aimed at the characterization of the VOCs emissions from soils amended with OWPs and to define the impact of different microbial dilution levels on the VOCs emissions from soil. In order to achieve the purpose, we performed three experiments. The first one was focused on the influence of the different type of OWPs in soil on the VOCs emissions. The second experiment concerned the study of the relationship between the VOCs emissions and the microbial diversity level in soils amended with the different type of OWPs. Finally, with the third experiment, we aimed to analyze the VOCs emissions short-term dynamics following OWP amendment, from different microbial dilution levels in soil and for a microbial community used to receive OWP and a one that never received OWPs amendment. Within these three experiments, three different timings between the OWPs application and sampling occurred: two years for the first experiment, one year for the second one and 49 hours for the third one. The results concerning the chapters as separate works have been discussed in detail in the previous single chapters. For this reason, in the following paragraphs, we will focus on giving a global overview of the whole dataset from the three experiments. This global overview will concern:

1. The effect of the timing following the OWPs amendment on VOCs emissions.

2. The effect of the soil microbial diversity on the VOCs emissions (the native soil, used for the first experiment, will be included as a non-manipulated soil)

3. The combined effects of OWPs amendment and microbial dilution levels on VOCs emissions.

The experimental conditions were similar in the three experiments, although some differences may arise due to soil manipulations: sterilization and incubation. However, samples for experiment two and three (Chapter V) were collected at the same time and manipulated following the same protocol for the microbial dilution diversity. The only differences between samples treatment of experiments two and three were: (i) the application of new OWP in

experiment three before the VOCs detection, and (ii) the number of different type of OWPs in soil, 4 for experiment two (GWS, FYM, BIOW and MSW), and 1 for experiment three (GWS).

In the following analysis, we first analyzed total VOCs emissions as a function of time following the OWP application. We then analyzed total VOCs emissions in response to microbial dilution levels comprising the first experiment where no microbial diversity manipulation was performed. In order to validate and compare the results, we performed ANOVA tests followed by a Tukey HSD test and a T-test.

VI.1 Effect of time following the OWPs application on total VOCs emissions

Results regarding the analysis of the timing following the OWPs application are showed in **Figure VI-** 1. Time h_0 is the timing just before the new application of the OWP which is equivalent to the VOCs emissions from experiment two (Chapter IV). The samples T1y showed high variability in the total VOCs emissions (from -30 nmol s⁻¹ g⁻¹ 10⁴ to 180 nmol s⁻¹ g⁻¹ 10⁴). This variability might be due to the higher number of OWPs analyzed crossed with the different microbial dilution levels.

The Tukey test showed that there was a significant difference between samples h_{-0} , Ty2, and h_{-49} (**Figure VI-** 1) in terms of total VOCs emissions. Total VOCs emissions at h_{-0} and Ty2 were statistically lower compared to h_{-49} total VOCs emissions. Besides, the total VOCs emissions 49 hours following OWPs amendment were two times higher than the total VOCs emissions before the amendment. A possible explanation of this total VOCs emissions increase during the 49 hours following the OWPs application could be the increase of the microbial activity caused by the priming effect. The priming effect is the enhanced decomposition of the existing soil organic matter due to a new C input (Chen et al., 2014; Fontaine et al., 2003). The quality of organic substances and their availability to decomposers affect the decomposition of soil organic matter (Six et al., 2002). In this case, availability is defined as the biochemical recalcitrance of organic compounds, that is, their susceptibility to microbial enzymatic degradation. The GWS has a high recalcitrance level, as shown by its high index of residual organic carbon (I_{roc}), that represents the portion of organic carbon potentially incorporated into soil organic carbon after the organic waste application

(Lashermes et al., 2009) which was equal to 75% org C (Obriot et al., 2016). We can hypothesize that an easy degradation of the GWS amendment may enhance the priming effect. Since VOCs production in the soil is related to the primary (Insam and Seewald, 2010; Stahl and Parkin, 1996) and secondary microbial metabolisms (Kai et al., 2009b), we can also hypothesize that high microbial activity enhanced by the priming effect might lead to higher VOCs total emissions rate. Furthermore, the priming effect is a short-term (maximum one month) boost of the soil organic matter degradation (Chen et al., 2014), which is not in contradiction with the observed return to lower VOCs emissions one year following the last OWPs application.

Since several studies reported that organic waste products are also abiotic sources of VOCs emissions (Kumar et al., 2011; Leff and Fierer, 2008; Mayrhofer et al., 2006), a second complementary hypothesis could be that the increase of VOCs emissions was in part due to the VOCs emitted by the GWS itself.



