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Abstract

Production of biogas through anaerobic digestion of organic wastes should play an important role in sustainable development of energy supply, and the environmental effects of digestates have to be assessed. We investigated the effect of anaerobic digestion of pig slurry (PS) on the molecular quality of the digestate produced. The consequences of digested (DPS) and undigested PS use as organic soil fertilizer on soil microbial and biochemical properties and C-gas emissions (CO_2 and volatile organic compounds) were studied during a two-month incubation. PS and DPS differed in the amount of volatile organic compounds (VOCs) emitted, in their organic C and lignin contents but not in their active microbial composition. Application of both types of slurry to the soil immediately increased the content of soil dissolved organic carbon (DOC) compared to the control soil. The application of DPS induced few changes in the biochemical composition of soil organic matter compared to its raw material (PS) that increased the amount of phenolic compounds. After 60 days, both amended and control soils contained similar amounts of DOC, amended soils presenting a more diverse biochemical composition of their soil organic matter. Application of both slurries to soil triggered a succession of different active microbial communities, which could be attributed to the introduction of new microorganisms and the input of new labile organic carbon. Changes in fungal communities were stronger than those of bacteria and archaea; however, only slight differences were observed between the slurries. Different fluxes and emission dynamics of five VOCs (methanol, acetone, DMS, 2-pentanone and phenol) were observed during the incubation time following application of PS or DPS to soil while no differences in CO_2 emissions were observed. The present study calls for long-term field studies with VOC analyses as a promising tool to differentiate organic fertilization practices.

Keywords

Soil archaea, bacteria and fungi; Anaerobic digestion; Digestate; Biochemical organic matter composition; Organic fertilizer; Soil quality.

1. Introduction

Production of biogas from organic wastes represents a major pillar of climate protection measures and the European Council endorsed the new target for 2030 to increase the proportion of renewable energy sources to 45% of the EU's final energy consumption (EREC, 2011). Bioenergy from biogas production will thus play an important role in facilitating a sustainable development of energy supply (Weiland, 2010). Biogas is produced through anaerobic digestion (AD) of wastes and leaves a liquid, nutrient-rich, fermentation by-product (digestate) (Insam et al., 2014). Anaerobic digestates are used as organic fertilizers applied on soil to improve its properties for crop production. Thus, before we can consider the production of biogas as a sustainable practice, the environmental effects of digestates have to be assessed.

During the AD process, the more labile organic fractions are rapidly degraded leading to an increase in the biological stability of the digestate that is concentrated in recalcitrant organic molecules compared with the initial raw material (Insam et al., 2015; Nkoa, 2014; Tambone et al., 2009). However, there is almost no research on the molecular changes induced to the organic matter (OM) during AD of slurry and their consequences once digestates are mixed with soil. Moreover, the digestate is a mixture of undigested material and a microbial consortium specific to the anaerobic process (Insam et al., 2015; Monlau et al., 2015), both capable of impacting the indigenous soil microbiota. While the quality of organic fertilizers is defined by its OM, C and N contents, the soil microbial compartment is the most commonly suggested biological indicator of soil quality. Bacteria, fungi and archaea are responsible for most of the soil biochemical processes, and changes in their communities can occur more quickly than changes in other soil characteristics (Doran and Parkin, 1994; Giacometti et al., 2013; Winding et al., 2005). Some literature studies reported differences in the responses (abundance, diversity, activity) of soil microorganisms to different kinds of organic fertilisers, included digestates, indicating a disturbance of soil biota (Abubaker et al., 2013; Barra Caracciolo et al., 2015; Odlare et al., 2008; Sapp et al., 2015; Singla et al., 2014).

The composition of digestate strongly depends upon the composition of the raw material from which they derived (Provenzano et al., 2011), but few studies compared the effects of digestate on soil to those of its raw material. Moreover, prior to the wide-promotion of using digestates as crop fertilizers, there is a need to assess their impact on C balance between soil and atmosphere. C balance is mainly monitored by measuring CO_2 and CH_4 emissions but it has been demonstrated that, by degrading soil OM, microorganisms also produce volatile organic compounds (VOCs) (Isidorov and Jdanova, 2002; Stahl and Parkin, 1996; Stotzky et al., 1976). VOCs could thus constitute an additional carbon loss from soil to the atmosphere and should be taken into account in the global C balance. It has previously been shown that soil organic amendments including digestates and N-mineral fertilization impact the VOC fluxes at the soil-atmosphere interface (Gray and Fierer, 2012; Seewald et al., 2010). Recently, using field monitoring, Potard et al. (2017) observed that digested pig slurry amendments differently impacted the soil bacterial communities and reduced the C-VOC fluxes from soil to the atmosphere compared to the amendments of undigested pig slurry. Thus by modifying the microbial decomposer communities and/or the soil OM status, undigested and digested organic amendments impact the VOC emissions from soil.

Despite growing literature aiming to evaluate the impact of digestates on C balance (CH_4 and CO_2 emissions) (*e.g.* Eickenscheidt et al., 2014; Johansen et al., 2013; Møller et al., 2009; Pampillon-Gonzalez et al., 2017; Pezzolla et al., 2012), there are still large uncertainties on how and to what extent soil microbiota and soil biochemical quality would be influenced in the long-term, and whether soil VOCs emissions would indicate such changes. The aim of the present study was thus to determine i) if anaerobic microbial digestion affects the biochemical composition of the organic substrate and ii) to what extent soil fertilisation with pig slurry digestate impacts soil microbial and biochemical properties which in turn could change the C balance between C stabilisation in soil and C loss through C-gas emissions. We compared our results using pig slurry digestate with its original raw material and a control treatment without any organic fertilizer. We hypothesized that OM quality of fertilizers changed due to the anaerobic digestion and that introduction of anaerobic microorganisms

through soil fertilization with digestate results in different patterns of VOCs emission from fertilized soils. The strength of the present study rests on the combined analyses of soil OM biochemical properties with the active soil microbial communities and the VOC emissions from soil. We observed that the fungal communities and the biochemical composition of soil OM were less modified following application of the digestate compared to that of the raw pig slurry. Overall, different dynamics of VOC emissions in the two-month study were observed between the two slurries.

2. Material and methods

2.1. Soil sampling and organic manures

The soil was collected from the top 10 cm of a long-term experimental observatory for environmental research (EFELE SOERE-PRO) of the French national institute of agronomic research (INRA) located near Rennes (France: 48°05'35.9"N, 1°48'49.5"W) and dedicated to study the environmental impact of the use of manures as soil fertilizers. The soil is a silt loam (71.3% silt, 12.6% clay, 16.1% sand) with 1.9% of OM content, an acidic water pH_w of 5.8 and a total nitrogen content of 1.3 g kg^{-1}. The pig slurry (PS) and the liquid digestate derived from its own anaerobic digestion (DPS) were collected from the Guernevez pig farming station and provided by the 'Chambre Régionale d'Agriculture de Bretagne' (Rennes, France). The DPS was collected from a 20 m^3 mesophilic (38°C) tank reactor after a retention time of 20 days.

