

Microbial communities from different soil types respond differently to organic waste input

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1 Microbial communities from different soil types respond

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22 Abstract

23 Using organic waste products (OWP) in agriculture has been reported to impact both the activity and composition of soil microbial communities. However, little information is 24 25 available on how the response of a soil microbial community to a given OWP may depend on 26 the physicochemical and microbial properties of the soil receiving the input. Here, we performed a microcosm experiment to compare the effect of 2 different OWPs (GWS: co-27 compost of Green Wastes and Sewage sludge or FYM: FarmYard Manure), each applied to 5 28 29 different soils, on the activity, abundance and diversity of the soil microbial communities. 30 Soils were selected to represent a range of physicochemical and climatic characteristics. CO₂ 31 and N₂O emissions, microbial biomass and taxonomic diversity were monitored for 28 days 32 following OWP input. The five soils presented different prokaryotic and fungal communities structures before OWP application. During the 28 days of incubation, those control soils 33 (without OWP) harboring the highest organic matter contents released the greatest CO₂ and 34 35 N₂O emissions, and had the highest soil microbial biomass. The impact of organic 36 amendments on soil activity and microbial diversity differed with the nature of the OWP. 37 FYM application increased CO₂ emissions 2-fold and delayed N₂O emissions compared to GWS. Major changes in prokaryotic genetic structures were also observed when GWS was 38 39 applied. The effect of OWPs was dependent on soil type and the five soils exhibited distinct 40 patterns of CO₂ and N₂O emission after a given input. This accorded with the fact that the 41 structure and composition of the microbial communities harbored by each soil type responded 42 differently to a given OWP application. To conclude, our results show that different soil 43 types, harboring distinct microbial community structures, responded differently to OWP application, leading to different patterns and rates of greenhouse gas emissions. This response 44 45 was also OWP-dependent.

46 1

Keywords: soil microbes; soils incubation; CO₂; N₂O; high throughput sequencing approach.

49 Organic waste products (OWP) resulting from human activities (e.g., sewage sludge, municipal solid waste composts or farmyard manure) are being increasingly applied to soils 50 51 because they facilitate the recycling of nutrients and improve soil fertility. Indeed, organic 52 amendment is known to enhance (i) physical soil fertility by improving soil porosity, 53 aggregation and structure stability, bulk density and water holding capacity (Abiven et al., 2009; Annabi et al., 2011; Eden et al., 2017) and (ii) chemical fertility through pH regulation, 54 55 as well as increased CEC and availability of nutrients (Diacono and Montemurro, 2010; 56 Chalhoub et al., 2013). Organic amendment also improves the biological components of soil, 57 especially the soil microbial communities. Application of organic materials led to an increase 58 of soil microbial biomass (Ros et al., 2006; Chaudhry et al., 2012; Francioli et al., 2016) and 59 changes in prokaryotic and fungal community structures (Ros et al., 2006; Chaudhry et al., 2012; Francioli et al., 2016; Sadet-Bourgeteau et al., 2018). The effect of OWP on soil 60 61 microbial communities may depend on: (i) the duration of repeated OWP application, (ii) the rate of OWP applied, (iii) the delay between OWP application and soil sampling, (iv) the 62 63 quality of the different OWP applied and (v) the soil type (Pérez-Piqueres et al., 2006; Sadet-Bourgeteau et al., 2018). In parallel to these microbial changes, several incubation studies 64 revealed an increase of CO₂, N₂O and CH₄ emissions (Saison et al., 2006; Pezzolla et al., 65 66 2013; Pezzolla et al., 2015; Ho et al., 2017). Thus, the use of organic materials on agricultural soils may also have unwanted side effects, such as increased greenhouse gas (GHG) 67 68 production.

69 Soil microorganisms (prokaryote and fungi) are key actors in ecosystem processes (Wagg 70 et al., 2014; Werling et al., 2014). Changes in the microbial community and biotic interactions 71 may therefore affect the processes mediated by soil microbes, with consequences on GHG 72 emission. In order to maximize the benefits of OWP by reducing soil GHG emissions, it is 73 therefore important to better characterize the impact of amendments on soil microbial

74 community composition and activity. Most studies have focused on the importance of the 75 quality of the applied OWP and very few have taken the importance of soil type into 76 consideration. However, soil type is the main driver of soil microbial abundance and diversity 77 (Dequiedt et al., 2011; Thomson et al., 2015).

78 Ho et al. (2017) and Pezzolla et al. (2013) investigated the impact of different organic 79 materials on several soil types and GHG emission in laboratory incubation experiments. Pezzolla et al. (2013) assessed the effect of OWPs on soil microbial community response by 80 81 using Phospholipid Fatty Acid Analysis (PLFA) analysis, and CO₂ emission only. Ho et al. 82 (2017) targeted the soil bacterial community using high throughout sequencing techniques 83 (16S rRNA gene), and measured both CO₂ and N₂O emissions. According to these authors, 84 the CO₂ and N₂O emission patterns and rates were amendment-dependent even though the 85 soils possessed different physicochemical parameters, textures and bacterial community 86 compositions and structures. To our knowledge, these two studies are the only ones to have 87 examined the impact of different organic materials on several soil types and GHG emission in the same experiment. However, only two different soil types were assessed, and different 88 89 methodological tools (PLFA vs 16S rRNA gene Ion Torrent amplicon sequencing) were used 90 in each study which makes general conclusions difficult.