Time following the last OWPs application

Figure VI- 1.Total VOCs emission rates against time following the OWPs amendment. h_0 = measure performed before the application of the OWP. h_1 = 1h after the application of OWPs, h_3 = 3h after the application of OWPs, h_2 = 25h after the application of OWPs, h_2 = 27h after the application of OWPs, h_3 = 30h after the application of OWPs, h_3 = 33h after the application of OWPs, h_3 = 30h after the application of OWPs, h_3 = 33h after the application of OWPs, h_4 = 49h after the application of OWPs, h_3 = 33h after the application of OWPs, h_4 = 49h after the application of OWPs. Bold line=median, boxes= interquartile, whiskers = minimum and maximum, white point = outliers. Letters indicate significant differences according to the Tukey test with p.value <0.05.

Extrapolating our results to a larger scale, we could thus conclude that the amendment with OWPs might increase the VOCs emissions in the short-term period following the OWPs amendment.

VI.2 VOCs emissions among the different microbial dilution levels

Pooling all soils together, results concerning the total VOCs emissions from the different microbial dilution levels as reported in both chapters IV and V showed an increase of the VOCs total emissions in the higher microbial dilution level. The addition of the results from non-manipulated soils showed that DX and D0 behaved similarly and both showed a significant difference in total VOCs emissions with medium and high microbial dilution levels (**Figure VI-2** and Erreur ! Source du renvoi introuvable.). In terms of percentages, the differences were significant (33% and 110%). We can conclude that there is a noticeable increase in the total VOCs emissions rate due to the microbial dilution diversity.



Figure VI-2. Total VOC emissions rate for the different microbial dilution levels. DX=nonmanipulated soil used in the first experiment, D0: microbial dilution equal to 1. D1: microbial dilution equal to 10^{-3} . D2: microbial dilution equal to 10^{-5} . Bold line=median, boxes= interquartile, whiskers = minimum and maximum, white point = outliers. Letters indicate significant differences according to the Tukey test with p.value <0.05.

The differences between the total VOCs emissions of the non-manipulated soils and the manipulated soils were probably due to the loss of microbial phyla correlated with the absorption of VOCs. Indeed, in experiments 2 and 3 we found that the increase in total VOCs

emissions was always accompanied by an increase of the *Proteobacteria* and *Bacteroidetes* relative abundance. Moreover, those phyla had a VOCs profile systematically positively correlated with VOCs compounds emissions. A positive correlation of a given VOC with a given phyla can occur through the emission from a specific VOC or its stimulated growth by this VOC. Other phyla than *Proteobacteria* and *Bacteroidetes* were found to be negatively correlated with VOCs emissions (chapter IV). A negative correlation may result from a given phyla using a particular VOC as carbon source (absorbing this VOC), or an inhibition of the given phyla by this particular VOC. Hence we could interpret the increase in total VOC emissions with increased dilution (**Figure VI-2** and Erreur ! Source du renvoi introuvable.), as a result of the loss of some phyla that were previously absorbing those VOCs together with the increase of *Proteobacteria* and *Bacteroidetes* that are emitting those VOCs.

We are aware that this study has been performed under laboratory conditions, by artificially manipulating the microbial diversity. Even though, reduced microbial diversity conditions, similar to our laboratory conditions, have been reported by several studies as a consequence of intensive soil management. Generally, high-input agricultural practices decrease microbial biodiversity while the low-input practices enhance microbial diversity in soil (Girvan et al., 2003; Munyanziza et al., 1997). Lupwayi et al., (2001) reported, for instance, higher microbial diversity in soils under conventional tillage than under zero tillage. Furthermore, Wolińska et al., (2017), compared cultivated soils with non-cultivated soils, reporting a decrease of the microbial diversity in soil up to 30% in the cultivated soil. Monoculture also reduces bacteria biodiversity in soil, while fungi biodiversity seems to be not affected (Liu et al., 2014). Furthermore, some studies reported soil microbial diversity decreasing with increasing latitude (Staddon et al., 1998). Soil microbial diversity may be lower in northern sites due to decreased productivity caused by nutrient limitation and higher acidity. The diversity and abundance of soil bacteria and fungi are also reduced in arid land. Indeed, aridity promoted shifts in the composition of soil bacteria, with increases in the relative abundance of Chloroflexi and Proteobacteria and decreases in Acidobacteria and Verrucomicrobia phyla (Maestre et al., 2015). We should further add that drylands are expected to expand in the global area by 11–23% by 2100 (Huang et al., 2016).