2.2. Experimental design and set up

The experimental design consisted in 27 microcosms corresponding to three treatments (control without any amendment, PS and DPS amendments) replicated three times and performed for three incubation times (initial time, 30 and 60 days). The fresh soil was sieved to < 4 mm, 2.6 kg (equivalent dry weight) were placed into glass microcosms (cylinders: 25 cm high, 12 cm of diameter) and compacted to a bulk density of 1.4 g cm^{-3}. Soil moisture content was adjusted to 30% by adding deionized water, a glass cover was placed on the top of each microcosm and they were incubated in

the dark at 15°C. The next day, the experiment was launched by adding 48 mL of PS, DPS or deionized water (control soil; CS) at the microcosm surface (0.0113 m^2), based on the recommended field application rate of 4.25 liters per m^2. To mimic agricultural practices of organic amendment burying, the top 4 cm of each microcosm was mixed with a sterile spatula and the microcosms were then incubated at 15°C in the dark for up to 60 days. To maintain the soil moisture at 30% throughout the incubation period, individual microcosms were weekly weighted and deionized water was added if necessary. Samples from the two slurries (PS and the DPS) were stored at -20°C for organic matter analyses and frozen in liquid nitrogen and kept at -80°C for further RNA extractions. Just after the start of the experiment (day 0) and after 30 and 60 days of incubation, the top 10 cm of the replicated soil microcosms were sampled and kept at -20°C for organic matter and microbial carbon biomass analyses and frozen in liquid nitrogen and kept at -80°C for further RNA extractions.

2.3. Gas sampling and CO_2 and VOCs measurements

Gases were quantified just after the slurries or water addition and after 1, 2, 7, 14, 21, 28, 35, 42, 49, 56 and 60 days of incubation. For gas measurements, a 2 L glass dome including an inlet and outlet airflow both composed of a gas-tight cap with silicone septum was placed at the top of each microcosm and tightened using a dedicated clamp and a Teflon O-ring joint located between both of them. The VOCs naturally emitted by the slurries (PS and the DPS) were also analyzed by pouring 48 mL of slurry in a glass beaker placed in an stainless steel base designed to be adapted to the glass dome. After flushing the glass dome volume with ultra-pure air (Air zero alphagaz 2, Air Liquide, France), the gases emitted by either the soil core or the slurries accumulated for one hour in the glass dome. Prior to each gas sampling, the air in the headspace was homogenized using a 50 mL gas-tight syringe and a charcoal cartridge air filter (Supelpure® HC Hydrocarbon trap, Sigma-Aldrich, Germany) was then placed at the inlet airflow in order to purify the entering air. Three different gas samplings and measurements were successively performed: CO_2 using a micro Gas Chromatograph (µGC, Agilent), VOCs using both a micro Gas Chromatograph – Mass Spectrometer (microGC–MS, SRA

Instrument, France) after concentration on a tenax cartridge and a Proton Transfer Reaction-Mass Spectrometer (PTR-MS; Ionicon GmbH, Innsbruck, Austria) (Lindinger et al., 1998) following gas sampling in a Tedlar bag.

Precisely, CO_2 concentrations were first measured by a µGC (Agilent) connected to the outlet airflow of the glass dome. Hourly CO_2 emission rates (F_{CO2}; mgC-CO_2 m^{-2} h^{-1}) were calculated based on the following equation:

$$F_{CO2} = \frac{CCO2 \text{ x } V \text{ x } Mc}{VM \text{ x } A}$$

where C_{CO2} is the concentration of CO_2 measured (ppm Mol), V is the volume of the headspace (0.002 m^3), M is the molecular weight of C (12.01 g mol^{-1}), VM is the molecular gas volume (23.233 L.mol^{-1} at 15°C and atmospheric pressure) and A is the soil area in the microcosms (0.0113 m^2). A micro Thermal Desorption unit (µTD, AIRSENSE Analytics, Germany) was then placed at the outlet airflow of the glass dome to trap the VOCs emitted in the headspace on a tenax cartridge at a flow rate of 200 mL.min^{-1} at 35°C for 450 sec. Compounds were released by the trap at a helium flow rate of 300 mL.min^{-1} at 180°C for 70 sec and then transferred to the micro gas chromatograph for 30 sec. Compounds were separated using a capillary GC column (Stabilwax 10m) heated at 55°C and detected by a 5975C mass detector Agilent (microGC–MS, SRA Instrument, France). The carrier gas was helium at a constant pressure of 25 psi. The chromatograms were analyzed using the ChemStation software (MSD ChemStation version).

Finally, 0.5 L of the headspace was sampled from the outlet airflow using a gas-tight syringe and injected in a 2 L Tedlar gas collection bag (Interchim, Montluçon, France) for further VOC measurements using a PTR-MS (QMS300, Ionicon GmbH, Innsbruck, Austria) (Lindinger et al., 1998). Each bag was cleaned with successive flushes of ultra pure air before collection.

For PTR-MS analysis, Tedlar bags were connected directly to the apparatus and, operating at 135 Townsends (Td) (1 Td = 10^{-17} V cm^2 $molecule^{-1}$) in order to reduce VOC fragmentation. The "MID mode" was selected during these experiments and consisted in scanning five masses (33, 59, 63, 87 and 95) in the range 21-95 amu at 0.2 s dwell time. These masses have been selected based on

literature, as they were previously detected as emitted by slurries (Feilberg et al., 2015; Sutton et al., 1999) and taking part in both C and S cycles. Thus, mass 33 was attributed to methanol (Aaltonen et al., 2013), mass 59 to acetone (Aaltonen et al., 2013), mass 63 to dimethyl sulfide (DMS) (Veres et al., 2014), mass 87 to 2,3-butanedione (Feilberg et al., 2015) and mass 95 to phenol (Feilberg et al., 2015). The ionization of VOCs either by O2+ or NO+ was negligible as the 02+:H3O+ and NO+:H30+ ratios were ≤3.5% and 5% respectively. Five measurements of each mass concentration were performed and averaged across treatments. To calculate the molecular density of each VOC, [R], the following equation was used, accounting for drift tube temperature (333 K) and drift tube pressure (2.20 mbar) (Zhao and Zhang 2004; de Gouw et al. 2003).

$$[RH^+] = k_1[H_3O^+][R]t + k_2[H3O^+(H_2O)][R]t$$

where $[H_3O^+]$, $[H_3O^+(H_2O)]$ and [RH+] are the ion signals (cps) at mass 21 (9500), mass 39 (9250), and the target VOC, respectively; t is the average time (t) spent by reactants in drift tube (105 ms); k_1 and k_2 are the rate coefficients between target VOC and H_3O^+ or $H_3O^+(H_2O)$. Including $H_3O^+(H_2O)$ into the reaction is a modification by Lindinger et al. (1998). Except for the slurries, each VOC flux (F_{VOC}) from soil was calculated using the following equation:

$$F_{VOCs} = \frac{CVOC \text{ x } V \text{ x } Mc}{VM \text{ x } A}$$

where C_{VOC} is the concentration of VOC measured (ppb), V is the volume of the headspace (0.002 m^3), M is the molecular weight of the VOC (Mass-1), VM is the molecular gas volume (23.233 $L.mol^{-1}$ at 15°C and atmospheric pressure) and A is the soil area in the microcosms (0.0113 m^2).

