



HAL
open science

The short-term response of soil microbial communities to digestate application depends on the characteristics of the digestate and soil type

F. Vautrin, P. Piveteau, M. Cannavacciuolo, P. Barre, C. Chauvin, C. Villenave, D. Cluzeau, K. Hoeffner, P. Mulliez, V. Jean-Baptiste, et al.

► To cite this version:

F. Vautrin, P. Piveteau, M. Cannavacciuolo, P. Barre, C. Chauvin, et al.. The short-term response of soil microbial communities to digestate application depends on the characteristics of the digestate and soil type. *Applied Soil Ecology*, 2024, 193, pp.art. 105105. 10.1016/j.apsoil.2023.105105. hal-04266661

HAL Id: hal-04266661

<https://hal.science/hal-04266661v1>

Submitted on 12 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

1 **The short-term response of soil microbial communities to digestate application depends**
2 **on the characteristics of the digestate and soil type**

3 **Authors:**

4 F. Vautrin¹, P. Piveteau², M. Cannavacciuolo³, P. Barré⁴, C. Chauvin⁵, C. Villenave⁵, D.
5 Cluzeau⁶, K. Hoeffner⁶, P. Mulliez⁷, V. Jean-Baptiste⁸, G. Vrignaud⁹, J. Tripied¹, S.
6 Dequiedt¹, P.A. Maron¹, L. Ranjard¹, S. Sadet-Bourgeteau¹

7 **Affiliations:**

8 ¹ Agroécologie, INRAE, Institut Agro, Univ. Bourgogne, Univ. Bourgogne Franche-Comté,
9 F-21000 Dijon, France

10 ² INRAE, UR OPAALE, Rennes, France

11 ³ USC 1432 LEVA, Ecole Supérieure d'Agricultures (ESA), INRAE, Angers, France

12 ⁴ Laboratoire de Géologie, Ecole normale supérieure, CNRS, PSL Univ., IPSL, Paris, France

13 ⁵ ELISOL Environnement, Congénies, France

14 ⁶ University of Rennes, CNRS, ECOBIO [(Ecosystèmes, biodiversité, évolution)] - UMR
15 6553, Rennes, France

16 ⁷ Chambre régionale d'agriculture des Pays de la Loire

17 ⁸ GRDF, Paris, France

18 ⁹ ACE méthanisation, Thouars, France

19 **E-mail addresses:**

20 F. Vautrin: florianvautrin@gmail.com; P. Piveteau: pascal.piveteau@inrae.fr; M.
21 Cannavacciuolo: m.cannavacciuolo@groupe-esa.com; P. Barré: barre@geologie.ens.fr; C.

22 Chauvin: camille.chauvin@elisol-environnement.fr; C. Villenave : cecile.villenave@elisol-
23 environnement.fr; D. Cluzeau: daniel.cluzeau@univ-rennes1.fr; K. Hoeffner:
24 kevin.hoeffner@univ-rennes1.fr; P. Mulliez: pierre.mulliez@pl.chambagri.fr; V. Jean-
25 Baptiste: vincent.jean-baptiste@grdf.fr; J. Tripied: julie.tripied@inrae.fr; S. Dequiedt:
26 samuel.dequiedt@inrae.fr; P.A. Maron: pierre-alain.maron@inrae.fr; G. Vrignaud:
27 gregory.vrignaud@gmail.com; L. Ranjard: lionel.ranjard@inrae.fr; S. Sadet-Bourgeteau:
28 sophie.bourgeteau-sadet@agrosupdijon.fr

29 **Keywords:** Digestate; soil types; high-throughput DNA sequencing; microcosm experiment;
30 microbial community

31

32 **Corresponding author:** Sophie Sadet-Bourgeteau, sophie.bourgeteau-
33 sadet@agrosupdijon.fr, Agroécologie, INRAE, Institut Agro, Univ. Bourgogne, Univ.
34 Bourgogne Franche-Comté, F-21000 Dijon, France

35

36 **Abstract**

37 Anaerobic digestion of organic waste is a key process to produce renewable energy and meet
38 the growing demand for sustainable energy. The residues of anaerobic digestion – called
39 digestates – can be used as soil amendments to improve crop yields. However, the effect of
40 digestates on the soil biota, especially on microorganisms, needs to be better documented
41 before a large scale use of digestates in agriculture. In addition, how the quality and
42 composition of the digestate may affect soil microbial communities has not been properly
43 addressed yet. We designed a microcosm experiment under controlled experimental
44 conditions to compare effects (42 days) of four digestates produced from varying intakes

45 (cattle manure and/or energy crop and/or food residues and/or slurry) on soil microbial
46 communities; a control microcosm made of undigested cattle manure was also used. Each
47 digestate was applied on three contrasting soils representing contrasted pedo-climatic
48 conditions (especially soil type and climate). These three soils presented different prokaryotic
49 and fungal communities structures. The effect of digestate inputs on the soil microbial
50 biomass and diversity was assessed using molecular DNA-based tools (quantification of
51 extracted soil DNA and high-throughput sequencing, respectively) in comparison to the
52 untreated cattle manure control condition. Our results show that 42 days after digestate
53 application, significant differences of soil microbial communities were observed according to
54 the digestate characteristics; these differences were soil-dependent. Thus, in the silty clay
55 loam soil, no effect of digestates was observed on soil microbial biomass or diversity ($P >$
56 0.05), as compared to the undigested cattle manure. In the two other soil types (loam and
57 sandy loam), soil microbial biomass decreased (around -40% , $P < 0.001$) when digestates
58 having a low total organic carbon content (from 0.61 to $3.3 \text{ g} \cdot 100\text{g}^{-1}$) were applied. None of
59 the digestates affected the soil prokaryotic diversity whatever the soil type ($P > 0.05$).
60 Digestate application resulted in higher fungal diversity (around $+35\%$; $P < 0.001$) in soils with
61 low C/N ratio (9.14 in average). The microbial community structure of coarse-textured soil
62 appeared more impacted by organic inputs than fine-textured soils. To conclude, our results
63 show that different soil types, harboring distinct microbial community structures, responded
64 differently to different digestates application. This response was also digestate-dependent.

65 **1. Introduction**

66 Biogas production has been rapidly growing in Europe for more than a decade (Appel et
67 al., 2016), in line with Europe's target to meet 32% of renewable energy production by 2030
68 (European Parliament, 2018). This increase may be further amplified by the current

69 geopolitical context raising questions about Europe's gas self-sufficiency. It is also an
70 alternative way to manage farmyard manure and/or industrial organic waste while producing
71 green energy (Panuccio *et al.*, 2021). In addition to biogas production, anaerobic digestion
72 produces digestate – a by-product used as an organic fertilizer (Pivato *et al.*, 2016). Thus,
73 biogas production contributes to promote circular bioeconomy by closing the nutrient loop, it
74 contributes also to the mitigation of greenhouse gas emissions from farm effluents and
75 increases carbon sequestration in soils (Nayal *et al.*, 2016; Tambone *et al.*, 2017).

76 According to the literature, the organic matter of digestates may improve the soil
77 structure, its infiltration rate and its water-holding capacity (Barlóg *et al.*, 2020; Makádi *et al.*,
78 2012; Möller and Müller, 2012), and thus increase productivity while lowering fertilization
79 costs. However, thorough investigations of possible side effects on the soil biota – especially
80 on microorganisms that play important roles in the soil ecosystem – are required to properly
81 assess the sustainability of large-scale land application of digestates in agricultural fields.