Contextualizing our results in the aforementioned scenarios (intensive agricultural practices, climatic changes leading to the dryland expansion, etc.), we hence hypothesized that total VOCs emissions from soil might increase. It is important to note that, apart from this study, and to our knowledge, no effects of the reduced microbial diversity dilution level on
Microbial dilution levels	Mean of the total VOCs emission rates nmol s ⁻¹ g ⁻¹ (DM)) x 10 ⁴	% of the Total VOC emissions rates increase from the non-manipulated soil	Tukey test Significance (p.value<0.5)		
DX	40.6		а		
D0	47.1	16%	а		
D1	54.2*	33%	а		
D2	85.4*	110%	b		

the VOCs emissions have been reported before. This hypothesis should hence require to be confirmed by field measurements.

Table VI-1.Mean of the total VOCs emissions rate for each microbial dilution levels and the nonmanipulated soil. DX=non-manipulated soil used in the first experiment, D0: microbial dilution equal to 1. D1: microbial dilution equal to 10^{-3} . D2: microbial dilution equal to 10^{-5} . *Total VOCs emissions means statistically different according to the T-test from DX total VOCs emissions mean (p.value<0.05).

VI.3 Coupled effect of the OWPs and microbial dilution on VOCs emissions

Analyzing the total VOCs emissions for the different type of OWPs without differentiating for the microbial dilution levels we could both find differences between OWPs total VOCs emissions rates (**Figure VI-3**). Abis et al., (2018) reported higher emissions from BIOW treatment which is not the case when the soil microbial manipulation occurs. This suggests that the effect of microbial dilution level was more important than the effect of the OWPs in soil on the VOCs total emissions. Once more, our results highlight the importance of the microbial diversity on the VOCs emissions.



Figure VI-4. Total VOC emissions rates for the different OWPs amendment in the soil. MSW: Municipal solid waste, GWS: Green waste and sludge, BIOW: Bio-waste, FYM: farmyard manure, CN: control without organic inputs. Bold line=median, boxes= interquartile, whiskers = minimum and maximum, white point = outliers. Letters indicate significant differences according to the Tukey test with p.value<0.05

Combining microbial dilution levels with the type of OWPs amendments in soil (**Figure VI- 5**), we can see that for CN and GWS soils, VOCs emissions increased with microbial dilution. For BIOW, MSW, and FYM the lower total VOCs emissions rate was recorded for the low microbial dilution level (D0). MSW soils showed the highest VOCs emissions (followed by BIOW and FYM samples) for the highest microbial dilution level. The MSW_D2 samples were found to have the higher presence of *Proteobacteria* and *Bacteroidetes* phyla (**Figure VI-6**). Those phyla were also found to be positively correlated with all VOCs compounds (chapter IV). We can, therefore, hypothesize that the observed emissions shift in MSW samples can be explained by the high relative abundance of *Proteobacteria* and *Bacteroidetes* phyla within the microcosm.

The BIOW_D0 reported the lower total VOCs emissions rate. Comparing this result with the relative bacterial abundance, we notice that the most abundant phylum was *Firmicutes*, representing 50% of the total bacterial abundance (**Figure VI-6**). Usually, for the other microcosms, *Firmicutes* relative abundance was between 20% and 7%. At the same time, in BIOW_D0 the relative abundance of *Proteobacteria* was around 20% while for the other samples, was always higher than 50%. Besides, *Bacteroidetes* were also less present in BIOW_D0 than in other samples: for the other OWPs and microbial dilution levels, we reported *Bacteroidetes* relative abundance between 20% and 40%, while in BIOW_D0 was around 4%. Once again, we can hypothesize that the lower presence of the *Proteobacteria* and *Bacteroidetes* are responsible for a lower VOCs total emission rate in BIOW_D0.

We can assume that the high microbial diversity dilution level in soil promotes the growth of *Proteobacteria* and *Bacteroidetes* phyla (**Figure VI-6**). Those pioneers bacteria phyla, in reduced competitive condition (i.e., high microbial diversity dilution level), colonized the environment quickly than other phyla. Since those phyla are positively correlated with VOCs emissions, we can thus conclude that the total VOCs emissions increase in the high microbial diversity dilution levels was due to the high presence of these two phyla. If we sum relative abundance of the *Bacteroidetes* and *Proteobacteria* phyla, we found that was always higher than 70% for all the microcosms except BIOW_D0, where they were less than 25%.