2.4. Soil water extractable organic matter (WEOM) composition analyses

The soil WEOM was extracted from 20 g of soil in 200 mL of deionized water. After orbital mixing during one hour, the extracts were filtered through a 0.7 µm glass microfiber filter (whatman International, Maidstone, England) and divided into two sub-samples. The first one was used for dissolved organic carbon (DOC) quantification on a Shimadzu TOC-5050A total carbon analyzer and the other 100 mL were lyophilized to analyze the composition of the WEOM. Pig slurry and its

digestate were filtered at 0.7 µm, aliquots were used for DOC quantification and 20 mL were lyophilized. The lyophilisates were analysed by thermally assisted hydrolysis and methylation using tetramethylammonium hydroxide coupled to a gas chromatograph and mass spectrometer (THM-GC-MS) as previously described by Jeanneau et al. (2014). The detected compounds were classified into five chemical classes: fatty acids (FA), carbohydrates biomarkers (CAR), lignin biomarkers (LIG), phenolic compounds (PHE) including benzoic, phenylacetic, methylphenylacetic and phenylpropanoic acids, and dimethyl indole (IND). Among the lignins, 8 compounds were detected: the vanillyl phenols (vanillic acid, acetovanillone and vanillaldehyde), syringyl phenols (syringaldehyde, syringic acid, acetosyringone) and cinnamyl phenols (*p*-coumaric acid and ferulic acid). The ratio of cinnamyl to vanillyl phenols (C/V) was calculated; it reflects either the botanic source of lignin or, for a given source of lignin, its biodegradation state (Hedges and Mann, 1979). FA can come from microbial and plant-derived OM and these contributions can be differentiated on the basis of the length of the aliphatic chain. FA with less than 19 C atoms mainly derived from microbial inputs, while FA with more than 19 C and an even-over-odd predominance are characteristic of plant-derived OM (Cranwell, 1974; Matsuda and Koyama, 1977). The proportion of microbial FA was calculated as the percentage of low molecular weight FA (< C19) by excluding C16:0 and C18:0, which can derive from both plant and microbial residues. The bacterial FA were thus composed of C12:0, C13:0, C14:0, C15:0, C17:0, *anteiso* and *iso* C15:0 and C17:0, *iso* C16:0, C16:1 and C18:1, which are commonly used as bacterial indicators (Frostegård et al., 1993). Among carbohydrates, the ratio of deoxyhexoses to pentoses (deoxyC6/C5) was used as a balance between plant-derived (< 0.5) and microbial-derived (> 2) carbohydrates (Murayama, 1984; Oades, 1984).

2.5. Soil microbial biomass and community analyses

Soil microbial C biomass was determined by the chloroform fumigation and extraction method (Vance et al., 1987) using a total organic carbon analyzer (1010 wet oxidation TOC - OI-Analytical - USA). The microbial biomass C was calculated using the following equation:

Microbial C biomass = (Cf – Cnf) x Kc

Where *Cf* is the DOC in fumigated soils, *Cnf* is the DOC in non-fumigated soil and *Kc* is a correction factor of 2.64.

RNA was extracted from 2 g of soil using Power Soil MoBio kit (MoBio laloratories Inc., NY) according to the manufacturer's instructions. The RNA extracts were electrophoresed over a 1% agarose gel to assess RNA quality. DNA was removed using RQ1 DNase treatment (Promega, Madison, WI) by mixing 35 µL of RNA extract, 15 units of DNase I, 5.5 µL of 10X DNase buffer, and the mixture was incubated at 37°C for 30 min. reactions were terminated by addition of 5.55 µL of DNase I stop solution and incubation at 65°C for 10 min.

Terminal restriction fragment polymorphism (T-RFLP) analyses were performed for each soil sample to determine the bacterial, archaeal and fungal community structures. Reverse transcription-polymerase chain reaction (RT-PCR) were carried out using the Titan one-tube reverse transcription-PCR kit (Roche Applied Science) to amplify the bacterial and archaeal 16S rRNA and the fungal ITS with the corresponding forward primer to which was attached the 6-carboxyfluorescein (FAM) label to the 5' end and reverse primer (Table 1). Reaction mixtures (25 µL) contained soil sample RNA (3 µL), 8 mM dNTPs, 1.25 µL dithiothreitol (DTT), 2.5 U RNase inhibitor, 5 µg bovine serum albumin (BSA), 0.8 µM of each primer and 0.5 µL of enzyme mix in 1X reaction buffer. The following program was used for the one step RT-PCR: 30 min at 51°C, 2 min at 94°C, 10 cycles of 30 s at 94°C, 30 s at the specific primer's pair hybridization temperature (Table 1) and 1 min at 68°C, followed by 10 cycles for bacteria, 15 for archaea and 25 for fungi of 30 s at 94°C, 30 s at the specific primer's pair hybridization temperature (Table 1) and 1 min added to 5 more seconds per cycle at 68°C, and finally 30 s at 94°C, 30 s at the specific primer's pair hybridization temperature (Table 1) and 7 min at 68°C. RT-PCR reactions were performed in duplicates for each sample, combined and the RT-PCR products were purified with the GenElute PCR Clean-Up kit (Sigma-aldrich). The Labeled PCR products were digested with the restriction enzyme MspI for bacteria and fungi and AluI (Promega, Madison, WI) for archaea in 50 µl separate reactions, at 37°C for 3 h. DNA was precipitated overnight and the T-RFs

were separated and accurately sized on the Biogenouest platform using an ABI system analyser (3730; Applied Biosystems).

Raw data from Peak Scanner 1.0 software (Applied Biosystems) were exported to T-REX online software (http://trex.biohpc.org) for the processing and analysis of T-RFLP data (Culman et al., 2009). A clustering threshold of 0.5 bp was applied and only the length of the T-RFs between 50 and 500 bp was taken into account for data analysis. Each T-RF was coded as a discrete variable (0 for its absence or 1 for its presence) and the matrix generated was used in principal component analysis (PCA) to compare the microbial community structures in the different treatments at the different incubation times. PCA were generated using R statistical software version 3.4.1 ("http://www.r-project.org/") and the ADE4 package (Dray and Dufour, 2007).

Microbial richness was estimated as the number of T-RFs for each sample and the richness proportion of each microbial taxa among the overall richness (sum of the richness of bacteria, archaea and fungi) was calculated for each sample and sampling time. These proportions were represented in a ternary plot based on Graham and Midgley (2000).

2.6. Statistical analyses

All the statistical analyses were conducted using R statistical software version 3.4.1 (http://www.r-project.org). Differences in i) the molecular composition of the DOM, ii) the amounts of microbial C biomass, iii) the C-CO_2 emissions of the soil with or without slurries and iv) the VOC emissions by the two slurries were analyzed using the non-parametric Kruskal-Wallis test (R package) followed by Dunn's post hoc tests for pairwise comparisons using the Pairwise Multiple Comparisons of Mean Rank Sums (PMCMR) package (http://CRAN.R-project.org/ package=PMCMR).

3. Results and discussion

3.1. Effects of anaerobic digestion on the biochemical and microbial properties of the slurries and their VOC emissions

278 The digested and undigested pig slurries differed from each other in some of their biochemical
279 properties (Fig. 1) but not significantly in their active microbial composition (Fig. 2). During the
280 anaerobic digestion, the slurry lost its C as CO_2 and CH_4 leading to a digestate with a half-reduced
281 organic C content (7.0 and 3.8 $mg.ml^{-1}$ in PS and DPS, respectively) (Fig. 1). Such a decrease is in
282 accordance with the value reported in the literature with a reduction from 40% to 82.5% of total C in
283 digested manures compared with undigested ones (Abubaker et al., 2012). The anaerobic digestion
284 resulted also in a higher proportion of lignin in the digested PS compared with the PS (4.5% and 0.7%,
285 respectively). Such concentration of recalcitrant molecules as lignin during anaerobic digestion has
286 been previously reported by Tambone et al. (2009).
287 Phenolic compounds were the main components of the dissolved fraction of both slurries (96.2%
288 and 91.2% in PS and DPS, respectively, Fig.1). They are derived from lignin degradation (Bugg et al.,
289 2011; Elder and Kelly, 1994) and among these aromatic acids, benzoic acids are used in porcine
290 breeding as dietary supplement to decrease ammonia emissions (Hansen et al., 2007) and have
291 previously been detected in pig urine (Yasuhara et al., 1984). PHE are known to be key metabolic
292 intermediates in the anaerobic digestion (Levén and Schnürer, 2005). For example, phenylpropanoic
293 acid is thought to come from the metabolic degradation of tyrosine in pig feces (Spoelstra, 1978) and
294 phenylacetic acid has been proposed as an indicator of the overall stability of the anaerobic process
295 (Iannotti et al., 1986).
296 During anaerobic digestion, the establishment of successive microbial consortia (Insam et al.,
297 2015; Manyi-Loh et al., 2013; Monlau et al., 2015) might lead to differences in the microbial
298 communities of PS and DPS. However, in the present study we only observed slight differences in the
299 active bacterial communities of PS and DPS using T-RFLP on RNA but surprisingly, no changes were
300 detected for fungi and archaea (Fig. 2). Although archaea are responsible for the production of CH_4
301 that takes place at the end of the anaerobic metabolic pathway of organic matter degradation, they
302 should have been stimulated and we could have expected changes in their community structure. It
303 has previously been shown that, based on DNA analysis, anaerobic digestion leads to an enrichment