91 The objective of the present study was to assess the effect of the application of different 92 types of organic material on five different soil microbial communities. Based on previous 93 studies, we hypothesized that whatever the soil microbial community considered, the respond 94 was amendment dependent. This work was performed using a laboratory microcosm 95 experiment involving the application of two different types of organic amendments to five soils each containing different microbial communities. The soils were sampled from five field 96 97 sites representing contrasted pedo-climatic conditions (especially soil type and climate). The sampled plots had never received any organic amendments. The organic amendments applied 98 99 to the microcosms differed mainly in their organic matter content and stability. One of the

100 novel point of this work was to assess in the same experiment the responses of prokaryotic 101 and fungal communities to these organic amendments by a high throughput sequencing 102 approach respectively targeting the 16S and 18S ribosomal genes. This approach is 103 appropriate to characterize the vast microbial diversity of soil and monitor microbial 104 communities' composition changes after amendment. Soil CO_2 and N_2O emission were also 105 measured in parallel.

106

107 **2. Materials and methods**

108 2.1. Soils and Organic waste products

109 The soil samples used in this experiment were collected in March 2015 from the 110 ploughed horizon (0-20 cm) of five french long-term observatories belonging to the SOERE-111 **PRO-network** (http://www6.inra.fr/valor-pro/SOERE-PRO-Presentation-de-l-112 observatoire/SOERE-PRO-Les-sites): QualiAgro (Qa; at Feucherolles), PROspective (Co; at 113 Colmar), Efele (Ef; at Rennes), Couhins (Ch; at Bordeaux) and La Bouzule (LB; at Nancy). 114 Choice of using these sites were to characterize the agronomic value of regular organic 115 residue application and their environmental impacts, in a long-term field experiments. The 116 main soils properties are given in Table 1.

117 For each observatory, a composite soil sample was made from a pool of five sub-samples 118 $(\emptyset 7 \text{ cm})$ in the diagonal section of plots that never received organic waste products. Samples 119 were then mixed and homogenized by passing them through a 4 mm sieve to remove aboveground plant debris, roots and stones. A portion of each soil was air-dried at room 120 121 temperature for physico-chemical analysis. Particle size distribution, pH, soil organic matter, soil organic carbon, soil total nitrogen (N), soil C/N ratio, Olsen P and Cation Exchange 122 Capacity (CEC), were determined by the Soil Analysis Laboratory at INRA Arras, France 123 124 (http://www.lille.inra.fr/las; Table 1). The rest of the sieved soils, was used for incubation.

125 The organic amendments used in the experiment included a farmyard manure (FYM) and 126 a compost issued from the co-composting of green wastes with sewage sludge (GWS). pH, 127 organic carbon, phosphorus and total N contents were determined for each of these two 128 organic waste products (OWP). Before analysis, the organic amendments were dried and ground to 1 mm. The total N and organic C contents were measured by elemental analysis 129 130 (NA 1500, Fison Instrument, San Carlos, CA, USA) after additional grinding to 200 µm (Retsch SM 2000, Haan, Germany). The mean OWP characteristics measured are represented 131 132 in Table 2. The index of residual organic carbon (I_{ROC}) that represents the proportion of 133 organic matter potentially incorporated into soil organic matter after OWP application 134 (Lashermes et al., 2009) was also determined (Table 2).

135

136 2.2. Microcosms set up and Incubation

137 The experimental design consisted of a factorial arrangement with five soils (Qa, Co, Ef, 138 Ch and LB), two amendments (a farmyard manure (FYM) and a co-compost of sewage 139 sludge, green wastes and wood chips (GWS)) plus unamended control for each soil (CON). 140 For each treatment, a series of 9 sterile plasma flasks (150 mL) were filled with 30 g of dried 141 and sieved soil to allow for 3 destructive samplings at 0 (d0), 7 (d7) and 28 (d28) days of incubation. These microcosms were supplemented with sterile water to attain 60 % soil water-142 143 holding capacity. After 2 weeks of pre-incubation (20°C), FYM or GWS were thoroughly 144 mixed with soil samples in their dry form (5 and 6.9 mg g^{-1} of soil respectively). The 145 sampling time d0 was considered immediately after amendment. The rest of plasma flasks 146 were then incubated for 7 and 28 days in the dark at 20°C and always 60 % water-holding 147 capacity to ensure good biomass activation. Every week, plasma flasks were aerated by flushing with air and at the same time, the water content was adjusted to correct for any soil 148 149 moisture lost through evaporation. At d0, d7 and d28, for each treatment, soil from 150 corresponding plasma flasks were sacrificed for molecular analyses. After sampling, each 151 sample was lyophilized and stored at -40 °C until use for molecular analyses based on soil
152 DNA extraction.

153

154 2.3. CO2 and N2O emissions

155 At 0, 3, 7, 14, 21 and 28 days of incubation, the gaseous phase was sampled in 10 mL 156 pre-vacuumed airtight flasks exetainers, to measure the CO₂ and N₂O concentrations using an 157 Agilent 7890B GC coupled to an automatic sampler (Agilent HS7697A Headspace). The 158 concentrations were measured on a thermal conductivity detector for CO₂ and on a plasma 159 emission detector (LDetek) for N₂O. The GC system was calibrated using multipoint CO₂ and 160 N₂O gas standards prepared by a GasMix (Alytech, France). Since the microcosms were 161 hermetically-sealed and aerated by flushing with air at each sampling date, the concentrations 162 measured corresponded to the CO₂ and N₂O accumulated in the microcosm headspace 163 between two sampling dates.