82 It is now well established that organic amendments improve soil microbial communities
83 by increasing the soil microbial biomass (Chaudhry *et al.*, 2012; Francioli *et al.*, 2016; Ros *et al.*,
84 2006) and inducing shifts of prokaryotic and fungal community structures (Chaudhry *et al.*,
85 2012; Francioli *et al.*, 2016; Ros *et al.*, 2006; Sadet-Bourgeteau *et al.*, 2018). The
86 response of microbial communities to organic waste inputs depends on the soil type and on
87 the nature of the organic materials (Sadet-Bourgeteau *et al.*, 2019). In addition, it is important
88 to consider the time lag between organic material application and their impact on the soil.
89 Transient changes in the bacterial functional and genetic structures occur within the first three
90 months following organic amendment. Then, a period of resilience is observed, eventually
91 leading to similar communities in both the amended and control plots (Calbrix *et al.*, 2007).
92 This strong transient alteration of soil microbial functioning may lead to modifications of the
93 carbon and nitrogen cycles (Sadet-Bourgeteau *et al.*, 2019). This could explain why the period

94 within days directly following land application of organic amendments is considered of the
95 highest importance for environmental hazards. However, the impact of digestates on the soil
96 microbial communities has not been extensively studied on the short term, *i.e.* up to eight
97 weeks after application. In addition, to our knowledge, no studies assessed this effect on soil
98 microbial communities from different soil types.

99 Reports on the impacts of digestates on the soil microbiota are scarce, and those on the
100 short-term effects of digestates on the soil microbial communities are rather contradictory, as
101 reviewed by Karimi *et al.* (2022). Some studies suggest that biogas digestates increase the soil
102 microbial biomass (Caracciolo *et al.*, 2015; Cattin *et al.*, 2021; García-Sánchez *et al.*, 2015;
103 Muscolo *et al.*, 2017; Panuccio *et al.*, 2021) and prokaryotic diversity (Caracciolo *et al.*, 2015;
104 García-Sánchez *et al.*, 2015) from 21 to 180 days after inputs compared to unfertilized soil.
105 Other studies show no significant effect of digestate inputs on the soil microbial biomass or
106 prokaryotic diversity from 1 to 10 weeks after application (Akari and Uchida, 2021; Barduca
107 *et al.*, 2021; Podmirseg *et al.*, 2019). To our knowledge, the short-term impact of digestates on
108 the soil fungal community has never been assessed. Interestingly, a short-term effect of
109 digestates on the microbial community structure is consistently observed whatever the
110 experimental conditions (Caracciolo *et al.*, 2015; Gómez-Brandón *et al.*, 2016; Hupfauf *et al.*,
111 2016; Johansen *et al.*, 2013; Sapp *et al.*, 2015), leading to growth of *Actinobacteria* and
112 *Acidobacteria*, a decrease of the *Bacteroidetes* (Sapp *et al.*, 2015) or a shift in the ammonia-
113 oxidizing bacteria (AOB) community (Gomez-Brandon *et al.*, 2016). Differences in
114 experimental setups and conditions could explain conflicting results observed for some
115 microbial indicators: the soil type, the digestate nature and dose, the delay between digestate
116 application and soil sampling, the presence and type of vegetation varied across studies. All
117 these factors make it difficult to reach generic conclusions about the impact of digestates on

118 the soil microbial quality. However, as previously mentioned, studies combining different
119 soil types and contrasting digestates are lacking (Karimi et al., 2022).

120 Therefore, thorough investigations of possible side effects on the soil biota – especially
121 on microorganisms that play important roles in the soil ecosystem – are required to properly
122 assess the sustainability of large-scale land application of digestates in agricultural fields.

123 Based on previous studies, we hypothesized that the responses of microbial communities to
124 digestate depend on the soil type and on the nature of digestate. Therefore, the objective of the
125 present study was to compare, under controlled experimental conditions, the short-term
126 impact of different types of digestates on soil microbial communities, and to assess if these
127 impacts depended on the type of soil. This laboratory microcosm experiment was designed
128 with four types of digestate and three soils, with a range of physicochemical and climatic
129 characteristics, selected to represent different microbial communities. Soils were sampled
130 from fields that had never received any digestate amendment. The four digestates mainly
131 differed in their physicochemical composition, especially their C/N ratio and nutrient content.
132 Digestates were applied to the microcosms at an agronomic dose of 25 t.ha⁻¹. The responses
133 of prokaryotic and fungal communities (microbial biomass, 16S and 18S ribosomal gene
134 inventories) were assessed 42 days after digestate application. The novelty of this work is to
135 address concomitantly the factors “type of digestate” and “type of soil” within the same
136 experimental setup to assess how these factors affect the responses of prokaryotic and fungal
137 communities upon digestate application to soil. High throughput sequencing respectively
138 targeting 16S and 18S ribosomal genes was used to characterize the vast microbial diversity
139 of soil and assess microbial communities’ composition changes after amendment.

140 **2. Materials and methods**

141 *2.1. Soils, biogas digestates and control manure used in this study*

142 Three soils were selected according to their pedoclimatic diversity. One silty clay loam
143 soil was collected from the continental Burgundy region (BOU, 47°38'86.88N; 5°06'29.01E).
144 One loam soil originated from the oceanic Pays de la Loire region (PDL, 47°72'86.90N;
145 0°49'71.88W). The third soil, of sandy loam texture, came from the Mediterranean Provence-
146 Alpes-Côte d'Azur region (PACA, 43°17'80.55N; 6°20'88.88E). These three soils, thereafter
147 called BOU, PDL and PACA did not have any record of digestate land application; BOU and
148 PDL were under annual cropping systems, while PACA was a vineyard soil.

149 The top layer (0-20 cm) of these soils was sampled in February 2021. Each soil sample
150 was homogenized and sieved to 4 mm to remove aboveground plant debris, roots and stones.
151 An aliquot was air-dried for physicochemical analysis. Particle size distribution, pH, soil total
152 organic carbon (TOC), soil total nitrogen (Total N), soil C/N ratio, Olsen P, Cation Exchange
153 Capacity (CEC) and exchangeable cations (calcium, magnesium, sodium and potassium) were
154 determined by the Soil Analysis Laboratory of INRAE (Arras, France,
155 <https://www6.hautsdefrance.inrae.fr/las>). The water-holding capacity (WHC; gravimetric
156 humidity) was estimated in the laboratory. The initial soil physicochemical parameters are
157 summarized in **Table 1**. The remaining soil was used for microcosm setup.

158 Whole digestates were collected from four commercial anaerobic digestors fed with
159 contrasting input organic materials: i) **FYM** :farmyard manure – 75% cattle manure, 10%
160 cattle slurry, 10% energy crop and 5% milk-derived matter, ii) **CMF** :cattle manure + fat –
161 45% duck slurry, 45% biowaste products, 10% fatty waste from agroindustry, iii) **MFW**
162 :manure + food waste – 52% manure, 17% vegetables, 19% waste from agroindustry, 10%
163 slurry and 2% blood and iv) **SMS** : silage maize and sorghum – 90% energy crop and 10%
164 maize silage. FYM underwent a sanitation stage at 70 °C for 1h. Undigested cattle manure
165 (**CM**) from a dairy farm was chosen as control to assess conventional agronomic practices.
166 All fresh organic amendments were sent to Aurea Co. (France, <https://aurea.eu/>) to determine

167 moisture, total nitrogen (Total N), pH, and exchangeable cations (phosphorus, potassium,
168 calcium, magnesium and sodium). The carbon fractions (total organic carbon (TOC), soluble
169 organic carbon (SOC), hemicellulosis, cellulosis and lignin-cutin) were assessed by the
170 Regional Laboratory of Analyses and Research (France, [https://aisne.com/a-votre-](https://aisne.com/a-votre-service/laboratoire-departemental)
171 [service/laboratoire-departemental](https://aisne.com/a-votre-service/laboratoire-departemental)). The physicochemical characteristics of the organic
172 amendments are summarized in **Table 2**.