304 of archaea from the genus *Methanosarcina* (Sun et al., 2015). By using RNA-based analysis, we did
305 not observe such changes suggesting that a common part of the community representing a core of
306 dominant microorganisms was active in both slurries. Even if the explained variations of the PCAs
307 were low (Fig. 2), we can speculate that the initial raw material rather than the AD process controlled
308 the diversity of active microorganisms in the digestate.

309 The emission profiles of five selected VOCs (methanol, acetone, DMS, 2-pentanone and phenol)
310 were determined for the slurries themselves and their concentrations varied from 1.8 up to 6.2 times
311 higher in PS than in DPS, excepting for the 2-pentanone which emissions were 1.2 times higher in
312 DPS than in PS (Table 2). The mineralization that occurred during the AD, by reducing the OM
313 available for microbial degradation, should have limited further emissions of VOCs in digestates.
314 Moreover, the emission of the studied VOCs might have been stimulated during the AD, as previously
315 observed during the composting of biowaste (Smet et al., 1999), and thus limiting their detection in
316 the digested slurry.

317

318 *3.2. Impact of slurry application on soil WEOM*

319 In the control soil receiving no organic fertilizer (CS), the amount of DOC increased with
320 incubation time at 15°C from 69 to 90 µg g^{-1} dry soil, although these differences were not significant
321 due to high variability between replicates after 60 days of incubation (Kruskal Wallis, H = 4.33, *P* =
322 0.12; Fig. 1). The WEOM in the CS at the beginning of the incubation was mainly composed of FA
323 (87.2%) from microbial origin (84.9%) with few proportions of CAR (4.9%), LIG (4.6%) and PHE (3.0%)
324 (Fig. 1). This initial composition varied through incubation time, the proportion of microbial fatty
325 acids being the highest at 30 days (93.4 %), and finally resulted, after 60 days of incubation, in an
326 enrichment in carbohydrates (10.7%) to the detriment of the initial microbial fatty acids content
327 (73.9%) (Fig. 1).

328 As expected, both slurry additions to soil immediately increased the soil DOC content that was 2.8
329 and 2.6 times higher in soils receiving PS and DPS than in the control soil at the start of the

incubation, respectively (Fig. 1). Moreover, the application of PS to soil was linked to an increase in the proportion of PHE (34.6%), LIG (7.8%) and IND (5.9%) associated with a decrease in FA (47%) (Fig.1). This was not observed following DPS application to soil since it had a weaker impact on the composition of the soil WEOM that only exhibited higher proportions of LIG (10.0%) and IND (2.2%) than those observed in CS. Interestingly, the proportion of PHE in the soil that received DPS (SDPS) at the start of the experiment was similar to the one in CS (3.6% vs 3.0% in SDPS and CS, respectively) (Fig. 1) while it was a main component of both slurries. Such differences between the two amended soils might be explained by the low initial content of DOC and thus PHE compounds observed in DPS (6.7 and 3.4 mg C-PHE. L^{-1} in PS and DPS, respectively).

The effect of PS application to soil was observed earlier than the one of DPS and, after 30 days, the amount of DOC was still 1.5 times higher in the soil that received PS (SPS) than in CS or DPS (SDPS)(Fig. 1). At that time, both slurries altered similarly the composition of soil DOC by increasing its content in carbohydrates, lignin and phenolic compounds to the detriment of the microbial FA. Finally, at the end of the experiment, the amended soils did not contain more DOC than the control soil but they exhibited a more diverse composition of their WEOM with less microbial fatty acids and higher carbohydrates and lignin contents (Fig. 1).

Both the lignin and carbohydrates in the WEOM of the unamended control soil mainly derived from degraded material and plant inputs, as indicated by the very low C/V lignin and deoxy C6/C5 ratios, respectively (Fig. 3). In accordance with a mixing between the inherent soil WEOM and the fine organic fraction (<0.7 µm) of PS (C/V lignin = 2.1 and deoxy C6/C5 = 2.3) and DPS (C/V lignin = 2.4 and deoxy C6/C5 = 1.6), these two indicators of lignin and carbohydrates sources temporarily increased significantly or not after the application of slurries (Fig. 3). This transient changes in the C/V lignin ratio reflected a new source of lignin brought by the slurries that was less degraded than the one already present in soil. The increase in the deoxy C6/C5 ratio clearly indicated a higher proportion of microbial carbohydrates (Rumpel and Dignac, 2006) linked to the ephemeral increase in the C biomass of soil microorganisms observed just after the application of both slurries (Fig. 4).

However, both C/V and deoxy C6/C5 ratios recovered similar low values as the one observed in the control soil after 60 days of incubation. Overall, the C/V lignin ratio was significantly correlated to both soil DOC and microbial C biomass (R^2 = 0.49, P < 0.001 ; R^2 = 0.47, P < 0.001, respectively) indicating the importance of the OM state of biodegradation in the dynamic of the soil C compartment.

Altogether our results indicate that the slurries brought a new microbial source that rapidly but briefly fueled the dissolved organic fraction of the soil. As the majority of soil microorganisms are heterotroph, they rely on the availability of organic C, addition of exogenous OM as PS and DPS should thus have supported a larger microbial biomass. In the present study no such stimulation was observed during the experiment lifespan (Fig. 4) while the labile organic C was initially up to three times higher in the amended soils than in the control soil (Fig. 1). The time scale of our experiment might not have been adapted and a short-term impact of the slurry application might have been visible by analyses performed within the first 30 days of incubation. Indeed, Potard et al. (2017), based on field monitoring and next generation sequencing analysis, detected changes in soil microbial communities seven days after either PS or DPS application that were not detected anymore after 36 days. However, Pezzolla et al. (2015) observed fluctuating trend in the C biomass of soil microorganisms up to 30 days following application of digestate from pig slurry to an agricultural soil in controlled conditions. The impact of organic amendments on soil microorganisms is thus not as obvious as expected and might also depend on the quantity and quality of the OM added. In the present study, the fractions of dissolved organic matter (< 0.7 µm fractions) of both slurries were mainly composed of phenolic compounds and also of lignin for the DPS that are recalcitrant to biodegradation and might have constrained microorganisms by stimulating only a few part of the community able to degrade such compounds.