164

165 2.4. DNA Extraction and purification

166 Microbial DNA was extracted from 1 g (dry weight) of soil from the 135 microcosms, 167 GWS (n=3) or FYM (n=3), using a single procedure standardized by the GenoSol platform 168 (INRA, Dijon, France, www.dijon.inra.fr/plateforme genosol) (Terrat et al., 2012). Since a 169 highly positive linear relationship has been shown between soil DNA recovery and C-biomass 170 measurement (Marstorp et al., 2000; Dequiedt et al., 2011), DNA concentrations of crude 171 extracts were determined by electrophoresis in 1% agarose gel stained with ethidium bromide 172 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 173 174 impurities, particularly humic substances, by centrifuging through two types of minicolumn. 175 Aliquots (100 µL) of crude DNA extract were first loaded onto polyvinyl polypyrrolidone

minicolumns (BIORAD, Marne-la-Coquette, France) and centrifuged at 1000 × 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). Purified DNA concentrations were finally measured
using the Quantifluor (Promega, Lyon, France) staining kit, according to the manufacturer's
instructions.

181

182 2.5. High throughput sequencing of 16S and 18S rRNA gene

For prokaryotic (bacterial-archaeal) diversity, a 440-base 16S rRNA gene fragment was amplified from each DNA sample with the following primers: F479 (5'-CAG CMG CYG CNG TAA NAC-3') and R888 (5'-CCG YCA ATT CMT TTR AGT-3') as previously described (Tardy et al., 2014). For each sample, 5 ng of DNA were used for a 25 μ L PCR conducted under the following 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.

189 For fungal diversity, a 350-base 18S rRNA gene fragment was amplified from each DNA 190 sample with the following primers: FF390 (5'-CGA TAA CGA ACG AGA CCT-3') and FR1 191 (5'-ANC CAT TCA ATC GGT ANT-3') (Chemidlin Prévost-Bouré et al., 2011). For each 192 sample, 5 ng of DNA were used for a 25 µL PCR conducted under the following conditions: 193 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 194 5 min at 72 °C. Prokaryotic and fungal PCR products were purified using the Agencourt® 195 AMPure® XP kit (Beckman Coulter, Italy, Milano) and quantified with the Quantifluor 196 (Promega, Lyon, France) staining kit according to the manufacturer's instructions.

A second PCR was performed with the purified PCR products (7.5 ng of DNA for bacteria and archaea, and 5 ng of DNA for fungi), with 10-bp multiplex identifiers added to the 5' end of the primers for the specific identification of each sample and the prevention of PCR biases. For bacteria and archaea, the second PCR conditions were the same as previously 201 described but with only seven cycles. For fungi, the second PCR conditions were optimized, 202 with the number of cycles being reduced to seven and the denaturation step processed at 94 203 °C during 1 min. PCR products were purified with the MinElute PCR purification kit (Qiagen 204 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 205 206 with the SPRI (Solid Phase Reverse Immobilization Method) using the Agencourt® AMPure® XP kit (Beckman Coulter, Italy, Milano). The pooled samples was finally 207 208 sequenced with a MiSeq Illumina instrument (Illumina Inc, San Diego, CA) operating with 209 V3 chemistry and producing 250 bp paired-reads.

210

211 2.6. Bioinformatic analysis of 16S and 18S rRNA gene sequences

212 Bioinformatic analyses were done using the GnS-PIPE, which was initially developed by 213 the Genosol platform (INRA, Dijon, France) (Terrat et al., 2012). Data from soils and OWP 214 were treated independently. First, all the 16S and 18S raw reads were sorted according to the 215 multiplex identifier sequences. The raw reads were then filtered and deleted based on their 216 length, their number of ambiguities (Ns), and their primer(s) sequence(s). More precisely, all 217 raw sequences were checked and discarded if: (i) they contained any ambiguous base (Ns), 218 (ii) if their length was less than 350 nucleotides for 16S reads or 300 nucleotides for 18S 219 reads, (iii) if the exact primer sequences were not found (for the distal primer, the sequence 220 can be shorter than the complete primer sequence, but without ambiguities). A PERL program 221 was then applied for rigorous dereplication (i.e. clustering of strictly identical sequences). The 222 dereplicated reads were then aligned using Infernal alignment (Cole et al., 2009), and 223 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 with a 224 225 threshold of 95% of sequence similarity. A filtering step was then carried out to check all singleton (reads detected only once and not clustered, which might be artifacts, such as PCR 226

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 (soils: 9 600 and 8 420 reads for 16S and 18S rRNA gene sequences, respectively; Organic Waste Products (OWP): 5 080 and 8 420 reads for 16S and 18S rRNA gene sequences, respectively).

The retained high-quality reads were used for: (*i*) taxonomy-independent analyses, determining several biodiversity indexes (Shannon, richness and evenness) using the OTU dataset and defining global OTU matrices, and (*ii*) taxonomy-based analysis using similarity approaches against dedicated reference databases from SILVA (Quast et al., 2012). The raw data sets are available in the EBI database system under project accession number PRJEB31513.