173 *2.2. Microcosm setup and incubation*

174 The experimental design consisted of a factorial arrangement with three soils (BOU,
175 PACA and PDL), five amendments (four different types of digestates (CMF, FYM, MFW,
176 SMS), one untreated organic amendment (CM) and one unfertilized control (sterile water).
177 Four replicate microcosms were prepared per condition. In total, 72 microcosms (3 soils × 6
178 treatments × 4 replicates) were prepared. Unfertilized control microcosms were added in
179 order to take into account the natural evolution of the microbial communities during
180 incubation of soil microcosms, as reported previously (Sadet-Bourgeteau et al., 2019; Tardy
181 et al., 2014). Microbial community results after 42 days of incubation of these unfertilized
182 control were subtracted to those of the treated samples. Thus, for each soil type, results were
183 expressed as percent differences between the test condition (CMF, FYM, MFW, SMS or CM)
184 and unfertilized control.

185 For each condition, four sterile flasks (250 mL) were filled with 60 g (dry weight) of soil.
186 After two weeks of pre-incubation at 15 °C, 0.682 g of organic waste products were
187 thoroughly mixed with a soil sample to reach the agronomic dose of 25 t.ha⁻¹ according to
188 agricultural practices in French cropping systems. Sterile water was added to these
189 microcosms to adjust the water content to 70 % soil water-holding capacity (WHC). The
190 flasks were incubated in the dark at 15 °C for 42 days to simulate spring weather conditions.

191 Every week, they were aerated under a biological safety cabinet, and the water content was
192 adjusted (70% of the soil WHC) when necessary to correct for any water loss through
193 evaporation. Germinating plants were removed when required. After incubation 42 days
194 incubation, samples were harvested, lyophilized and stored at -40 °C until use for molecular
195 analyses based on soil DNA extraction. Aliquots of each of the four replicates were pooled,
196 and soil chemical analyses (except particle size distribution) were performed by the Soil
197 Analysis Laboratory at the INRAE of Arras (France) as described for the initial stage of the
198 experiment.

199 *2.3. DNA Extraction, purification and molecular microbial biomass determination*

200 Microbial DNA was extracted from 1 g (dry weight) of soil according to a standardized
201 procedure of the GenoSol platform (INRAE, Dijon, France,
202 https://www2.dijon.inrae.fr/plateforme_genosol/) (Terrat et al., 2012). Since a highly positive
203 linear relationship has been shown between soil DNA recovery and C-biomass measurement
204 (Dequiedt et al., 2011; Marstorp et al., 2000), DNA concentrations of crude extracts were
205 determined by electrophoresis in 1% agarose gel stained with ethidium bromide using a calf
206 thymus DNA standard curve, and used as estimates of microbial biomass (Dequiedt et al.,
207 2011). After quantification, residual impurities were separated from nucleic acids by
208 centrifuging 100 µL of crude DNA through two types of minicolumns (Macherey-Nagel,
209 Hoerd, France) according to the manufacturer's instructions. Purified DNA concentrations
210 were finally measured on a 5-µL load using the Quantifluor staining kit (Promega, Lyon,
211 France) according to the manufacturer's protocol.

212 *2.4. High-throughput sequencing of 16S and 18S rRNA gene sequences*

213 For prokaryotic diversity (16S), the V3-V4 region of the bacterial chromosome was
214 targeted using primers F479 (5'-CAG CMG CYG CNG TAA NAC-3') and R888 (5'-CCG

215 YCA ATT CMT TTR AGT-3') on a 440-bp fragment (Tardy et al., 2014). For each sample, 5
216 ng of DNA were used in a final PCR volume of 25 μ L, and amplified under the following
217 conditions: 94 °C for 2 min, 35 cycles of 30 s at 94 °C, 30 s at 52 °C and 1 min at 72 °C,
218 followed by 7 min at 72 °C (Tardy *et al.*, 2014).

219 As demonstrated by George *et al.*, 2019, soil fungal richness follows the same trends
220 across land use, irrespective of primer set (18S rRNA or ITS (internal transcribed spacer)).
221 Thus, as proceeded in previous studies (Chemidlin Prévost-Bouré *et al.*, 2011, Sadet-
222 Bourgeteau *et al.*, 2019) we used a 350-bp 18S rRNA fragment to target fungi with primers
223 FR1 (5'-ANC CAT TCA ATC GGT ANT-3') and FF390 (5'-CGA TAA CGA ACG AGA
224 CCT-3') (Chemidlin Prévost-Bouré et al., 2011). For each sample, 5 ng of DNA were used in
225 a final PCR volume of 25 μ L and amplified under the following conditions: 94 °C for 3 min,
226 35 cycles of 30 s at 94 °C, 1 min at 52 °C and 1 min at 72 °C, followed by 5 min at 72 °C
227 (Sadet-Bourgeteau *et al.*, 2019).

228 All PCR products were purified using the ProNex® Size-Selective DNA Purification
229 System (Promega, Lyon, France) and quantified with the Quantifluor staining kit (Promega,
230 Lyon, France) according to the manufacturer's instructions.

231 Series of 10-bp multiplex identifiers were added to the 5' end of the primers to identify
232 each sample and prevent PCR biases. For bacteria and archaea, 7.5 ng of DNA were used and
233 amplified with seven cycles under the same conditions as those of the first PCR. For fungi, 5
234 ng of DNA were used under optimized PCR conditions with a denaturation step at 94 °C for 1
235 min and seven cycles.

236 The PCR products were purified with the MinElute gel extraction kit (Qiagen NV) and
237 quantified with the Quantifluor staining kit (Promega, Lyon, France) according to the
238 manufacturer's instructions. All samples were pooled in equal amounts and cleaned with the

239 solid-phase reverse immobilization method (SPRI) using the ProNex[®] kit (Promega, Lyon,
240 France). The pool was finally sequenced with a MiSeq Illumina instrument (Illumina Inc, San
241 Diego, CA) operating with V3 chemistry and producing 2x300 bp paired-reads by
242 GenoScreen (<https://www.genoscreen.fr/fr/>).

243 *2.5. Bioinformatic analysis of the 16S and 18S rRNA gene sequences*

244 All paired-end Illumina MiSeq reads were processed using the BIOCUM-PIPE, as
245 described by Djemiel *et al.* (2020). The parameters retained for each step can be found in
246 **Supplementary Table S1**. The resulting high-quality reads were used for (i) taxonomy-
247 independent analysis, determining a biodiversity richness index, and (ii) taxonomy-based
248 analysis using similarity approaches against dedicated reference databases from SILVA v132
249 (Quast *et al.*, 2012). The raw datasets are available in the EBI database system under project
250 accession PRJEB53389.

251 *2.6. Statistical analysis*

252 All datasets were analyzed with R software (R version 4.1.3) (Verzani, 2004). As
253 previously mentioned, in order to take into account the natural evolution of the soil microbial
254 community throughout the incubation (molecular microbial biomass and microbial richness),
255 the results at the end of the experiment (42 days) from the soils that only received sterile
256 water (un-fertilized control) were subtracted from those of the treated samples. Then, the
257 results were expressed as percent differences. Normality was tested for each parameter using
258 Shapiro-Wilk's test followed by Bartlett's test of the homogeneity of variances. Then, an
259 analysis of variance (ANOVA) and a *post-hoc* Tukey's honestly significant difference (HSD)
260 test ($P < 0.05$) were performed to test the effect of soil type, organic materials type and soil
261 type \times organic material type on physicochemical properties and microbial community

262 indicators (microbial biomass, prokaryotic and fungal richness). The effects with p-values <
263 0.05 were considered statistically significant.