3.3. Response of soil microbial communities

381 The community structures of active bacteria, archaea and fungi were analyzed by T-RFLP just after
382 slurry application to soil and after 30 and 60 days of incubation (Fig. 2). Specific changes in the soil
383 microbial communities were observed for each microbial taxa and slurry following slurry application
384 to soil. At the start of the experiment, just after the application of slurries to soil (SPS0, SDPS0),
385 changes observed in fungal, archaeal and to a lesser extent in bacterial communities are likely due to
386 the introduction of microbial communities specific to PS and DPS (Fig. 2) that also increased the
387 microbial C-biomass (Fig. 4). Cropped soils are well known to sustain bacteria-dominant microbial
388 communities (Hendrix et al., 1990; Strickland and Rousk, 2010) that might have buffered the impact
389 of the introduction of active bacteria from slurries. Interestingly, the active fungal communities in the
390 soil receiving both slurries (SPS0, SDPS0) were closer to the one in the slurries themselves (PS, DPS)
391 than to one in the initial soil (CS0) (Fig. 2C). This might be due to the low abundance of fungi usually
392 observed in cropped soils (Hendrix et al., 1990; Strickland and Rousk, 2010), allowing their
393 community to be greatly shaped by the application of slurries (as substrates) and their proper fungal
394 communities. Indeed, while fungal communities have been poorly studied during anaerobic
395 digestion, a recent study highlighted their high diversity in several digesters supplied with different
396 plants and livestock manures (Langer et al., 2019).
397 Together with temporal dynamics of soil microbial communities observed in the control soil,
398 slurry application to soil led to successive active microbial communities. Several studies observed
399 differences in the response of soil microorganisms following the application of slurries and digestates
400 (Abubaker et al., 2013; Barra Caracciolo et al., 2015; Chen et al., 2012; Walsh et al., 2012). These
401 differences were mainly attributed to the composition and availability of the OM added, the digested
402 material being enriched in more stable compounds while the undigested one was concentrated in
403 labile organic C easily degradable by microorganisms (Abubaker et al., 2013; Marcato et al., 2009).
404 Such differences in the two slurries may induce shifts in the structure of the microbial community,
405 the digestate stimulating the slow-growing microorganisms compared with application of undigested
406 organic matter (Chen et al., 2012). In the present study, the application of both slurries greatly

impacted the composition of the active bacterial and archaeal communities after 30 days of incubation (SPS30 and SDPS30), the disturbance generated by DPS being higher than by PS. At 60 days, the composition of active bacterial and archaeal communities in amended soils was close to the one observed in control soils with the exception of active archaea in soil amended with DPS, the effect of the digestate being subtle but still detected (Fig. 2A and 2B). The application of PS strongly constrained the fungal community that was specific and stable with time in this treatment (SPS0, SPS30 and SPS60) whilst it was highly variable in the SDPS treatment (Fig. 2C). The differences observed in the fungal community in SPS and SDPS might result from their interaction with the bacterial community. It is suggested that bacteria tend to dominate the ecological interactions with fungi in conditions of rich resources such as in the SPS were labile organic C is available, whilst fungi grow when conditions are unfavorable for bacteria (Rousk and Bååth, 2011; Walsh et al., 2012). Moreover, fungi are more efficient for the degradation of complex substrates that should make them better competitors in SDPS treatments (Meidute et al., 2008).

The species richness, estimated as the number of T-RFs, within the three microbial taxa was investigated (Fig. 5). The disturbance observed in microbial community structures 30 days after slurry application corresponded to a homogeneous distribution of the richness across the microbial taxa whatever the treatment, with no taxa representing more than 50% of the total T-RFs (Fig. 5B). At the end of incubation, the slurry application induced changes in the distribution of species richness that finally led to distinct communities, CS being enriched in fungi and depleted in archaea, soils amended with SPS being depleted in archaea and SDPS being slightly enriched in bacteria.

3.4. Soil volatile organic compounds and Carbon dioxide emissions

C-CO_2 fluxes were measured in the different soil microcosms during the incubation time. The respiration kinetics over 60 days showed a clear time effect (Kruskal Wallis test, $H = 42.8$, $p < 0.001$) but no significant impact of the slurry application despite it represented an additional C source to soil compared with the control soil (Kruskal Wallis test, $H = 1.4$, $p = 0.49$). The C-CO_2 fluxes similarly

433 varied, from 11.7 ± 1.1 to 18.4 ± 0.4 in CS, from 11.0 ± 3.2 to 20.6 ± 0.1 in SPS and from 12.3 ± 0.1 to
434 19.3 ± 1.3 mg C-CO_2 m^{-2} h^{-1} in SDPS, leading to cumulative emissions of 3.7, 3.9 and 4.0 g C-CO_2 m^{-2}
435 after 60 days of incubation in CS, SPS and SDPS, respectively (Fig. 6). The loss of C by aerobic
436 respiration was thus similar whatever the treatment, while different contents of DOC were observed
437 for amended soils and control soil (Fig. 1). It is likely that the amount of DOC in soil does not reflect
438 the available C to be mineralized, probably due to limitation in the availability of other nutrients for
439 soil microorganisms.

440 The present study demonstrates changes in both microbial communities and WEOM composition
441 following application of either PS or DPS to soil. We hypothesized that such modifications of soil
442 biochemical properties induced changes in the emissions of the VOCs through SOM biodegradation
443 by microorganisms. It is known that VOCs produced by soil microorganisms strongly depend on both
444 quality of the substrate degraded and on microbial diversity and activity (McNeal and Herbert, 2009;
445 Peñuelas et al., 2014; Stotzky et al., 1976). The VOC emissions were thus measured using both a µGC-
446 MS and a PTR-MS.

447 Due to its high detection limit and to our experimental conditions providing a restricted volume
448 of soil, the µGC-MS did not detect any VOC in CS whatever the incubation time. However, up to two
449 days after the application of both PS and DPS, emissions of acetone, 2-butanone, dichloromethane,
450 2-pentanone and toluene were identified. These molecules have already been described as emitted
451 directly by different kinds of manures or by soil following their application (Abis et al., 2018; Feilberg
452 et al., 2015; Trabue et al., 2010; Woodbury et al., 2006). Moreover, we found that these molecules
453 were emitted by the slurries (Table 2) and thus we cannot exclude their emissions by the slurries
454 themselves following their application to soil.

455 Thanks to the lower detection limit of the PTR-MS we measured the emission of five masses all
456 along the incubation time and corresponding to methanol (m33), acetone (m59), DMS (m63), 2-
457 pentanone (m87) and phenol (m95) (Fig.7). Contrarily to CO_2, distinct fluxes and dynamics of VOC

458 emissions were observed after application of slurries to soil, but we mainly observed marginal
459 responses and no specific VOC emission patterns were identified.

460 Acetone, 2-pentanone and DMS emissions were the most abundant C-VOC fluxes. Just after slurry
461 application, the soil amended with PS systematically emitted higher concentrations of any VOC
462 compared to the SDPS and C-VOCs fluxes from SDPS were even lower than those from control soil for
463 2-pentanone and methanol (Fig. 7). One day after both slurry applications, the C-VOC fluxes from the
464 microcosms with slurries were higher than those in CS, except for the phenol emission, and, this
465 increase lasted from one day for the acetone in SDPS up to 49 days for the DMS. Distinctive VOC
466 emissions following the application of PS and DPS to soil have already been observed, especially for
467 methanol fluxes that were induced after PS fertilization of soil compared with DPS fertilization
468 (Potard et al., 2017). Interestingly, for all the VOCs measured, the emissions could be lower than
469 those detected in CS (Fig. 7). Once produced, the VOCs can either be degraded by soil
470 microorganisms that use them as C source (Owen et al., 2007; Ramirez et al., 2009) or adsorbed on
471 soil that acts as a sink of VOCs (Asensio et al., 2007), two processes that reduce their emission from
472 soil to the atmosphere and make it difficult to identify specific VOC emission patterns depending on
473 amendment practices. This specificity between VOC emission patterns and amendment practices
474 have been already observed on soils from long-term experimental sites, suggesting that repeated
475 inputs are needed to allow to differentiate fertilization practices according to the VOC spectra
476 emitted by soil (Abis et al., 2018; Seewald et al., 2010).