Figure VI- 5. Total VOC emissions rate for the different OWPs amendment in the soil. MSW: Municipal solid waste, GWS: Green waste and sludge, BIOW: Bio-waste, FYM: farmyard manure, CN: control without organic inputs. Bold line=median, boxes= interquartile, whiskers = minimum and maximum, white point = outliers. Letters indicate significant differences according to the Tukey test with p.value < 0.05



Figure VI-6. Bacterial relative abundance for the different OWPs and microbial dilution rate in soil samples. MSW: Municipal solid waste, GWS: Green waste and sludge, BIOW: Bio-waste, FYM: farmyard manure, CN: control without organic inputs.

Chapter VII

Conclusions and future perspectives

VII. Conclusions and future perspectives

This work aimed at the characterization of the VOCs emissions from soils amended with OWPs and to determine the impact of the different microbial dilution levels on VOCs emissions. Within the three experiments performed in order to achieve this aim, three different timings between the OWPs application and sampling were studied: two years for the first experiment (chapter III), one year for the second one (chapter IV) and 49 hours for the third one (chapter V).

The first experiment (chapter III) concerned the characterization of the VOCs emissions from soils amended with OWPs for almost 20 years. We found that the total VOCs emissions rate was affected by the type of OWPs amendment in the soil. In particular, we showed that the BIOW compost in soil had the largest VOCs emissions rate, while MSW compost had the lowest VOCs emission rate. Furthermore, in chapter III, we showed that the organic matter content and pH jointly influenced total VOCs emissions with higher emissions for higher joint pH and OM content.

Chapter III, further revealed that the most emitted compounds were acetone, butanone, and acetaldehyde whatever the OWPs amendment. We finally found that 21 compounds had statistically different emissions between the OWPs amendment.

In chapter IV, we showed by microbial dilution manipulation that VOCs emissions increased when soil microbial diversity decreased. We further showed that the most present bacteria benefited from dilution diversity: bacteria from *Proteobacteria* and *Bacteroidetes* phyla. A correlation analysis revealed that these bacteria we probable emitters of most VOCs compounds detected while other bacteria which presence decreased with dilution were probable absorbers of those compounds.

Additionally, in chapter IV we found that the VOC compounds most emitted by these manipulated soils were different from those from soils in Chapter IV. We also showed that microbial dilution affected both the quantity and the composition of emitted VOCs much stronger than the soil historical OWPs amendment.

With chapter V we aimed at characterizing GWS application effects on soil VOCs emissions and microbial community over the short-term following GWS application. This work led to the conclusion that the GWS amendment boosted *Bacteroidetes* growth while penalized *Proteobacteria* colonization. Furthermore, the addition of GWS in soil increased soil VOCs emissions in soils accustomed to GWS amendment but not in soils that did not

receive organic matter for 20 years. This may be attributed to increased activity of microbes specialized in degrading that OWP. This work finally suggested that the VOCs emissions induced by the GWS amendment might be used as a carbon source by microorganisms.

In order to give a global overview of the results found during these three years' work, we merged all the results in a unique dataset (chapter VI). This overview allows ordering factors affecting VOCs emissions. The effect of the microbial diversity was indeed stronger than the effect of the long-term OWPs application. At the same time, we demonstrate that the microbial diversity also affected the microbial community structure with consequences in term of composition and quantity on VOCs emissions. In contrast, in the short-term period following the last OWPs application (49h), the effect of the new OWPs application on total VOCs emissions was stronger than the microbial dilution level (chapter V).

With this work, we just began the investigation of the microbial diversity and the OWPs in soil effects on VOCs emissions. We demonstrated that the VOCs emissions were widely affected by the microbial diversity level and the microbial structure in soil. Since VOCs emissions are also influenced by several abiotic factors, we can also propose to study the effect of the microbial dilution levels as a function of temperature, soil pH, soil moisture, soil porosity, and organic matter content. Furthermore, VOCs emissions by soil are strictly related to the microbial enzymatic activity (i.e., sugar degradation). It could be thus interesting to perform enzymatic soil analysis. The analysis of the enzymatic activities allows the evaluation of soil processes for the degradation of cellulose, hemicelluloses, lignin, chitin, etc. by microorganisms. Furthermore, enzymes, give information about cycles and mobilization of the principal nutrient such as N, P and S. The complementary information obtained with the study of the enzymatic activity in soil will help to draw a bigger picture of the microorganisms role on the nutrient cycles in soil and the link with the VOCs emissions.

With the characterization of the emissions by the OWPs in soil we can raise awareness on the role played by the flux of VOCs emitted from soil on the atmospheric reactions involving VOCs. Hence, an interesting follow up of the present work will be the evaluation of the impact on atmospheric chemistry of the VOCs emitted from OWPs in soil. We can propose an analysis of the role played by the OWPs emissions on the SOA and O_3 formation. We can also imagine that a similar work could be carried out for the different levels of microbial diversity in soil in order to clarify the real impact of these VOCs emissions on atmospheric reactions.