264 In order to highlight the organic amendment or soil physicochemical parameters involved
265 in changes in the microbial community indicators, a redundancy analysis (RDA) was
266 performed, based on the following parameters: soil granulometry, total nitrogen (Total N),
267 total organic carbon (TOC), C/N ratio, pH, Olsen P, together with the following organic
268 amendment variables: total nitrogen (Total N), total organic carbon (TOC), SOC, C/N ratio,
269 pH, Olsen P. These variables were used to evaluate whether the soil and organic inputs
270 physico-chemical characteristics discriminated the different treatments. To determine the
271 abiotic effects on the microbial community indicators, the *rda* and *ordistep* functions (*vegan*
272 package) were used in R software (R version 4.1.3) (Verzani, 2004).

273 The prokaryotic and fungal community structures were also compared using a taxonomic
274 abundance matrix at the phylum level. Non-metric multidimensional scaling (nMDS) was
275 performed on raw data (no subtraction of the unfertilized control) to graphically depict the
276 distribution patterns of the microbial community structures across treatments, constructed
277 with the 1% most abundant phyla. Differences between treatments were assessed by non-
278 parametric PERMANOVA tests ($P < 0.05$) run with the *adonis* function in the *vegan* package.
279 Explanatory phyla and physicochemical parameters were fitted on the graphs (Total N, TOC,
280 SOC, C/N ratio, pH and Olsen P for the organic amendments; Total N, TOC, C/N ratio, pH,
281 Olsen P, clay and silt for the soils).

282 **3. Results**

283 *3.1. Soil physicochemical characteristics*

284 Before organic material application, the three soils exhibited contrasting textures, with a
285 greater clay content in BOU (386 g clay.kg⁻¹ dry soil) and a greater sand content in PACA

286 (666 g sand.kg⁻¹ dry soil), whereas PDL was considered as a loamy soil (392 g silt kg⁻¹ dry
287 soil) (**Table 1**). Forty-two days after organic material application, the Principal Component
288 Analysis (PCA) of the soil physico-chemical characteristics highlighted that soil samples
289 were not discriminated according to organic treatments, but remained mainly discriminated by
290 their type (**Fig. 1**). Thus, at the end of the experiment, BOU was characterized by a higher pH
291 (7.9), higher TOC (20.57 g.kg⁻¹ dry soil) and nitrogen (1.79 g.kg⁻¹ dry soil) contents (**Fig. 1**).
292 PACA was characterized by lower TOC (5.99 g.kg⁻¹ dry soil) and nitrogen (0.63 g.kg⁻¹ dry
293 soil) contents (**Fig. 1**). PDL was characterized by a lower pH (6.8).

294 *3.2. Effects of digestate inputs on soil microbial community indicators*

295 Forty-two days after organic material application, an effect of soil, digestate and
296 soil*digestate interaction was observed for all measured soil microbial indicators (molecular
297 microbial biomass, prokaryotic and fungal richness; **Fig. 2, 3 and 4**; $P < 0.001$). More
298 precisely, in BOU, no significant digestate-related difference was observed ($P > 0.05$; **Fig. 2,**
299 **3 and 4**). All digestates (CMF, FYM, MFW and SMS) induced the same effect on the soil
300 microbial biomass and the microbial diversity indexes (prokaryotic and fungal richness) as
301 cattle manure did.

302 In PACA, 42 days after application, most of digestates (CMF, FYM and SMS) inputs induced
303 lower microbial biomass compared to CM (-48%, -53% and -37%, respectively; $P < 0.001$;
304 **Fig. 2**). No significant difference in soil microbial biomass was observed between MFW- and
305 CM-amended PACA ($P > 0.05$; **Fig. 2**). In PACA soil, digestate application induced no
306 differences of prokaryotic richness in comparison to the CM control, excepted for MFW-
307 treated PACA, which exhibited 20% lower prokaryotic diversity compared to CM treatment
308 ($P < 0.001$, **Fig. 3**). Regarding PACA soil fungal richness, no significant differences were

309 observed between digestates (CMF, FYM, MFW and SMS)- and CM-treatments ($P > 0.05$;
310 **Fig. 4**).

311 In PDL, 42 days after application, significant lower soil microbial biomass was observed
312 when CMF and SMS were applied compared to CM (-44% and -48%, respectively; $P < 0.001$;
313 **Fig. 2**). Regarding soil prokaryotic diversity, no difference was noted between CM- and
314 digestates-amended PDL ($P > 0.05$; **Fig. 3**). On the opposite, all digestates induced 21%
315 higher fungal richness in PDL soil compared with cattle manure input ($P < 0.001$; **Fig. 4**).

316 The redundancy analysis (RDA) approach allowed us to decipher the determinants of these
317 variations of soil microbial parameters. Whatever the soil considered, the total organic carbon
318 content of the inputs (29% of the variance due to the TOC) and the silt content (30% of the
319 variance) of soil explained the significant variations of soil molecular microbial biomass
320 (**Supplementary Table S2**). Concerning the prokaryotic diversity, none of the
321 physicochemical characteristics tested could explain observed variance. Fungal diversity
322 variation was explained by the soil C/N ratio (67% of the variance) (**Supplementary Table**
323 **S2**).

324 *3.3. Soil microbial community structure*

325 The nMDS ordination highlighted distinct prokaryotic and fungal community structures
326 among the three unfertilized soils at the end of the 42-day incubation (**Supplementary Fig.**
327 **S1 A and B**). Consequently, the microbial structures of each soil, 42 days after organic
328 amendment application, were analyzed separately. In BOU, the prokaryotic and fungal
329 community structures differed among treatments at the end of the 42-day incubation (**Fig. 5A**
330 and **Fig. 6A**). The prokaryotic community structure of CMF-, FYM- and MFW-amended
331 BOU seemed to differ from those of CM- and SMS-amended BOU (**Fig. 5A**). However,
332 PERMANOVA analysis highlighted that prokaryotic community structure of BOU soil

333 amended with digestates or undigested cattle manure was similar 42 days after organic
334 materials inputs ($P > 0.05$, **Supplementary Table S3**). The fungal community structure of
335 CMF- and MFW-amended BOU differed from those of CM-amended BOU (**Fig. 6A**).
336 PERMANOVA analysis confirmed these observations highlighting significant differences
337 between fungal community structures from BOU soil amended with CM and those amended
338 with the following digestates: CMF, FYM and MFW ($P < 0.05$, **Supplementary Table S4**). A
339 greater abundance of *Ascomycota* was found in CM-amended BOU. BOU soil amended with
340 digestates such MFW and CMF recorded a greater abundance of *Mucoromycota*,
341 *Cryptomycota* and *Basidiomycota* (**Fig. 6A**). FYM-amended BOU soil was supported by a
342 greater abundance of *Chytridiomycota* (**Fig. 6A**).

343 The prokaryotic community structure of CM-amended PACA significantly differed from
344 FYM and CMF treatments (**Fig. 5B**, PERMANOVA, **Supplementary Table S3**, $P < 0.05$).
345 To note that PERMANOVA analysis highlighted that the prokaryotic structure from PACA
346 amended with CMF differed from those amended with SMS (**Supplementary Table S3**, $P <$
347 0.05). These differences were supported by a greater abundance of *Bacteroidetes*, *Chloroflexi*
348 and *Spirochaetes* and a lower abundance of *Firmicutes*, *Actinobacteria* and *Cloacimonetes* in
349 CM-amended PACA than in CMF-, FYM-, MFW- and SMS-amended PACA (**Fig. 5B**).
350 Regarding the fungal community structure, SMS-amended PACA differed from all other
351 amended PACA, CM included (**Fig. 6B**, PERMANOVA, **Supplementary Table S4**, $P <$
352 0.05). PACA amended with SMS recorded a greater abundance of *Basidiomycota* (**Fig. 6B**).