477

478 **4. Conclusions**

479 Anaerobic digestion of pig slurry increased dissolved organic C by a factor of two, without affecting
480 microbial community structures. The application of both slurries to soil led to a similar increase in soil
481 DOC contents, microbial C-biomass and soil respiration, and disturbed soil microbial communities.
482 However, instantaneous changes in the quality of the WEOM were mainly observed following the
483 application of PS only. After 60 days, the biochemical composition of the soil WEOM was more

484 diverse in the amended soils but the DOC content was not affected anymore by the two slurry
485 applications. This disappearance of soil DOC could not be attributed to either an enhanced
486 mineralization or an increase in VOC emissions. It is likely that the amount of DOC in soil did not
487 reflect the available C to be mineralized. Further analyses of soil available nutrients (e.g. nitrogen,
488 phosphorus…) might have helped to better understand the effect of slurry application on C balance
489 and the associated biogeochemical cycles. Overall, the application of PS induced greater changes in
490 the biochemical composition of the soil organic matter and fungal communities compared to its
491 digestate (DPS), and different fluxes and emission dynamics of VOCs were observed following the
492 application of the two slurries. The present study i) calls for strengthening long-term on-field studies
493 to better predict the future impact of slurry and digestate amendments on soil quality and C-balance
494 and ii) supports the use of VOC analyses to differentiate organic fertilization practices.
495

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705

706 Table 1

707 Description of primer sets and hybridization temperatures used for RT-PCR

708

Target group	primer	sequence	hydridization temperature	References
Bacteria	63F	5'-CAGGCCTAACACATGCAAGTC-3'	58°C	Marchesi et al., 1998; Osborn et al., 2000
	1389R	5'-ACGGGCGGTGTGTACAAG-3'		Marchesi et al., 1998; Osborn et al., 2000
Archaea	Ar109F	5'-ACKGCTCAGTAACACGT-3'	56°C	Lueders and Friedrich, 2000
	Ar912R	5'-CTCCCCCGCCAATTCCTTTA-3'		Lueders and Friedrich, 2000
Fungi	ITS1F	5'-CTTGGTCATTTAGAGGAAGTAA-3'	55°C	White et al. 1990; Gardes and Bruns, 1993
	ITS4	5'-TCCTCCGCTTATTGATATGC-3'		White et al. 1990; Gardes and Bruns, 1993

709 Table 2

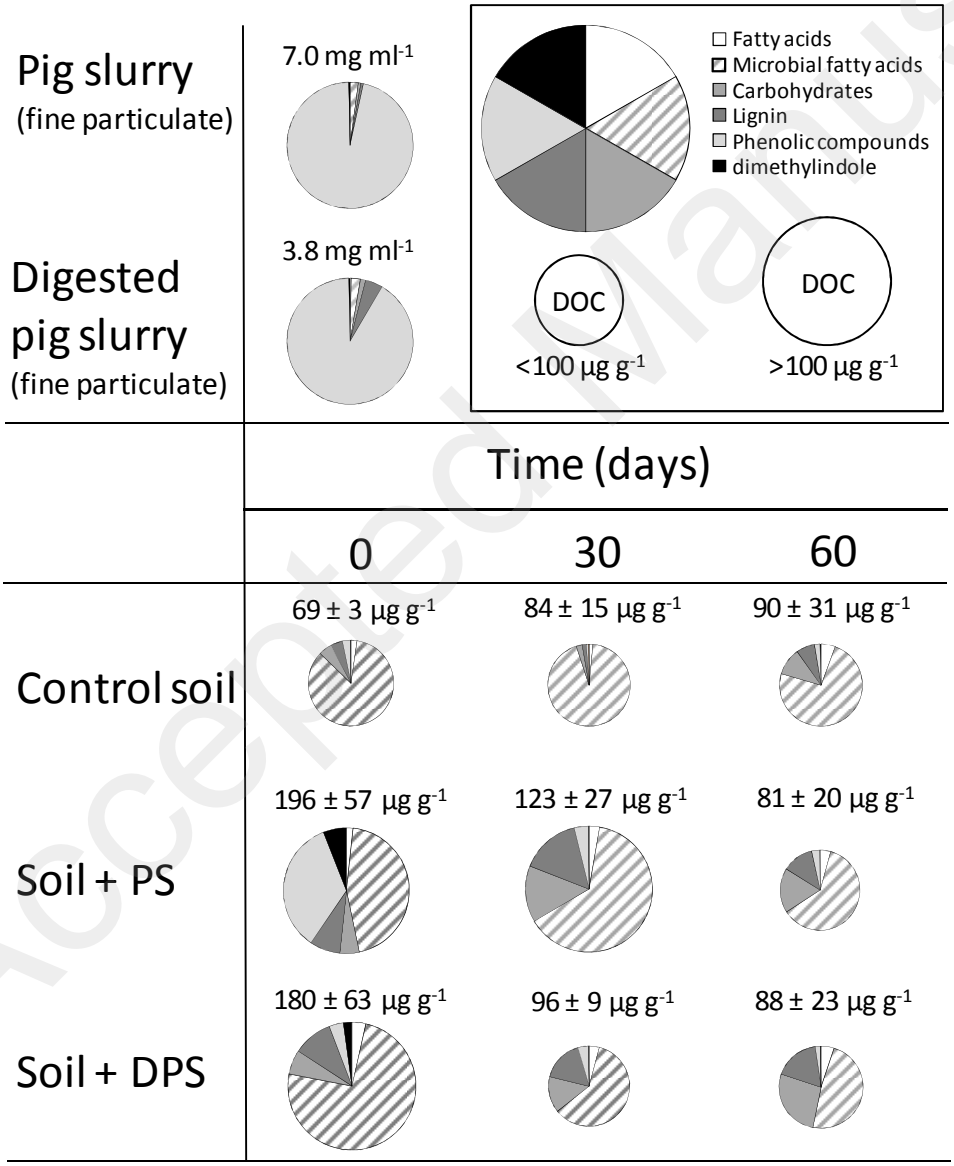
710 Concentrations (ppb) of the main VOC emitted by the pig slurry (PS) and digested pig slurry (DPS)
711 themselves (mean of three replicates with standard deviation, asterisks represent significant
712 differences between slurries, Kruskal Wallis test).

	PS	DPS
Methanol (m33)	33.6 ± 2.7	5.4 ± 1.4
Acetone (m59)	399.7 ± 176.2	88.9 ± 59.2
DMS (m63)*	19.1 ± 10.6	6.5 ± 1.9
2-Pentanone (m87)	12.8 ± 6.3	16.0 ± 9.7
Phenol (m95)	4.8 ± 3.7	2.6 ± 0.5