Annex A – Supplementary material Chapter III - Profiles of VOCs emissions from soil receiving differing organic waste products

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Table III-S1. Isotopes and fragments found in the m	hass spectra by correlation analysis.
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m/z	correlated m/z		m/z difference
Likely isotopes			
37.01	39.03		2.02
44.99	45.99		1.00
106.07	108.08		2.01
107.08	108.08		1.00
120.10	121.10	122.10	1.00
127.10	128.11		1.01
135.11	136.11		1.00
137.12	138.13		1.01
151.14	152.14		1.00
165.16	166.16	167.16	1.00
179.17	180.18		1.01
297.08	299.05		1.97
355.07	356.08		1.01
371.10	372.10		1.00
Likely fragment	S		
167.17	179.17		12.00
167.17	181.18		14.01

m / z	TR	$TR_{H_3O^+}/TR_{R^+}$
< 21	0.018	1.000
33	0.40	0.045
59	0.65	0.028
79	0.75	0.024
107	0.86	0.021
146	0.96	0.019
> 181	1.00	0.018

Table III-S2. Standard Ionicon transmission curve used for computing the mixing ratio in equations (2) and (3)

Table III-S3. Total VOC emission rate and mixing ratio (summed up over all identified VOCs) for each treatment. Mean and standard deviation for each treatment calculated from replicates. BIOW= bio-waste compost, MSW= municipal solid waste compost, CN= control without organic input, FYM= farmyard manure, GWS= green waste and sludge compost.

	units	CN	BIOW	FYM	GWS	MSW
Total emission rate	nmol s ⁻¹ g ⁻¹ DW × 10 ⁴ μ g C m ⁻² s ⁻¹	42 ± 8	52 ± 12	43 ± 8	39 ± 4	36 ± 8
Total mixing ratio at chamber outlet	ppb	1700 ± 310	2100 ± 470	1700 ± 330	1600 ± 150	1500 ± 300

Figure III-S1. Emission rates (in nmol s⁻¹ g⁻¹ DM x 10⁴) per treatment for VOCs identified with the ANOVA test. MSW: Municipal solid waste, GWS: Green waste and sludge, BIOW: Bio-waste, FYM: farmyard manure, CN: control without organic inputs. Each plot label shows m/z of the VOC. Letters indicate significant differences according to the Tukey test with p.value < 0.05. Only VOCs with significant differences are shown.





Figure III-S2. Relative abundance of chemical classes found for each treatment.



Figure III-S3. Total VOC emissions as a function of the product of the soil pH (or $-\log[H^+]$) with the organic matter.

Annex B – Supplementary material Chapter IV - Volatile organic compounds emissions from soils are linked to the loss of microbial diversity

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Figure IV-S1. Shannon index for fungi in soil per dilution level. Bold line=median, boxes= interquartile, whiskers = minimum and maximum. Grey point= value the Shannon Index for each sample, white point = outliers. Letters indicate significant differences according to the Tukey test with p.value >0.05. D1= microbial dilution diversity equal to 10^{-3} , D2= microbial dilution diversity equal to 10^{-5} .



Figure IV-S2. Fungi/Bacteria ratio. Bold line=median, boxes= interquartile, whiskers = minimum and maximum. Letters indicate significant differences according to the Tukey test with p.value >0.05.



Figure IV-S3. Total VOCs emission rates per soil treatment. BIOW= bio-waste compost, MSW= municipal solid waste compost, CN= control without organic input, FYM= farmyard manure, GWS= green waste and sludge compost, D0= microbial diversity pure or 10^{0} , D1= microbial dilution diversity equal to 10^{-3} , D2= microbial dilution diversity equal to 10^{-5} .



Figure IV-S4. Shannon index for VOCs emissions in soil. D0= microbial diversity pure or 10^{0} , D1= microbial dilution diversity equal to 10^{-3} , D2= microbial dilution diversity equal to 10^{-5} .