353 The prokaryotic community structure of CM-amended PDL seemed to differ from all
354 digestates-amended PDL (**Fig. 5C**), but this was not confirmed by PERMANOVA analysis,
355 (**Supplementary Table S3**, $P > 0.05$). Regarding the fungal community structure, CM-
356 amended PDL seemed to differ from FYM-, MFW- and CMF-amended PDL (**Fig. 6C**). This
357 was not confirmed by PERMANOVA analysis, (**Supplementary Table S4**, $P > 0.05$),

358 however, CMF-amended PDL differed from MFW- and SMS-amended PDL
359 (PERMANOVA, **Supplementary Table S4**, $P < 0.01$). As observed in BOU soil, PDL soil
360 amended with CMF recorded a greater abundance of *Mucoromycota* and *Cryptomycota* (**Fig.**
361 **6C**).

362 **4. Discussion**

363 Widespread development of anaerobic digestion is expected in the coming years to meet the
364 rising demand for green energy. As a result, agronomic use of digestates may be generalized.
365 However, impacts of digestates on soil biodiversity still need to be evaluated to prevent
366 potential environmental side effects associated to the use of these inputs across a wide range
367 of biogeographic regions and soil contexts. To our knowledge, the comparison of different
368 digestates in different soil types has never been addressed simultaneously under the same
369 controlled experimental conditions. Contrasting digestates were selected and raw cattle
370 manure was added as control treatment to illustrate the most common organic materials used
371 as crop amendments. A dose of digestate of $25 \text{ t}\cdot\text{ha}^{-1}$ was applied to mimic common
372 agronomic practices in French fields (Monard *et al.*, 2020). Application of the same
373 agronomic dose of digestates with contrasting composition resulted in different doses of
374 elements brought, especially nitrogen. Interestingly, the total nitrogen and C/N ratio of CMF
375 differed from those of the other digestates: the nitrogen content was two times higher in CMF
376 (the largest amount) than in SMS, and TOC was seven times lower in CMF than in FYM.
377 These differences in carbon and nitrogen amounts induced a high variability of the C/N ratio
378 across treatments, probably leading to different fates of nitrogen in the amended soils. A C/N
379 ratio > 15 (*e.g.* in CM) leads to nitrogen immobilization in the soil, whereas a low C/N ratio
380 leads to NO_x emissions, so that a value $5 \leq \text{C/N ratio} \leq 6$ was proposed to be optimal

381 (Abubaker *et al.*, 2015; Cheng *et al.*, 2020). The differences of C/N ratios of the applied
382 organic materials led us to expect differences among the microcosms.

383 Differences in molecular microbial biomass were observed depending on the soil type
384 and the digestate. Microbial biomass remained unchanged in the silty clay loam soil (BOU)
385 regardless of the treatment, but decreased in the sandy loam (PACA) and loam (PDL) soils
386 down to 48% under CMF and SMS treatment compared to the CM treatment. The effects of
387 digestate on soil microbial biomass reported in the literature are often conflicting, mainly due
388 to great variations between the experimental systems. Thus, several authors observed an
389 increase of soil microbial biomass (assessed by fumigation extraction or soil DNA
390 measurement) after digestate application (Cattin *et al.*, 2021; García-Sánchez *et al.*, 2015;
391 Muscolo *et al.*, 2017; Panuccio *et al.*, 2021), whereas others reported no change (Barduca *et al.*,
392 2021; Podmirseg *et al.*, 2019). In the present study, redundancy analysis highlighted silt
393 content as a major driver of the observed differences according to the soil type. This suggests
394 that the soil texture can influence the response of microbial biomass to digestate application.
395 This hypothesis is supported by Panuccio *et al.* (2021), who found greater changes of
396 microbial biomass in sandy soil than in silty soil both amended with digestate. Fine-textured
397 soil is indeed known to be a favourable habitat for microbes because of a better supply of
398 organic resources and protection from desiccation, gas diffusion, toxic exogenous compounds
399 and predation by protozoa (Dequiedt *et al.*, 2011; Ranjard *et al.*, 2003).

400 Our work also highlighted total organic carbon of digestates as a second major driver
401 of soil microbial biomass variations. Thus, the lower microbial biomass quantified in some
402 digestate-amended soils than in CM-amended soils can be explained by the low total organic
403 carbon content of the digestates, hence a lower amount of organic carbon available for
404 microbial growth.

405 As observed for the soil microbial biomass, 42 days after organic material application,
406 microbial diversity variations depended on the characteristics of the digestate applied and the
407 soil type. This was consistent with previous results collected from different soil microbial
408 communities amended with other types of organic waste products (Sadet-Bourgeteau et al.,
409 2019). o increase of prokaryotic richness could be observed but a significant decrease was
410 observed in MFW-amended sandy loam soil (PACA) (20% less than in CM-amended soil).
411 These results are not in accordance with the literature available where an increase of soil
412 prokaryotic diversity (Shannon) was reported after digestate application (Caracciolo et al.,
413 2015; García-Sánchez et al., 2015; Hupfauf et al., 2016). These conflicting results could be
414 due to the sensitivity of the method used (fingerprint technics, PLFA... versus 16S
415 metabarcoding), on the difference of metric used (Shannon vs Richness), and, more largely,
416 on the differences between experimental designs (soil type, digestate...).

417 In the present work, the prokaryotic community structures of the three unfertilised
418 soils (control microcosms) were significantly different (Supplementary Fig. S1). Whatever the
419 treatment and soil type, at the end of the experiment the prokaryotic community structures at
420 the phylum level were dominated by *Proteobacteria*, *Bacteroidetes*, *Thaumarchaeota* and
421 *Actinobacteria*, major taxa found in French soils (Karimi *et al.*, 2018), but changes of
422 microbial community structure were detected depending on the soil type and the quality of the
423 amendment applied (Fig. 4 and 5). In comparison with manure amendment, differences in
424 prokaryotic community structures were significant only in sandy loam soil (PACA) amended
425 with FYM and CMF digestates. Similar trends were recorded in the loam (PDL) and silty clay
426 loam (BOU) soils, but differences were not significant. As already mentioned for the soil
427 microbial biomass, the coarse texture also influenced the prokaryotic community structure
428 more than the fine texture did. This is consistent with other studies highlighting an effect of
429 digestate on prokaryotic community structure limited to coarse-textured soils (Gómez-

430 Brandón et al., 2016; Hupfauf et al., 2016; Johansen et al., 2013; Sapp et al., 2015). This was
431 confirmed by Abubaker *et al.* (2013) who observed greater variability of community structure
432 in sandy soil than in clay soil. To summarize, our results highlighted that the observed effects
433 on distinct prokaryotic community structures were digestate- and soil-dependent. However, in
434 the three studied soils, the CM and CMF treatments consistently led to the most different soil
435 prokaryotic community structures, with *Spirochaetes* more abundant in all CM-amended soils
436 and *Actinobacteria* in all CMF-amended soils. This can be explained by the abundance of
437 *Spirochaetes* in manure because *Spirochaetes* are known free-living, anaerobic symbionts in
438 the stomachs of cows and other ruminants (Gupta et al., 2013). Thus, they may have come
439 from non-sanitized livestock manure. CMF was the digestate with the largest amount of
440 nitrogen and NH_4^+ . High amounts of ammonium ions lower the soil pH and modify the
441 microbial community structure (Chen et al., 2020). Furthermore, *Actinobacteria* are
442 stimulated in acidic soils (Karimi et al., 2018). In CMF, these conditions probably favoured
443 the abundance of *Actinobacteria*. This is also in accordance with a previous study
444 highlighting that the proportion of *Actinobacteria* was significantly higher in inorganically
445 fertilized (NPK) soils (Francioli *et al.*, 2016).