238

239 2.7. Statistical analyses

All statistical analyses were achieved with R studio (RStudio, Version 0.98.501, RStudio 240 241 Inc., Boston, Massachusetts, USA) with the free statistics software R (R version 3.0.2). A 242 three-way analysis of variance (ANOVA) was used to compare the CO₂ and N₂O cumulative 243 fluxes and microbial community indicators. The statistical model included soil type, OWP 244 type, incubation time, soil type \times OWP type and soil type \times OWP type \times incubation time. For 245 all parameters, all significant effects were assessed by Tukey's Honestly Significant 246 Difference (HSD) post hoc test (P < 0.05). All the variables were tested for normality using 247 Shapiro-Wilk test. The prokaryotic and fungal communities from all samples were also 248 compared by using taxonomical abundance matrix. Principal Component Analysis (PCA) was 249 then used to graphically depict any differences in the microbial structure at phylum level 250 between the samples. For each sample, the coordinates of both principal axes were analyzed 251 by calculating the Euclidean distance (Eudist) between the treatment and the corresponding controls for each incubation time and OWP type. This genetic distance was interpreted as the 252

magnitude of genetic structure modifications induced by OWP application and was calculated
as follows. Magnitude of modifications, example of calculation for one OWP type and each
incubation time = [Average of Inter Eudist (treated microcosms/control microcosms)][((Average of Intra Eudist treated microcsms) + (Average of Intra Eudist control
microcosms))/2] (Bressan et al., 2008).

258

259 **3. Results**

260 3.1. CO2 emissions

261 Variations in the cumulative emissions of CO₂ in unamended and amended soils are 262 shown in Fig. 1. Basal respiration differed significantly between the five control soils, the greatest cumulative emissions of CO₂ being observed with LB (94.1 µg C g⁻¹ of soil). and the 263 lowest with Co (34.2 μ g C g⁻¹ of soil, P < 0.001). Addition of organic amendment (GWS or 264 265 FYM) resulted in greater cumulative emissions of CO₂ in all five soils with respect to the unamended controls (Fig.1, P < 0.001). Regardless of the soil, GWS application led to lower 266 cumulative emissions of CO₂ (on average 156.9 μ g C g⁻¹ of soil) than FYM (on average 304.8 267 $\mu g C g^{-1}$ of soil, P < 0.001), particularly at the end of incubation (from day 14 to 28). In 268 269 amended soils (GWS or FYM), the cumulative emissions of CO₂ also differed according to the soil type, greater values being observed for soil from Ch (188.1 and 369.7 µg C g⁻¹ of soil 270 271 for GWS and FYM respectively) and the lowest values being observed for soil from Co (104.9 and 217.1 μ g C g⁻¹ of soil for GWS and FYM respectively, P < 0.001). 272

273

274 3.2. N2O emissions

Variations in the cumulative emissions of N_2O in unamended and amended soils are reported in Fig. 2. Only control soil from LB, Ch and Ef soils showed a significant N_2O release throughout the incubation period (from 0 to 3.7 ng N g⁻¹ of soil in average, P<0.001).

The addition of organic amendment (GWS or FYM) resulted in greater cumulative emissions 278 of N₂O in all five soils with respect to unamended controls ($P \le 0.001$). Regardless of the 279 280 soil, GWS and FYM application induced equivalent cumulative emissions of N₂O (23.6 and 26.4 ng N g⁻¹ of soil respectively). GWS led to more rapid N₂O emission (until day 3) than 281 282 FYM. In amended soils, the cumulative emissions of N₂O differed according to the site 283 considered. For GWS, the highest cumulative emissions of N₂O were observed for Co (35.9 ng N g⁻¹ of soil, P < 0.001). LB and Ef soils emitted significantly less (~1.3 fold) N₂O than 284 285 Co (23.7 and 30.3 ng N g⁻¹ of soil respectively). Still compared to Co soil, Qa emitted ~2 fold less (17.5 ng N g⁻¹ of soil, P < 0.001) and Ch ~4 fold less (10.3 ng N g⁻¹ of soil, P < 0.001). 286 287 The soil emission classification after FYM application differed from that observed with GWS, the lowest emission rates of N₂O being observed for Co and Qa soils (4.5 and 16.2 ng N g⁻¹ of 288 soil respectively). Ch emitted ~5 fold more N₂O (22.1 ng N g⁻¹ of soil, P < 0.001) than Co. 289 Soils Ef and LB emitted ~10 fold more N_2O than Co (52.2 and 36.9 ng N g⁻¹ of soil 290 respectively, P < 0.001). 291

292

293 3.3. Microbial biomass

294 The dynamics of the microbial biomass during incubation of unamended and amended 295 soils are shown in Fig. 3. The microbial biomass in control soils generally remained constant throughout the incubation (P > 0.05). The LB soil had the highest biomass (on average 73.6 296 μ g DNA g⁻¹ of dry soil) and Ch the lowest (on average 16.9 μ g DNA g⁻¹ of dry soil; P < 297 0.001). The biomass values for Co, Qa and Ef soils were similar and significantly lower than 298 LB, and greater than Ch (on average 29.8 μ g DNA g⁻¹ of dry soil; P < 0.001). Addition of 299 300 GWS or FYM enhanced the microbial biomass only in LB soil, from 7 days of incubation (106.7 and 123.7 µg DNA g⁻¹ of dry soil respectively; $P \le 0.001$). A significantly greater 301 302 amount of microbial biomass was observed when FYM was applied on LB soil with respect 303 to GWS (123.7 and 106.6 μ g DNA g⁻¹ of dry soil respectively; P < 0.001).