Figure IV-S5. (a) Shannon index for bacteria in soil per OWPs. Bold line=median, boxes= interquartile, whiskers = minimum and maximum. Point= value the Shannon Index for each sample. Letters indicate significant differences according to the Tukey test with p.value >0.05. (b) Shannon index for VOCs emissions in soil per OWPs. D0= microbial diversity pure or 10⁰, D1= microbial dilution diversity equal to 10⁻³, D2= microbial dilution diversity equal to 10⁻⁵.

m/z Most likely formula		Tontotive identification	Close compounds	Emission rates ± sd (nmol s ⁻¹ g ⁻¹ (DW)) x 10 ⁴			Average ± sd emission rates	Averaged percentage contribution (%)		
111/ Z	Wost fikely for mula	Tentative mentilication	Class compounds	D0	D1	D2	(nmol s ⁻¹ g ⁻¹ (DW)) x 10 ⁴	D0	D1	D2
121.097	C ₉ H ₁₂	Propylbenzene cumene mesytilene 1,3,5-trimethylbenzene	Aromatic	7.83±13.04	14.33±11.04	49.59±8.2	23.92±17.12	35.57	33.47	37.17
93.066 107.081 135.113	C_7H_8 C_8H_{10} $C_{10}H_{14}$	Toluene Xylenes/ethyl benzene/benzaldehyde p-cymene	Aromatic	5.66±8.2	11.24±6.88	32.69±6.19	16.53±10.77	25.72	26.25	24.5
135.113	$C_{10}H_{14}$	p-cymene	Aromatic	2.04±2.77	4.39±2.44	10.45±2.35	5.63±3.22	9.27	10.25	7.83
73.062	C ₄ H ₈ O	Butanone, MEK	Carbonyl/ketone	0.82±1.02	0.96±1.49	2.49±1.36	1.42±0.71	3.73	2.24	1.87
122.100	C ₈ H ₁₁ N	Benzenamine/2,6-Xylidine		0.75±1.31	1.37±1.12	5.23±1.09	2.45±1.85	3.41	3.2	3.92
44.996	CO ₂	Carbon dioxide		0.67±0.38	0.62±0.63	0.74±0.5	0.68 ± 0.04	3.04	1.45	0.55
79.049 94.070 105.066	C ₆ H ₆ NA C ₈ H ₈	Benzene NA Styrene	Aromatic	0.63±0.93	1.24±0.85	4.24±0.81	2.04±1.47	2.86	2.9	3.18
57.068	C_4H_8	Butene	Alkene	0.51±0.77	2±0.81	3.11±0.55	1.87±0.91	2.32	4.67	2.33
119.078 136.118	C ₉ H ₁₁ NA	NA	Hydrocarbon	0.47±0.7	0.96±0.61	2.75±0.6	1.39±0.9	2.14	2.24	2.06
41.037	C ₃ H ₄	Propyne	Hydrocarbon (Alkyne)	0.35±0.37	0.94±0.55	2.19±0.47	1.16±0.69	1.59	2.2	1.64
				0.31±0.48	0.61±0.42	1.88±0.39	0.93±0.63	1.41	1.42	1.41
120.090	C ₈ H ₉ N		Aromatic heterocyclic compound	0.29±0.53	0.52±0.45	2.03±0.53	0.95±0.72	1.32	1.21	1.52
105.066				0.26±0.43	0.47±0.35	1.66±0.34	0.8±0.58	1.18	1.1	1.24
136.021	C ₇ H ₅ NS	Benzothiazole	Aromatic heterocyclic compound	0.23±0.07	0.3±0.15	0.4±0.12	0.31±0.06	1.04	0.7	0.3
134.108 136.118	C ₉ H ₁₁ N NA	NA NA	Amine	0.22±0.31	0.47±0.27	1.15±0.29	0.61±0.36	1	1.1	0.86
91.072	$C_4H_{10}S$	Diethyl sulphide	Organosulfur	0.19±0.31	0.33±0.27	0.88 ± 0.25	0.47 ± 0.28	0.86	0.77	0.66
136.118				0.16±0.23	0.35±0.2	0.87±0.21	0.46±0.27	0.73	0.82	0.65
106.073 108.084	NA C7H9N		Amine	0.15±0.23	0.26±0.18	0.98±0.21	0.46±0.34	0.68	0.61	0.73
94.070				0.14±0.21	0.27±0.18	$0.89{\pm}0.17$	0.43±0.3	0.64	0.63	0.67
79.071				0.12±0.16	0.27±0.18	0.9±0.15	0.43±0.31	0.55	0.63	0.67
108.084				0.1±0.15	0.18±0.12	0.66±0.14	0.31±0.23	0.45	0.42	0.49
33.033	CH ₄ O	Methanol	Alcohol	0.06±0.04	0.08±0.05	0.18±0.12	0.11±0.05	0.27	0.19	0.13