446 Increases of fungal richness were captured but only in loam soil (PDL) amended with
447 CMF and FYM (21% more than in CM-amended soil). To our knowledge, this is the first
448 report of the effect of digestates on soil fungal diversity. In the present study, the redundancy
449 analysis did not explain prokaryotic richness variation, but a 67% negative effect of the soil
450 C/N ratio of digestates on fungal richness was captured. This is in accordance with previous
451 works reporting that the soil fungal richness was negatively correlated with the soil C/N ratio
452 (George et al., 2019; Yang et al., 2017).

453 The fungal community structure was influenced by the input type, but the soil type
454 balanced this effect. To our knowledge, the impact of digestate application on soil fungal

455 community structure had never been assessed before. In the present study, the sandy loam soil
456 (PACA) exacerbated the effect of organic inputs on soil fungal community structure
457 compared to the silty clay loam soil (BOU). In the three soils, the communities were
458 dominated by *Ascomycota*, *Mucoromycota* and *Basidiomycota* whatever the condition, as
459 frequently observed in French soils (Maron *et al.*, 2018). Contrary to what was observed with
460 the soil prokaryotic communities, different fungal phyla could be stimulated by the same
461 organic material depending on the type of soil in which it was applied. This suggests that
462 different soil types, harbouring distinct fungal community structures, responded differently to
463 digestates application.

464 The present work monitored under controlled conditions enabled us to address the
465 impact of the characteristics of digestates on the response of soil microbial communities.
466 Moreover, high throughput sequencing was appropriate to characterize the vast microbial
467 diversity of soil but it provides limited information on soil microbial functioning. In order to
468 better characterize the environmental impacts of digestates, further research is needed under
469 field conditions. Future field studies should be implemented with repeated applications of
470 digestates over several years, in order to address holistically the sustainability of digestate
471 application. A special focus on microbial communities involved in biogeochemical cycles
472 would be of interest in combination with measurements of GHG emissions.

473

474 **5. Conclusions**

475 The forthcoming increase of the amounts of anaerobic digestates produced requires a proper
476 assessment of the environmental safety of land application of digestates, notably for soil
477 health and the telluric microorganisms that play key roles in biogeochemical cycles. This
478 study provided new insights regarding the short-term effects of contrasting anaerobic

479 digestates on soil microorganisms. Our results highlighted that the response of soil microbial
480 communities to the application of digestate depended firstly on the characteristics of the
481 digestate and secondly that these effects of digestate on soil microbial community depended
482 on the soil type. Indeed, coarse-textured soil was most affected than the finest ones.
483 Importantly, when applied on coarse-textured soil, digestates characterized by a low C/N ratio
484 (high levels of ammonium and low levels of organic carbon) induced the greatest impact on
485 soil microbial communities (lower microbial biomass and a shift of microbial structure and
486 composition) compared to untreated manure amendment. This pattern suggests that digestates
487 with low C/N ratios could be recommended for fertilisation of heavy soils with high clay and
488 C content while digestates with higher C/N ratios seem more appropriate for lighter, sandier
489 soils, containing less organic carbon.

490

491 **6. Acknowledgments**

492 The authors thank the GenoSol platform for technical support
493 <https://doi.org/10.15454/L7QN45> in molecular biology. The authors are grateful to the
494 OPAALE (Optimisation des Procédés en Agriculture, Agroalimentaire et Environnement) and
495 LEVA (Légumineuses, Ecophysiologie Végétale, Agroécologie) technical staff for their
496 technical support for soil and biogas digestate harvesting and microcosm experiment setup,
497 and Aurélien Cottin for his help with the bioinformatic tools. Calculations were performed
498 using HPC resources from DNUM CCUB (Centre de Calcul de l'Université de Bourgogne).
499 They also thank Annie Buchwalter for correction and improving English language in the
500 manuscript.

501 **Funding**

502 This work was supported by grants GRAINE from the ADEME, the CASDAR Innov et Part
503 Metha-BioSol and GrDF.

504 **Declaration of competing interest**

505 The authors declare that they have no known competing financial interests or personal
506 relationships that could have appeared to influence the work reported in this paper.

507 **7. References**

508 Abubaker, J., Cederlund, H., Arthurson, V., Pell, M., 2013. Bacterial community structure
509 and microbial activity in different soils amended with biogas residues and cattle
510 slurry. *Applied Soil Ecology* 72, 171–180.
511 <https://doi.org/10.1016/j.apsoil.2013.07.002>

512 Abubaker, J., Risberg, K., Jönsson, E., Dahlin, A.S., Cederlund, H., Pell, M., 2015. Short-
513 Term Effects of Biogas Digestates and Pig Slurry Application on Soil Microbial
514 Activity. *Applied and Environmental Soil Science* 2015, e658542.
515 <https://doi.org/10.1155/2015/658542>

516 Akari, M., Uchida, Y., 2021. Survival Rates of Microbial Communities from Livestock Waste
517 to Soils: A Comparison between Compost and Digestate. *Applied and Environmental*
518 *Soil Science* 2021, e6645203. <https://doi.org/10.1155/2021/6645203>

519 Appel, F., Ostermeyer-Wiethaup, A., Balmann, A., 2016. Effects of the German Renewable
520 Energy Act on structural change in agriculture – The case of biogas. *Utilities Policy*
521 41, 172–182. <https://doi.org/10.1016/j.jup.2016.02.013>

522 Barduca, L., Wentzel, S., Schmidt, R., Malagoli, M., Joergensen, R.G., 2021. Mineralisation
523 of distinct biogas digestate qualities directly after application to soil. *Biology and*
524 *Fertility of Soils* 57, 235–243. <https://doi.org/10.1007/s00374-020-01521-5>

525 Barłóg, P., Hlisnikovský, L., Kunzová, E., 2020. Effect of Digestate on Soil Organic Carbon
526 and Plant-Available Nutrient Content Compared to Cattle Slurry and Mineral
527 Fertilization. *Agronomy* 10, 379. <https://doi.org/10.3390/agronomy10030379>

528 Barra Caracciolo, A., Bustamante, M.A., Nogues, I., Di Lenola, M., Luprano, M.L., Grenni,
529 P., 2015. Changes in microbial community structure and functioning of a semiarid soil
530 due to the use of anaerobic digestate derived composts and rosemary plants.
531 *Geoderma* 245–246, 89–97. <https://doi.org/10.1016/j.geoderma.2015.01.021>

532 Calbrix, R., Barray, S., Chabrierie, O., Fourrie, L., Laval, K., 2007. Impact of organic
533 amendments on the dynamics of soil microbial biomass and bacterial communities in
534 cultivated land. *Applied Soil Ecology* 35, 511–522.
535 <https://doi.org/10.1016/j.apsoil.2006.10.007>

536 Cattin, M., Semple, K.T., Stutter, M., Romano, G., Lag-Brotons, A.J., Parry, C., Surridge,
537 B.W.J., 2021. Changes in microbial utilization and fate of soil carbon following the
538 addition of different fractions of anaerobic digestate to soils. *European Journal of Soil
539 Science* 1–16. <https://doi.org/10.1111/ejss.13091>

540 Chaudhry, V., Rehman, A., Mishra, A., Chauhan, P.S., Nautiyal, C.S., 2012. Changes in
541 Bacterial Community Structure of Agricultural Land Due to Long-Term Organic and
542 Chemical Amendments. *Microbial Ecology* 64, 450–460.
543 <https://doi.org/10.1007/s00248-012-0025-y>