305 *3.4. Prokaryotic and fungal diversity indices*

306 The dynamics of the microbial diversity indices during incubation of unamended and 307 amended soils are shown in Fig. 4. In general, the control soils did not show any variations in 308 prokaryotic and fungal diversity indices (richness and Shannon) throughout the study period. 309 Only the control soils from LB showed, at 28 days of incubation, an increase in fungal 310 richness and microbial Shannon indices ($P \le 0.001$). The effect of organic amendment on 311 microbial diversity indices was dependent on the soil and the type of amendment. Thus, the 312 addition of GWS led to a decrease of prokaryotic richness and Shannon index in Qa soil after 313 7 days of incubation (P < 0.001), and an increase of the prokaryotic Shannon index in LB soil 314 after 28 days of incubation (P < 0.001). Concerning fungal diversity indices, the addition of 315 GWS led to a decrease of richness and Shannon index in Co and Ch soils (P < 0.001), after 7 316 days of incubation, but an increase of richness in Ef soil ($P \leq 0.001$). The addition of FYM 317 increased the prokaryotic richness in Ch soil and the fungal richness in Co soil after 7 days of 318 incubation (P < 0.001). For Co soil, from day 7 to 28, the fungal Shannon index decreased to 319 reach, at the end of incubation, a value lower than that observed before the application ($P \leq$ 320 0.001). This suggests that the fungal community from Co soil would be resilient 28 days after 321 FYM application. The addition of FYM also led to a decrease of the fungal Shannon index in 322 Ef soil throughout the entire incubation (P < 0.001).

323

324 *3.5. Prokaryotic and fungal community structures and taxonomic composition*

Principal Component Analysis (PCA) achieved on phyla taxonomical abundance matrix revealed that before the OWP application, the microbial community structures in the five soils differed significantly from each other (Supplementary Fig. S1A and B). The addition of GWS or FYM induced, from 7 days of incubation, a change in the prokaryotic and fungal (Fig. 5A and B, Supplementary Fig. 1A and 1B) community structure in all the soils. Same results 330 were obtained on genera taxonomical abundance matrix (data not shown). OWP (FYM or 331 GWS) application impacted prokaryotic community structure whatever the sampling site, as 332 evidenced by the Euclidian distances calculated at D7 and 28 (Fig. 5). However, the 333 amplitude of the modifications depended on the type of OWP applied, with major changes 334 observed when GWS, rather than FYM, was used (Fig. 5). The prokaryotic genetic structures 335 seemed to be more impacted in Ch than in the other soils, 28 days after OWP application (FYM or GWS) (P < 0.05). Analysis of the taxonomic composition, following FYM 336 337 application to Ch soil, revealed that the observed changes in prokaryotic genetic structures 338 were mainly due to an increase in the relative abundance of Firmicutes and a decrease of 339 Acidobacteria and Planctomycetes groups (P < 0.05). GWS led only to a decrease of the 340 Planctomycetes group in that soil. Twenty-eight days after GWS application, the prokaryotic 341 genetic structure showed resilience in the Ef, LB and Qa soils, but not in the Ch and Co soils 342 (Fig. 5). This was also observed after FYM amendment, but only in Co soil (Fig. 5). OWP 343 (FYM or GWS) application also impacted the fungal community structure whatever the sampling site (Fig. 5). Fungal genetic structure changes were due to a decrease of 344 Chytridiomycota in Ef soil 28 days after FYM application (P < 0.05). FYM input also led to 345 346 an increase of *Glomeromycota* in Ch soil 28 days after application (P < 0.05). A decrease of 347 *Chytridiomycota* was observed in LB soil 28 days after GWS application (P < 0.05). Twenty-348 eight days after GWS application, the fungal genetic structure in Ch, Co and Qa soils showed 349 resilience (Fig. 5). This was not observed after amendment with FYM (Fig. 5). Interestingly, 350 in the present study, the prokaryotic and fungal communities appeared more resilient after 351 GWS application than after FYM application.

352

353 **4. Discussion**

The addition of OWP (FYM or GWS) led to a rapid increase of CO₂ emission in all the soil samples tested. This confirmed the role of labile C (provided by OWP) in inducing a 356 rapid increase of soil microbial respiration after amendment application. Among the different 357 soil types studied, those with the greatest basal respiration (Ch, LB and Ef) were also the 358 greatest emitters when OWPs were applied. Soil CO₂ emissions were not correlated with any 359 of the soil physicochemical characteristics measured in the present study. From 14 days of 360 incubation, and whatever the type of soil considered, CO₂ emission was ~50 % greater in soils 361 amended with FYM than in those amended with GWS. As previously observed by Brenzinger et al. (2018), this could be explained by the quality of OWP applied. In the present study, the 362 363 different OWP varied in their organic matter quality. FYM was characterized by a lower 364 potential ability to increase soil stable pool of exogenous organic matter - total organic 365 carbon, as shown by its smaller I_{ROC} (66.6 %), compared to GWS (77.6 %) (Lashermes et al., 366 2009). This suggests that FYM was subjected to a greater biodegradability of organic carbon 367 and a greater CO₂ emission compared to GWS.