713

714 Fig. 1

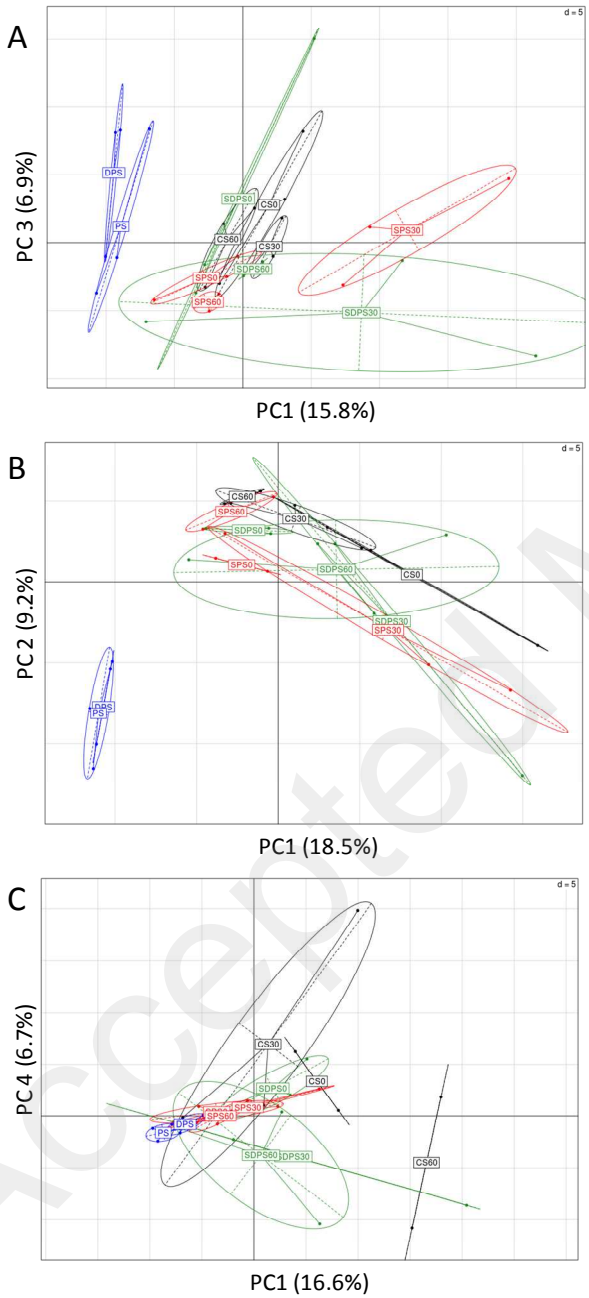
715 Soil dissolved organic carbon concentration (DOC per mL of slurry or g of dry soil - values on top of
716 the circles, mean of three replicates with standard deviation) and water extractable organic matter
717 (WEOM) composition in fatty acids, carbohydrates, lignin, phenolic compounds and methylindole
718 (proportion of analyzed compounds) in the < 0.7 µm fractions of the pig slurry and digestate pig
719 slurry and in the top 10 cm soil of the microcosms that received either water (control soil), pig slurry
720 (soil + PS) or digested pig slurry (soil + DPS) just after (0), 30 and 60 days following the application of
721 slurries.



722

Fig. 2

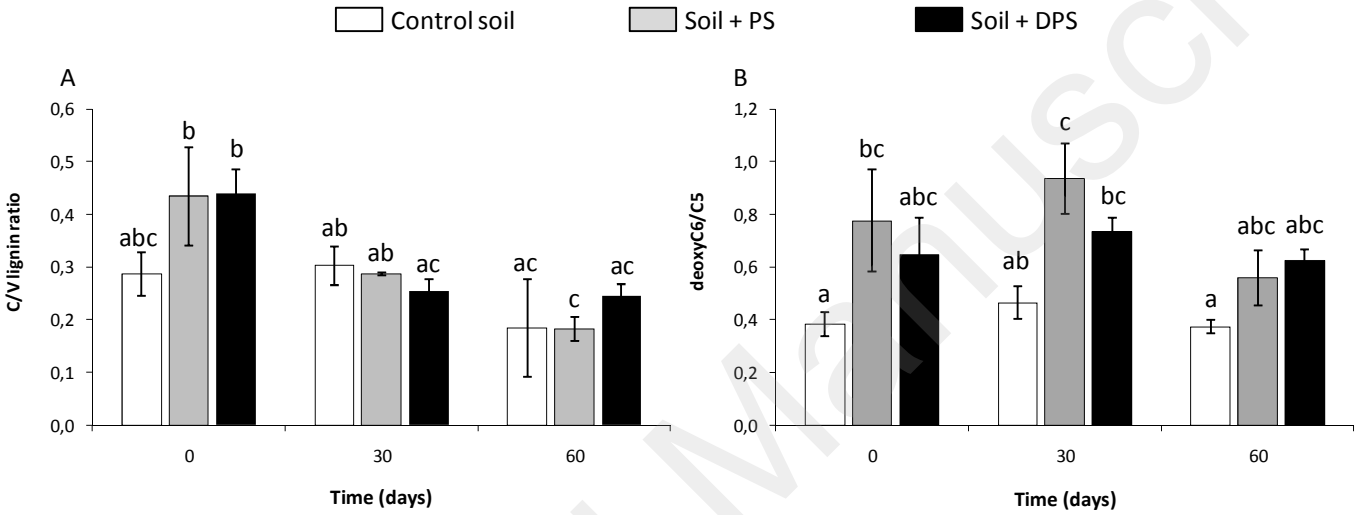
Principal component analysis of T-RFs of bacterial (A), archaeal (B) and fungal (C) communities in the pig slurry (PS) and digested pig slurry (DPS) in blue, in the control soils in black (CS0, CS30, CS60), the soils with pig slurry in red (SPS0, SPS30, SPS60) and with digested pig slurry in green (SDPS0, SDPS30, SDPS60) at the initial time and after 30 and 60 days of incubation, respectively.



730 Fig. 3

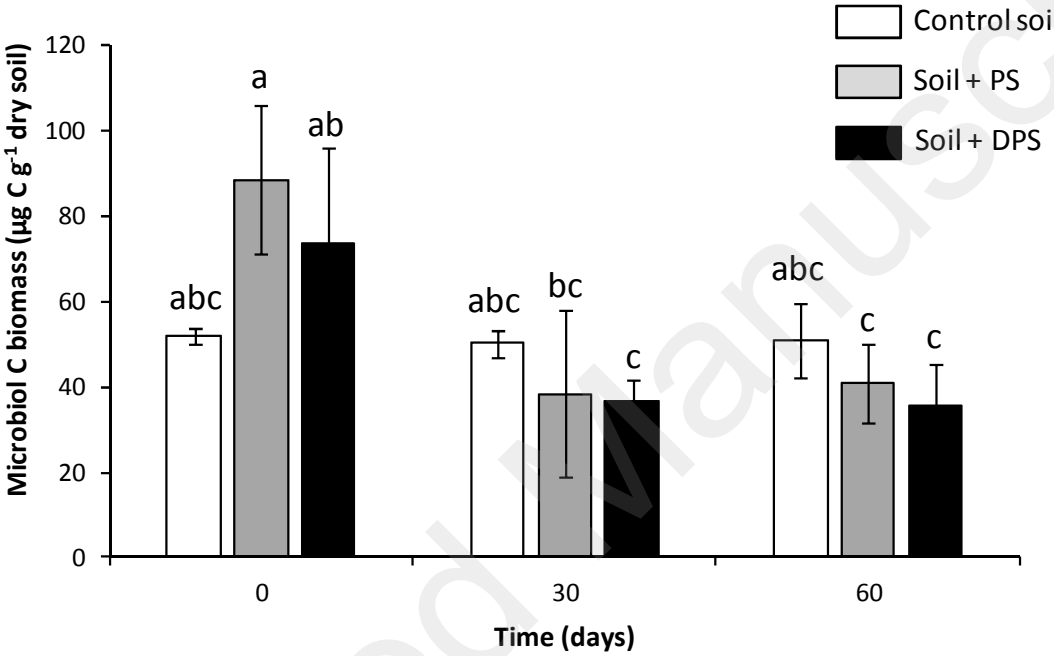
731 C/V lignin (A) and deoxy C6/C5 (B) ratios in the top 10 cm of soil in the microcosms that received
732 either water (control soil CS), pig slurry (SPS) or digested pig slurry (SDPS) just after (0), 30 and 60
733 days following application of the slurries. Error bars represent the standard deviation of mean values
734 (three replicates). Different letters correspond to significant differences between samples (Kruskal
735 wallis post hoc Dunn's tests).