134.108	$C_9H_{11}N$		Amine	0.05 ± 0.08	0.12±0.07	0.28 ± 0.08	0.15±0.09	0.23	0.28	0.21
55.051	C_4H_6	1,3 butadiene	Hydrocarbon (diene)	$0.05 {\pm} 0.07$	0.08±0.13	0.27 ± 0.14	0.13±0.09	0.23	0.19	0.2
106.073				$0.05 {\pm} 0.08$	0.08 ± 0.06	0.32 ± 0.07	0.15±0.11	0.23	0.19	0.24
133.108	C10H13/C5H12N2O2H+		Hidrocarbon/Amine	$0.04{\pm}0.05$	0.09 ± 0.04	0.17±0.03	0.1 ± 0.05	0.18	0.21	0.13
122.064	C7H8NO		Amide	$0.04{\pm}0.06$	0.08 ± 0.05	0.14 ± 0.02	0.09 ± 0.04	0.18	0.19	0.1
95.052				0.03±0.04	0.06 ± 0.04	0.2±0.03	0.1 ± 0.07	0.14	0.14	0.15
74.065				0.03±0.04	0.04 ± 0.06	0.1±0.05	0.06±0.03	0.14	0.09	0.07
95.043	С6Н7О		Oxygenated compound	0.03±0.04	0.06 ± 0.04	0.18±0.03	0.09±0.06	0.14	0.14	0.13
123.073	C8H11O		Oxygenated compound	0.03±0.04	0.05 ± 0.04	0.17 ± 0.02	0.08 ± 0.06	0.14	0.12	0.13
45.033	C_2H_4O	Acetaldehyde	Carbonyl/aldehyde	0.03±0.29	0.06±0.47	0.42 ± 0.45	0.17±0.17	0.14	0.14	0.31
122.062				0.03±0.04	0.06±0.03	0.1±0.02	0.06±0.02	0.14	0.14	0.07
92.056	C ₇ H ₈		Hydrocarbon	0.03±0.04	0.05 ± 0.04	0.14±0.03	0.07 ± 0.04	0.14	0.12	0.1
58.071				0.02±0.03	0.08±0.03	0.12±0.02	0.07 ± 0.04	0.09	0.19	0.09
83.047	С5Н7О		Oxygenated compound	0.02 ± 0.01	0.03±0.02	0.17±0.12	0.07 ± 0.06	0.09	0.07	0.13
158.162				0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02±0	0.09	0.05	0.01
109.099	C8H13		Hydrocarbon	0.01±0.01	0.02±0.01	0.05 ± 0.01	0.03±0.02	0.05	0.05	0.04
149.130	C11H17		Hydrocarbon	0.01±0.01	0.03±0.01	0.05 ± 0.01	0.03±0.01	0.05	0.07	0.04
95.083	C7H11	Monoterpene fragment	Monoterpene	0.01±0.01	0.02±0.01	0.06 ± 0.01	0.03±0.02	0.05	0.05	0.04
111.041	$C_6H_6O_2$			0.01±0.01	0.02±0.01	0.02±0.01	0.02±0	0.05	0.05	0.01
95.028	$C_2H_6S_2$	Dimethydisulfide	Organosulfur	0.01±0.01	0.03±0.02	0.1±0.02	0.05 ± 0.04	0.05	0.07	0.07
108.953				0.01±0	0.01±0	0.01±0	0.01±0	0.05	0.02	0.01
117.084	C6H13O2		Oxygenated compound	0.01±0.01	0.02±0.01	0.06 ± 0.01	0.03±0.02	0.05	0.05	0.04
61.049				0.01±0.01	0.01±0.02	0.05 ± 0.05	0.02±0.02	0.05	0.02	0.04
80.048	C5H6N	Pyridine	Aromatic heterocyclic compound	0.01±0.02	0.03±0.02	0.09±0.02	0.04±0.03	0.05	0.07	0.07
105.031				0.01±0.02	0.02±0.01	0.06±0.01	0.03±0.02	0.05	0.05	0.04
53.037	C4H5		Hydrocarbon	0.01±0.02	0.03±0.02	0.1±0.02	0.05 ± 0.04	0.05	0.07	0.07
89.056	$C_4H_8O_2$	Acetoin	Ketons	0.01±0.02	0.08±0.07	1.96±1.68	0.68±0.85	0.05	0.19	1.47

Table IV-S1. Most emitted compounds for the different microbial dilution levels D0, D1 and D2

Annex C – Supplementary material Chapter V - Microbial VOCs dynamics after green waste and sludge amendment

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Figure V-S1.Comparaison of the microbial structure before (CN samples) and after GWS amendment (CN+GWS). a) Microbial community structure of CN samples. b) Microbial structure for the CN+GWS. D0= microbial dilution diversity equal to 1, D1= microbial dilution diversity equal to 10⁻³, D2= microbial dilution diversity equal to 10⁻⁵.