544 Chemidlin Prévost-Bouré, N.C., Christen, R., Dequiedt, S., Mougél, C., Lelièvre, M., Jolivet,
545 C., Shahbazkia, H.R., Guillou, L., Arrouays, D., Ranjard, L., 2011. Validation and
546 Application of a PCR Primer Set to Quantify Fungal Communities in the Soil
547 Environment by Real-Time Quantitative PCR. *PLOS ONE* 6, e24166.
548 <https://doi.org/10.1371/journal.pone.0024166>

549 Chen, X., Henriksen, T.M., Svensson, K., Korsæth, A., 2020. Long-term effects of
550 agricultural production systems on structure and function of the soil microbial
551 community. *Applied Soil Ecology* 147, 103387.
552 <https://doi.org/10.1016/j.apsoil.2019.103387>

553 Cheng, J., Chen, Y., He, T., Liu, R., Yi, M., Yang, Z., 2020. Effects of biogas slurry irrigation
554 DOC/N ratios on the fate of soil nitrogen and GHG emissions: A laboratory study.
555 *Geoderma* 375, 114458. <https://doi.org/10.1016/j.geoderma.2020.114458>

556 Dequiedt, S., Saby, N.P.A., Lelièvre, M., Jolivet, C., Thioulouse, J., Toutain, B., Arrouays,
557 D., Bispo, A., Lemanceau, P., Ranjard, L., 2011. Biogeographical patterns of soil
558 molecular microbial biomass as influenced by soil characteristics and management.
559 *Global Ecology and Biogeography* 20, 641–652. [https://doi.org/10.1111/j.1466-](https://doi.org/10.1111/j.1466-8238.2010.00628.x)
560 [8238.2010.00628.x](https://doi.org/10.1111/j.1466-8238.2010.00628.x)

561 Djemiel, C., Dequiedt, S., Karimi, B., Cottin, A., Girier, T., El Djoudi, Y., Wincker, P.,
562 Lelièvre, M., Mondy, S., Chemidlin Prévost-Bouré, N., Maron, P.-A., Ranjard, L.,
563 Terrat, S., 2020. BIOCOT-PIPE: a new user-friendly metabarcoding pipeline for the
564 characterization of microbial diversity from 16S, 18S and 23S rRNA gene amplicons.
565 *BMC Bioinformatics* 21, 492. <https://doi.org/10.1186/s12859-020-03829-3>

566 European Parliament, 2018. Energy: new target of 32% from renewables by 2030 agreed by
567 MEPs and ministers. URL [https://www.europarl.europa.eu/news/en/press-](https://www.europarl.europa.eu/news/en/press-room/20180614IPR05810/energy-new-target-of-32-from-renewables-by-2030-agreed-by-meps-and-ministers)
568 [room/20180614IPR05810/energy-new-target-of-32-from-renewables-by-2030-agreed-](https://www.europarl.europa.eu/news/en/press-room/20180614IPR05810/energy-new-target-of-32-from-renewables-by-2030-agreed-by-meps-and-ministers)
569 [by-meps-and-ministers](https://www.europarl.europa.eu/news/en/press-room/20180614IPR05810/energy-new-target-of-32-from-renewables-by-2030-agreed-by-meps-and-ministers) (accessed 17.2.23)

570 Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F., Reitz, T., 2016. Mineral vs.
571 Organic Amendments: Microbial Community Structure, Activity and Abundance of
572 Agriculturally Relevant Microbes Are Driven by Long-Term Fertilization Strategies.
573 *Frontiers in Microbiology* 7. <https://doi.org/10.3389/fmicb.2016.01446>

574 Garcia-Sánchez, M., Garcia-Romera, I., Cajthaml, T., Tlustoš, P., Száková, J., 2015. Changes
575 in soil microbial community functionality and structure in a metal-polluted site: The
576 effect of digestate and fly ash applications. *Journal of Environmental Management*
577 162, 63–73. <https://doi.org/10.1016/j.jenvman.2015.07.042>

578 George, P.B.L., Creer, S., Griffiths, R.I., Emmett, B.A., Robinson, D.A., Jones, D.L., 2019.
579 Primer and Database Choice Affect Fungal Functional but Not Biological Diversity
580 Findings in a National Soil Survey. *Frontiers in Environmental Science* 7.

581 Gómez-Brandón, M., Juárez, M.F.-D., Zangerle, M., Insam, H., 2016. Effects of digestate on
582 soil chemical and microbiological properties: A comparative study with compost and
583 vermicompost. *Journal of Hazardous Materials* 302, 267–274.
584 <https://doi.org/10.1016/j.jhazmat.2015.09.067>

585 Gupta, R.S., Mahmood, S., Adeolu, M., 2013. A phylogenomic and molecular signature based
586 approach for characterization of the phylum Spirochaetes and its major clades:
587 proposal for a taxonomic revision of the phylum. *Frontiers in Microbiology*. 4, 217.
588 <https://doi.org/10.3389/fmicb.2013.00217>

589 Ho, A., El-Hawwary, A., Kim, S.Y., Meima-Franke, M., Bodelier, P., 2015. Manure-
590 associated stimulation of soil-borne methanogenic activity in agricultural soils.
591 *Biology and Fertility of Soils* 51, 511-516.
592 <https://doi.org/10.1007/s00374-015-0995-2>

593 Hupfauf, S., Bachmann, S., Fernández-Delgado Juárez, M., Insam, H., Eichler-Löbermann,
594 B., 2016. Biogas digestates affect crop P uptake and soil microbial community
595 composition. *Science of The Total Environment*, Special Issue on Sustainable
596 Phosphorus Taking stock: Phosphorus supply from natural and anthropogenic pools in
597 the 21st Century 542, 1144–1154. <https://doi.org/10.1016/j.scitotenv.2015.09.025>

598 Johansen, A., Carter, M.S., Jensen, E.S., Hauggard-Nielsen, H., Ambus, P., 2013. Effects of
599 digestate from anaerobically digested cattle slurry and plant materials on soil
600 microbial community and emission of CO₂ and N₂O. *Applied Soil Ecology* 63, 36–
601 44. <https://doi.org/10.1016/j.apsoil.2012.09.003>

602 Karimi, B., Chemidlin-Prevost Boure, N., Dequiedt, S., Terrat, S., Ranjard, L., 2018. Atlas
603 français des Bactéries du sol. Biotope, Mèze, Muséum national d'histoire naturelle,
604 Paris.

605 Karimi, B., Sadet-Bourgeteau, S., Cannavacciuolo, M., Chauvin, C., Flamin, C., Haumont,
606 A., Jean-Baptiste, V., Reibel, A., Vrignaud, G., Ranjard, L., 2022. Impact of biogas
607 digestates on soil microbiota in agriculture: a review. *Environmental Chemistry*
608 *Letters* 20, 3265-3288. <https://doi.org/10.1007/s10311-022-01451-8>

609 Makádi, M., Tomócsik, A., Orosz, V., 2012. Digestate: A New Nutrient Source – Review.
610 *Biogas* 14, 295-312. <https://doi.org/10.5772/31355>

611 Maron, P.-A., Sarr, A., Kaisermann, A., Lévêque, J., Mathieu, O., Guigue, J., Karimi, B.,
612 Bernard, L., Dequiedt, S., Terrat, S., Chabbi, A., Ranjard, L., 2018. High Microbial
613 Diversity Promotes Soil Ecosystem Functioning. *Applied and Environmental*
614 *Microbiology*. <https://doi.org/10.1128/AEM.02738-17>