The addition of OWP (FYM or GWS) led to an increase of N₂O emission. Twenty-eight 368 369 days after application, both OWP led to equivalent cumulative emissions of N₂O. However, 370 the trend of N₂O emission differed according to the amendment applied. Indeed, whatever the 371 type of soil considered, the application of GWS led to earlier N₂O emission with \sim 50 % of the 372 total N₂O emission observed after 3 days of incubation. After the same period of incubation, 373 FYM application had induced only 10 % of the total N₂O emission. Increased N₂O emission 374 from soil is commonly reported after organic fertilization (Shah et al., 2016; Ho et al., 2017); 375 however, the effect of the type of OWP amendment on the kinetics of N₂O emission is poorly 376 documented (Shah et al., 2016). According to Shah et al. (2016), the kinetics of N₂O emission 377 would depend on the C/N ratio of the applied OWP. More precisely, a high C/N ratio means 378 that N will be exhausted before the C has been digested by the bacteria as an energy source, hence leading to a lower N₂O emission. Conversely, a low C/N ratio results in high NH4⁺ 379 380 concentrations and finally a greater emission of N₂O (Shah et al., 2016). Thus, the lower C/N 381 ratio measured for GWS (13.2 \pm 0.5), as compared to FYM (18.1 \pm 0.1), could explain the 382 earlier N₂O emission observed with this amendment.

383 Interestingly, an effect of soil type on N₂O emission was also observed. Soil with the 384 highest pH before amendment (Co; pH=8.51) emitted the greatest amount of N₂O after 385 amendment with GWS, but the lowest after amendment with FYM. As previously mentioned, 386 the cumulative emissions of N₂O from other soil types were equivalent after GWS and FYM 387 applications. According to the literature, the relationship between soil pH and denitrification 388 remains unclear. In the present study, soil pH had not been measured throughout the 389 incubation. However, based on previous study (Shah et al. 2016), for a same soil type, the 390 dynamic of soil pH would be different according to the quality of OWP applied. Some authors 391 indicated that the N₂O fraction could be larger at low soil pH (Focht, 1974; Goodroad and 392 Keeney, 1984; Eaton and Patriquin, 1989; Brumme and Beese, 1992; Thomas et al., 1994), whereas others reported lower N₂O emissions in acidic compared to neutral or slightly 393 394 alkaline soils (ŠImek and Cooper, 2002). Thus, it is difficult to explain the effect of soil type on N₂O emission as being determined by soil pH and other interpretations are needed. 395

396 In the present work, before the amendments, the prokaryotic and fungal community 397 structures and compositions of the five soils were distinctly different (Supplementary Fig. 398 S2). Moreover, as previously observed by Ho et al. (2017), the application of OWP led to 399 changes in the microbial community that were dependent on the soil type and the quality of 400 OWP applied (Fig. 5 and Supplementary Fig. S1). Nitrous oxide (N₂O) is mainly generated 401 via microbial processes of nitrification and denitrification in soils (Pajares and Bohannan, 402 2016). Thus, as proposed by Ho et al. (2015), we hypothesized that, in the case of Co soil, 403 GWS application stimulated the soil-borne microbes involved in denitrification (resulting in 404 earlier N₂O emission), whereas FYM favored those involved in the nitrification process 405 (leading to later N₂O emission). The effect of OWP quality on N₂O emission was not so 406 marked for the other soil types probably due to the differences between the soil-borne 407 microbial communities.

408 From 7 to 28 days of incubation, and only in LB soil, the addition of OWP (FYM or 409 GWS) led to a ~40 % increase in microbial biomass. Previous soil incubation studies had 410 already highlighted an increase in microbial biomass within 10 days after the addition of 411 OWP (Saison et al., 2006; Pezzolla et al., 2013; Pezzolla et al., 2015). However, in the present 412 study, OWP had an effect on microbial biomass in only one soil (LB), which is surprising. As 413 proposed by Marschner et al. (2003), this could be due to the higher organic carbon content (17.9 g kg⁻¹) of LB soil before OWP application. Indeed, the organic carbon content is known 414 415 to determine the response of microbial communities to external inputs, soils with higher 416 organic carbon being more prone to an increase in microbial biomass and changes in the 417 microbial community (Marschner et al., 2003). Further studies also reported a correlated 418 increase of soil CO₂ emission and microbial biomass in the first days after OWP application 419 (Saison et al., 2006; Pezzolla et al., 2013). This was not observed in the present study, except 420 for the LB soil. As proposed by other studies, this suggests that the modifications in activity 421 highlighted would more likely be due to a modification in microbial community structure and 422 composition, rather than in its size (Zogg et al., 1997; Zhang et al., 2005).

423 No overall tendency was observed regarding the effect of OWP on soil microbial diversity 424 indices. However, changes in microbial richness and diversity occurred depending both on the type of soil and OWP. The effects of OWP on microbial diversity reported in the literature 425 426 are often ambiguous, mainly due to great variations between the experimental systems and 427 management definitions (Chaudhry et al., 2012; Hartmann et al., 2015; Chen et al., 2016; 428 Sadet-Bourgeteau et al., 2018). As reported by several authors (Saison et al., 2006; Pezzolla et 429 al., 2013; Pezzolla et al., 2015; Federici et al., 2017), OWP addition (GWS or FYM) led to a 430 rapid shift in microbial community structure, in all soils, within 7 days. The observed changes in microbial structure were dependent on the soil type and the quality of OWP applied. It 431 432 cannot be excluded that these early changes might be due to microorganisms present in the 433 organic materials applied (Supplementary Fig. S3). This hypothesis is reinforced by the different microbial genera composition present in unamended soils and OWPs 434