736



737 Fig. 4

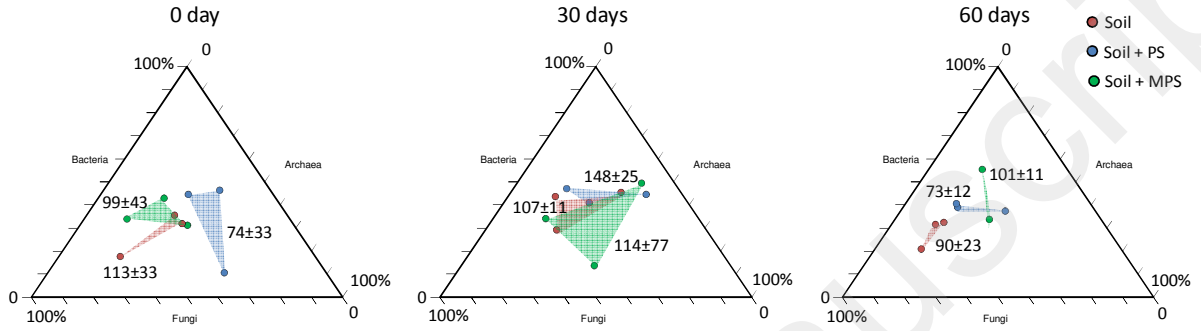
738 Microbial carbon biomass (µg g^{-1} dry soil) in the top 10 cm soil of the microcosms that received either
739 water (control soil), pig slurry (Soil + PS) or digested pig slurry (Soil + DPS) just after (0), 30 and 60
740 days following application of the slurries. Error bars represent the standard deviation of mean values
741 (three replicates). Different letters correspond to significant differences between samples (Kruskal
742 wallis post hoc Dunn's tests).



743

744 Fig. 5

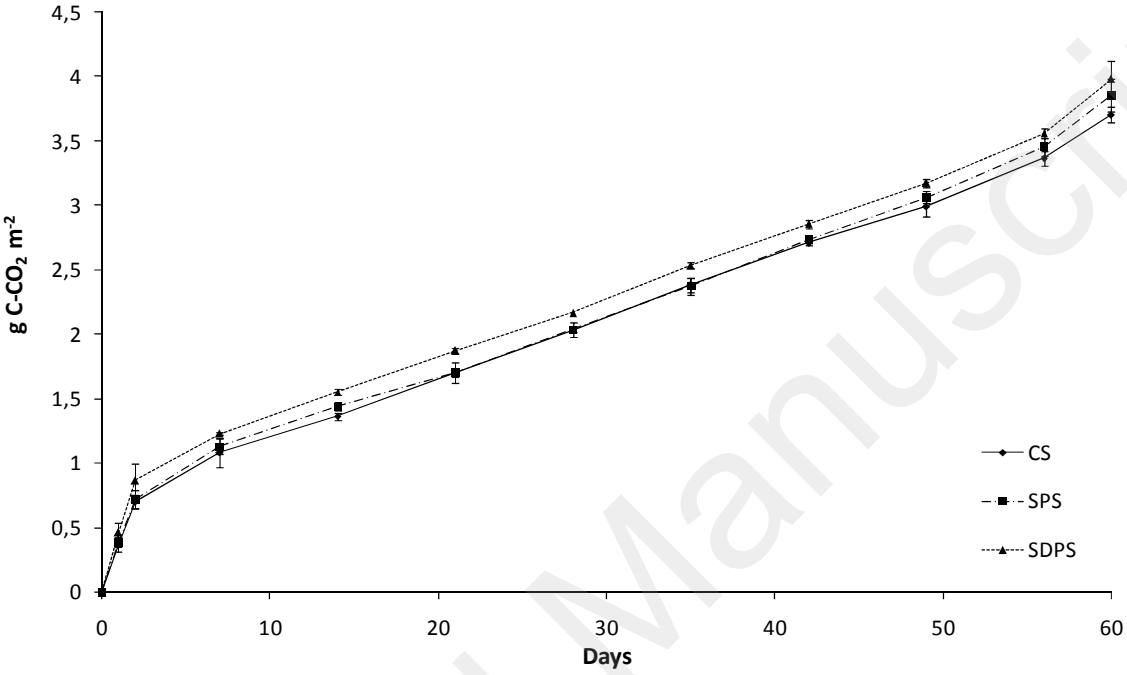
745 Microbial richness and its proportion within the active bacterial, archaeal and fungal taxa in the
746 control soil (in blue) and in soils that received pig slurry (in orange) or digested pig slurry (in green)
747 just after (0 day) and 30 and 60 days after application of the slurries. Values in the ternary plots
748 correspond to the total species richness (mean of three replicates ± standard deviation).



749

750 Fig. 6

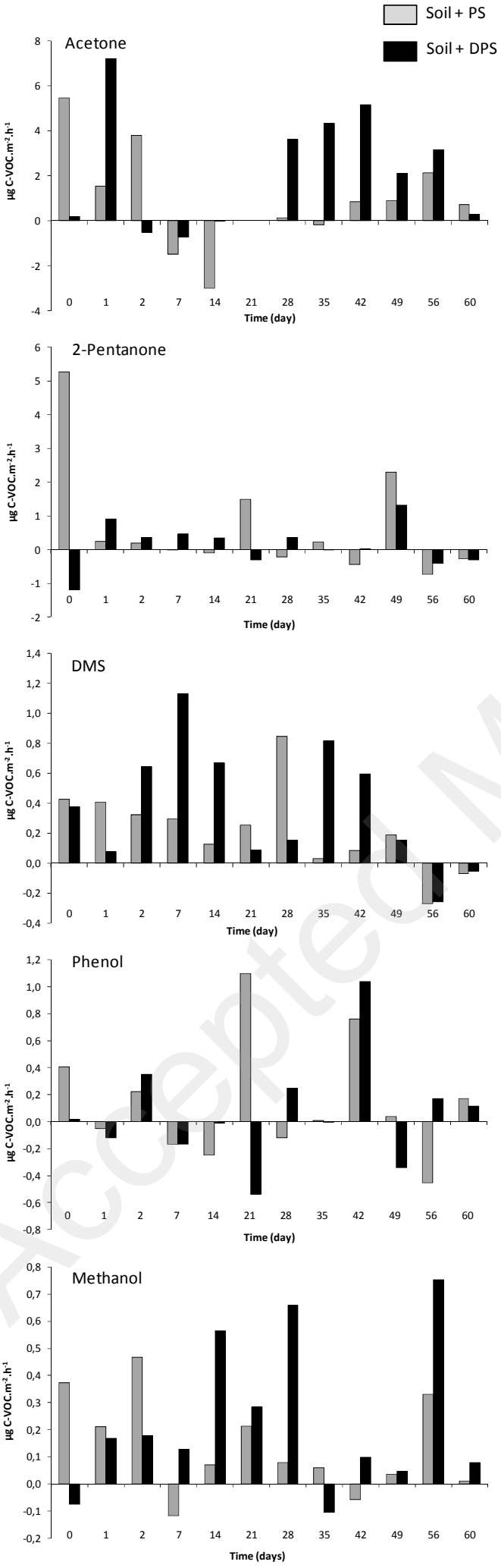
751 Cumulative C-CO_2 emissions from the microcosms with control soil (CS), with soils that received pig
752 slurry (SPS) and digested pig slurry (SDPS) just after (0 day) and 30 and 60 days after application of
753 the slurries. Error bars indicate the standard deviation (n = 3)



754
755

Fig. 7

Net C-VOCs fluxes of the main VOCs detected in soil with pig slurry (Soil + PS) and digested pig slurry (Soil + DPS) compared to the control microcosms soil just after (T0) and up to 60 days after application of the slurries.



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