Figure V-S2. Figure V-S1.Comparaison of the microbial structure before (GWS samples) and after GWS amendment (GWS+GWS). a) Microbial community structure of GWS samples. b) Microbial structure for the GWS+GWS. D0= microbial dilution diversity equal to 1, D1= microbial dilution diversity equal to 10⁻³, D2= microbial dilution diversity equal to 10⁻⁵.



Figure V-S3.Shannon index of the different dilution levels calculated for CN and GWS. D0= microbial dilution diversity equal to 1, D1= microbial dilution diversity equal to 10⁻³, D2= microbial dilution diversity equal to 10⁻⁵. Blacks vertical bars= standard deviation, black points= outliers, Bold line=median, boxes= interquartile.

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Supplementary material

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Abis Letizia – Thèse de doctorat – 2018

Etude de l'effet des épandages avec des produits résiduaires organiques (PROs) et de la diversité microbienne sur les émissions des composés organiques volatiles (COVs) par les sols.

Résumé :

Les émissions de COVs jouent un rôle central sur la pollution atmosphérique. Les sources biogéniques des COVs sont entre 10 et 11 fois plus élevées que les émissions des COVs provenant par des sources anthropiques. Récemment, l'importance de la caractérisation des flux de COVs par les sols et les microorganismes a été soulignée. En effet, les émissions des COVs provenant du sol et des microorganismes sont des possibles précurseurs des particules atmosphériques et de la formation d'O₃. En particulier, ce travail est centré sur la caractérisation des émissions de COVs par des sols amendés avec des PROs en détectant les émissions toute de suite après l'apport, 1 an et 2 ans après l'apport des PROs. De plus, l'influence de la diversité microbienne du sol sur les émissions de COV a également été analysée. Les émissions de COVs ont été détectées à l'aide de la technique PTR-QiTOF-MS et la totalité des expériences a été réalisées dans des conditions de laboratoire contrôlées en utilisant des chambres dynamiques pour la détection des COVs émis par les échantillons. Les résultats ont montré que les différentes PROs émettent des quantités COVs variables et que les propriétés chimiques et physiques du sol influençaient également les émissions. L'analyse de l'influence de la biodiversité microbienne sur les émissions de COVs a montré que si la diversité microbienne est plus élevée, les émissions de COVs par les sols sont plus faibles. En outre, la diversité des COVs diminue lorsque les émissions de COVs par le sol sont plus élevées. Enfin, l'étude de la dynamique des émissions de COVs par de microcosmes récemment amendés avec du PRO, a montré que le flux des émissions de COV augmentait dans les premières 49 heures après l'apport des PRO en raison d'une perturbation de la communauté microbienne dans le sol.

Mots clés : COVs, COVs microbiens, dilution de diversité microbienne, PTR-QiTOF-MS, produits résiduaires organiques

Study of the effect of organic waste products amendments (OWPs) and microbial diversity on volatile organic compounds (VOCs) emissions by soil

Abstract :

VOCs emissions play a pivotal role on the atmospheric pollution. Biogenic sources of VOCs are between 10 and 11 times higher than VOC emissions from anthropogenic sources. Recently, the importance of the characterization of the VOC fluxes by soils and microorganisms has been highlighted. Instead, VOCs emissions from soil and microorganisms are possible precursors of the particulate matter and the O₃ formation. This work is focused on the characterization of the VOCs emissions by soils amended with OWPs over the long and short terms application. The influence of the microbial diversity in soil on VOCs emissions was also analysed. VOC emissions were detected using the PTR-QiTOF-MS technique and all the experiments were performed under controlled laboratory condition using dynamic chambers for the detection of the VOCs emissions from samples. The results showed that different OWPs released different quantity of VOCs emissions and also the chemical and physical properties of the soil were linked to the emissions. Analyses on the influence of microbial biodiversity on VOCs emissions have shown that while the microbial diversity was higher VOC emissions by soils were lower. Furthermore, the diversity of the VOCs decreases when the VOCs emissions by soil are higher. Finally, the study of the dynamics of VOC emissions from microcosms amended with fresh OWPs, showed that the VOC emission flux increased in the first 49 hours after the OWP amendment, due to a disturbance of the microbial community in the soil.

Keywords : VOCs, microbial VOCs, microbial dilution diversity, PTR-QiTOF-MS, organic waste products