435 (Supplementary Fig. S2 and S3). However, as mentioned by Saison et al. (2006), the OWP 436 microbiota added to soil is rapidly outcompeted by the soil-derived microorganisms and its 437 effect on community structure is likely to be marginal. In addition, as proposed by Ho et al. 438 (2016), the changes in microbial community structure observed in the present study is more 439 likely due to the stimulation of soil-borne microbes by chemical composition of OWP 440 applicated than microbes present in the organic materials applied. The early shift in structure 441 of the soil microbial community seems in close agreement with the initial increase of 442 microbial respiration observed immediately after applying the amendments and could be 443 ascribed to the stimulation of microbial populations with great ability to rapidly metabolize 444 the labile organic C provided by OWP (Blagodatskaya et al., 2007). This was corroborated by 445 the fact that, in the present study, the application of FYM led to a stimulation of *Firmicutes* in 446 Ch soil and also the highest CO₂ emission. Carbon additions appear to strongly stimulate 447 organisms within the Firmicutes clades (Cleveland et al., 2007). The dominance of organisms 448 within the Firmicutes groups, and their relative increase in abundance in response to C 449 additions, suggested that members of these groups quickly respond to the addition of organic 450 material to the soil, leading to rapid increases in soil respiration (Cleveland et al., 2007). In 451 parallel, the decrease of Acidobacteria and Planctomycetes, suggested that these organisms 452 exhibit poor competitiveness and colonization ability, which could explain their decrease in 453 the present experiment. The impact on these *phyla* generally becomes apparent 3 days after 454 amendment and has already been highlighted in previous studies of the impact of digestate 455 application on soil microbial communities (Fierer et al., 2007; Cruz-Martínez et al., 2009; 456 Pezzolla et al., 2015). Interestingly, contrary to FYM, the addition of GWS to soils only led to 457 a decrease in *Planctomycetes* 3 to 28 days after the application. As mentioned above, the lack 458 of increase in the proportion of Firmicutes in GWS-amended soils may help to explain the 459 lower CO₂ emissions, as compared to FYM.

460 The fungal community also responded by a decrease of *Chytridiomycota* 28 days after
461 FYM or GWS application, and an increase in *Glomeromycota* 28 days after FYM application.

462 Chytridiomycota have been recovered as saprophytes and can degrade polymers from a 463 variety of plant and animal debris (Czeczuga and Mazalska, 2000; Czeczuga et al., 2005). 464 This fungal phylum is probably included within the group of k-strategists, which could 465 explain its decrease in the present experiment. It has been reported in the literature that 4 466 months after the last application, OWP tended to increase Basidiomycota, one of the main soil 467 decomposers (Ma et al., 2013; Weber et al., 2013) and to decrease *Glomeromycota* (Chen et 468 al., 2016; Sadet-Bourgeteau et al., 2018). This did not occur in the present study, where no 469 effect on Basidiomycota was apparent and an increase of Glomeromycota was observed 28 470 days after OWP application. As mentioned in a previous study, this emphasizes the 471 importance of taking the delay between OWP application and soil sampling into account 472 when studying the effect of OWP on the soil microbial community (Sadet-Bourgeteau et al., 473 2018).

474 Despite the above-described overall response of soils to OWP application, in the present 475 work, the microbial communities of some soils responded differently. This concords with observations by Pérez-Piqueres et al. (2006) that the changes in microbial community 476 477 structure induced by amendments were related both to the soil and to the type of OWP used. 478 The particular behavior of the prokaryotic community in response to OWP application in the 479 Ch soil could be related to the soil's physico-chemical characteristics. Indeed, Ch soil has a 480 sandy loam texture, unlike the other soils in the present study (silty clay loam or silty loam 481 texture). In a recent study, Ho et al. (2017) highlighted that the composition of the bacterial 482 community from sandy loam soil responded differently to a same type of OWP application 483 than a bacterial community from clay soil. Thus, we suggest that, contrary to the other soils in 484 this study, the sandy loam texture of Ch soil would be an important determinant of its 485 microbial community composition, and lead to a different impact of the amendment on soil 486 prokaryotic community composition. As previously observed by Procter et al. (2014), the 487 effect of OWP on the fungal community was also soil type-dependent. Some of the microbial 488 communities impacted by OWP application, tended to revert to their initial structure (before

amendment). This resilience was dependent on soil type, and globally more marked after
GWS input. This accords with reports by Saison et al. (2006) that the resilience of the soil
microbial community was OWP-quality dependent.

492

493 **5.** Conclusions

The present study indicated that soils with different physico-chemical properties and microbial community structures responded differently to organic fertilization. This response appeared to depend on the type of OWP and suggests that GHG emission could be modulated by spreading specific types of amendment on specific types of soil. Further analyses are needed to best determine which type of amendment should be applied to which type of soil in order to limit soil GHG emission.

500

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Table 1

T.	Long-Term Observatories							
Item	QualiAgro (Qa)	PROspective (Co)	EFELE (Ef)	La Bouzule (LB)	Couhins (Ch)			
Location	Feucherolles	Colmar	Rennes	Nancy	Bordeaux			
	North West of France	Est of France	North West of France	North Est of France	South West of France			
Geographical coordinates	48°53'49"N, 1°58'21"E	48°03′33"N, 7°19′41"E	48°05′35.9"N, 1°48′53.1"W	48°44'23.64"N, 6°19′20.16"E	44°45′06.45"N, 0°33′34.37"W			
Clay (<2 µm) g kg ⁻¹	199	243	181	359	83			
Fine silt (2/20 μ m) g kg ⁻¹	263	292	241	398	45			
Coarse silt (20/20 μ m) g kg ⁻¹	442	353	423	127	21			
Fine sand (50/200 μ m) g kg ⁻¹	87	69	103	44	64			
Coarse sand (200/2000 $\mu m)$ g kg^-1	9	43	52	72	787			
Organic Carbon g kg ⁻¹	9.11	13.4	11.3	17.9	9.33			
Total N g kg ⁻¹	0.87	1.18	1.17	1.76	0.64			
C/N	10.4	11.4	9.64	10.2	14.6			
Organic Matter g kg ⁻¹	15.8	23.1	19.6	31	16.1			
pH	6.88	8.51	6.4	6.93	6.06			
CEC cmol+kg ⁻¹	8.26	10.8	6.79	14.3	2.78			
P ₂ O ₅ g 100g ⁻¹	0.13	0.23	0.25	0.35	0.06			

509 Soil physico-chemical parameters from control plots of different Long-Term observatories

Table 2

Treatment		FYM	GWS
Dry Matter (DM^{\dagger})	%	33.9 ± 0.7	67.2 ± 1.3
Organic Carbon	g.kg ⁻¹ DM	355 ± 14	257 ± 2
Total N	g.kg ⁻¹ DM	19.7 ± 0.6	22.5 ± 0.5
P ₂ O ₅ (Olsen)	g.kg ⁻¹ DM	2.6 ± 0.1	0.8 ± 0.1
C:N		18.1 ± 0.1	13.2 ± 0.5
pH (water)		8.8 ± 0.3	7.7 ± 0.1
${\rm I_{ROC}}^{\ddagger}$	(% organic C)	66.6 ± 7.5	77.6 ± 9.3
Microbial Biomass	µg of DNA.g ⁻¹ of OWP	515.5 ± 87.0	54.9 ± 19.1

511 Main characteristics of the different organic waste products (OWP) used

[†]DM: Dry Matter; FYM: farmyard manure; GWS: green wastes with sewage sludge

 ${}^{\ddagger}I_{ROC}$: index of residual organic carbon

525	GWS	green	wastes	with	sewage	sludge)
524	amended or not	(control) with t	wo different orga	nic materials (FYM: farmyard 1	nanure or
523	(QualiAgro (Qa	ı), PROspective	(Co), Efele (Ef), Couhins (Cł	n) and La Bouz	ule (LB))
522	Fig 1. The dyn	namics of cumul	ative emission o	f CO ₂ from fir	ve different type	s of soils

526	Fig. 2. The d	ynamics of	cumulative emission	n of N ₂ O from	n five differen	t types of soils
527	(QualiAgro (Q	Qa), PROsp	ective (Co), Efele	(Ef), Couhins	(Ch) and La	Bouzule (LB))
528	amended or no	ot (control)	with two different o	organic materia	lls (FYM: farm	yard manure or
529	GWS:	green	wastes	with	sewage	sludge).

- 530 Fig. 3. The dynamics of microbial biomass from five different types of soils (QualiAgro (Qa),
- 531 PROspective (Co), Efele (Ef), Couhins (Ch) and La Bouzule (LB)) amended or not (control)
- with two different organic materials (FYM: farmyard manure or GWS: green wastes withsewage sludge).

Fig. 4. The dynamics of Prokaryotic (A) and fungal (B) diversity indices from five different
types of soils (QualiAgro (Qa), PROspective (Co), Efele (Ef), Couhins (Ch) and La Bouzule
(LB)) amended or not (control) with two different organic materials (FYM: farmyard manure
or GWS: green wastes with sewage sludge).

538	Fig. 5. Magn	itude of the	proka	aryotic	and fungal	geneti	ic sti	ructure of	five dif	ferent t	ypes of
539	soils (QualiA	agro (Qa), P	ROsp	ective	(Co), Efele	(Ef), (Couł	nins (Ch)	and La	Bouzule	e (LB))
540	modifications	s during 28	days	after	amendment	with 1	two	different	organic	materia	als: (A)
541	farmyard	manure	or	(B)	green	was	stes	with	sewa	ige	sludge.

542 Supplementary Figure S1

543 Principal Component Analysis of the evolution of microbial community composition over
544 time (A: Prokaryote and B: Fungi) of five different types of soils (QualiAgro (Qa),
545 PROspective (Co), Efele (Ef), Couhins (Ch) and La Bouzule (LB)) amended or not (CON)
546 with two different organic materials (FYM: farmyard manure or GWS: green wastes with
547 sewage sludge).

548 Supplementary Figure S2

- 549 Relative abundances of prokaryotic (A) and fungal (B) *phyla* and prokaryotic (A') and fungal
- 550 (B') genera in the different sampling sites (QualiAgro (Qa), PROspective (Co), Efele (Ef),
- 551 Couhins (Ch) and La Bouzule (LB)) before the application of organic waste products (T0).

552 Supplementary Figure S3

- 553 Relative abundances of prokaryotic (A) and fungal (B) *phyla* and prokaryotic (A') and fungal
- (B') genera in the two organic waste products applied on soils (GWS and FYM).

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-A-Ch

-X-Qa

-O-LB

→ Ef

Co

Magnitudue of prokaryotic genetic structure modification from control