615 Marstorp, H., Guan, X., Gong, P., 2000. Relationship between dsDNA, chloroform labile C
616 and ergosterol in soils of different organic matter contents and pH. *Soil Biology and*
617 *Biochemistry* 32, 879-882. [https://doi.org/10.1016/S0038-0717\(99\)00210-2](https://doi.org/10.1016/S0038-0717(99)00210-2)

618 Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability
619 and crop growth: A review. *Engineering in Life Sciences* 12, 242–257.
620 <https://doi.org/10.1002/elsc.201100085>

621 Monard, C., Jeanneau, L., Le Garrec, J.-L., Le Bris, N., Binet, F., 2020. Short-term effect of
622 pig slurry and its digestate application on biochemical properties of soils and

623 emissions of volatile organic compounds. *Applied Soil Ecology* 147, 103376.
624 <https://doi.org/10.1016/j.apsoil.2019.103376>

625 Muscolo, A., Settineri, G., Papalia, T., Attinà, E., Basile, C., Panuccio, M.R., 2017.
626 Anaerobic co-digestion of recalcitrant agricultural wastes: Characterizing of
627 biochemical parameters of digestate and its impacts on soil ecosystem. *Science of the*
628 *Total Environment* 586, 746–752. <https://doi.org/10.1016/j.scitotenv.2017.02.051>

629 Nayal, F.S., Mammadov, A., Ciliz, N., 2016. Environmental assessment of energy generation
630 from agricultural and farm waste through anaerobic digestion. *Journal of*
631 *Environmental Management* 184, 389–399.
632 <https://doi.org/10.1016/j.jenvman.2016.09.058>

633 Panuccio, M.R., Romeo, F., Mallamaci, C., Muscolo, A., 2021. Digestate Application on Two
634 Different Soils: Agricultural Benefit and Risk. *Waste and Biomass Valorization* 12,
635 4341-4353. <https://doi.org/10.1007/s12649-020-01318-5>

636 Pastorelli, R., Valboa, G., Lagomarsino, A., Fabiani, A., Simoncini, S., Zaghi, M., Vignozzi,
637 N., 2021. Recycling Biogas Digestate from Energy Crops: Effects on Soil Properties
638 and Crop Productivity. *Applied Sciences* 11, 750.
639 <https://doi.org/10.3390/app11020750>

640 Pivato, A., Vanin, S., Raga, R., Lavagnolo, M.C., Barausse, A., Rieple, A., Laurent, A.,
641 Cossu, R., 2016. Use of digestate from a decentralized on-farm biogas plant as
642 fertilizer in soils: An ecotoxicological study for future indicators in risk and life cycle
643 assessment. *Waste Management* 49, 378–389.
644 <https://doi.org/10.1016/j.wasman.2015.12.009>

645 Podmirseg, S.M., Waldhuber, S., Knapp, B.A., Insam, H., Goberna, M., 2019. Robustness of
646 the autochthonous microbial soil community after amendment of cattle manure or its

647 digestate. *Biology and Fertility of Soils* 55, 565–576. <https://doi.org/10.1007/s00374->
648 019-01371-w

649 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner,
650 F.O., 2013. The SILVA ribosomal RNA gene database project: improved data
651 processing and web-based tools. *Nucleic Acids Research* 41, D590–D596.
652 <https://doi.org/10.1093/nar/gks1219>

653 Ranjard, L., Lejon, D.P.H., Mougel, C., Schehrer, L., Merdinoglu, D., Chaussod, R., 2003.
654 Sampling strategy in molecular microbial ecology: influence of soil sample size on
655 DNA fingerprinting analysis of fungal and bacterial communities. *Environmental*
656 *Microbiology* 5, 1111–1120. <https://doi.org/10.1046/j.1462-2920.2003.00521.x>

657 Ros, M., Pascual, J.A., Garcia, C., Hernandez, M.T., Insam, H., 2006. Hydrolase activities,
658 microbial biomass and bacterial community in a soil after long-term amendment with
659 different composts. *Soil Biology and Biochemistry* 38, 3443–3452.
660 <https://doi.org/10.1016/j.soilbio.2006.05.017>

661 Sadet-Bourgeteau, S., Houot, S., Dequiedt, S., Nowak, V., Tardy, V., Terrat, S., Montenach,
662 D., Mercier, V., Karimi, B., Chemidlin Prévost-Bouré, N., Maron, P.A., 2018. Lasting
663 effect of repeated application of organic waste products on microbial communities in
664 arable soils. *Applied Soil Ecology* 125, 278–287.
665 <https://doi.org/10.1016/j.apsoil.2018.02.006>

666 Sadet-Bourgeteau, S., Houot, S., Karimi, B., Mathieu, O., Mercier, V., Montenach, D.,
667 Morvan, T., Sappin-Didier, V., Watteau, F., Nowak, V., Dequiedt, S., Maron, P.-A.,
668 2019. Microbial communities from different soil types respond differently to organic
669 waste input. *Applied Soil Ecology* 143, 70–79.
670 <https://doi.org/10.1016/j.apsoil.2019.05.026>

671 Sapp, M., Harrison, M., Hany, U., Charlton, A., Thwaites, R., 2015. Comparing the effect of
672 digestate and chemical fertiliser on soil bacteria. *Applied Soil Ecology* 86, 1–9.
673 <https://doi.org/10.1016/j.apsoil.2014.10.004>

674 Semenov, M.V., Krasnov, G.S., Semenov, V.M., Ksenofontova, N., Zinyakova, N.B., van
675 Bruggen, A.H.C., 2021. Does fresh farmyard manure introduce surviving microbes
676 into soil or activate soil-borne microbiota? *Journal of Environmental Management*
677 294, 113018. <https://doi.org/10.1016/j.jenvman.2021.113018>

678 Tambone, F., Orzi, V., D’Imporzano, G., Adani, F., 2017. Solid and liquid fractionation of
679 digestate: Mass balance, chemical characterization, and agronomic and environmental
680 value. *Bioresource Technology* 243, 1251–1256.
681 <https://doi.org/10.1016/j.biortech.2017.07.130>

682 Tang, Y., Luo, L., Carswell, A., Misselbrook, T., Shen, J., Han, J., 2021. Changes in soil
683 organic carbon status and microbial community structure following biogas slurry
684 application in a wheat-rice rotation. *Science of The Total Environment* 757, 143786.
685 <https://doi.org/10.1016/j.scitotenv.2020.143786>

686 Tardy, V., Mathieu, O., Lévêque, J., Terrat, S., Chabbi, A., Lemanceau, P., Ranjard, L.,
687 Maron, P.-A., 2014. Stability of soil microbial structure and activity depends on
688 microbial diversity. *Environmental Microbiology Reports* 6, 173–183.
689 <https://doi.org/10.1111/1758-2229.12126>

690 Terrat, S., Christen, R., Dequiedt, S., Lelièvre, M., Nowak, V., Regnier, T., Bachar, D.,
691 Plassart, P., Wincker, P., Jolivet, C., Bispo, A., Lemanceau, P., Maron, P.-A., Mougél,
692 C., Ranjard, L., 2012. Molecular biomass and MetaTaxogenomic assessment of soil
693 microbial communities as influenced by soil DNA extraction procedure. *Microbial*
694 *Biotechnology* 5, 135–141. <https://doi.org/10.1111/j.1751-7915.2011.00307.x>

- 695 Verzani, J., 2004. Using R for Introductory Statistics. Chapman and Hall/CRC, New York.
696 <https://doi.org/10.4324/9780203499894>
- 697 Yang, T., Adams, J.M., Shi, Y., He, J.-S., Jing, X., Chen, L., Tedersoo, L., Chu, H., 2017.
698 Soil fungal diversity in natural grassland of the Tibetan Plateau: associations with
699 plant diversity and productivity 215, 756-765. <https://doi.org/10.1111/nph.14606>

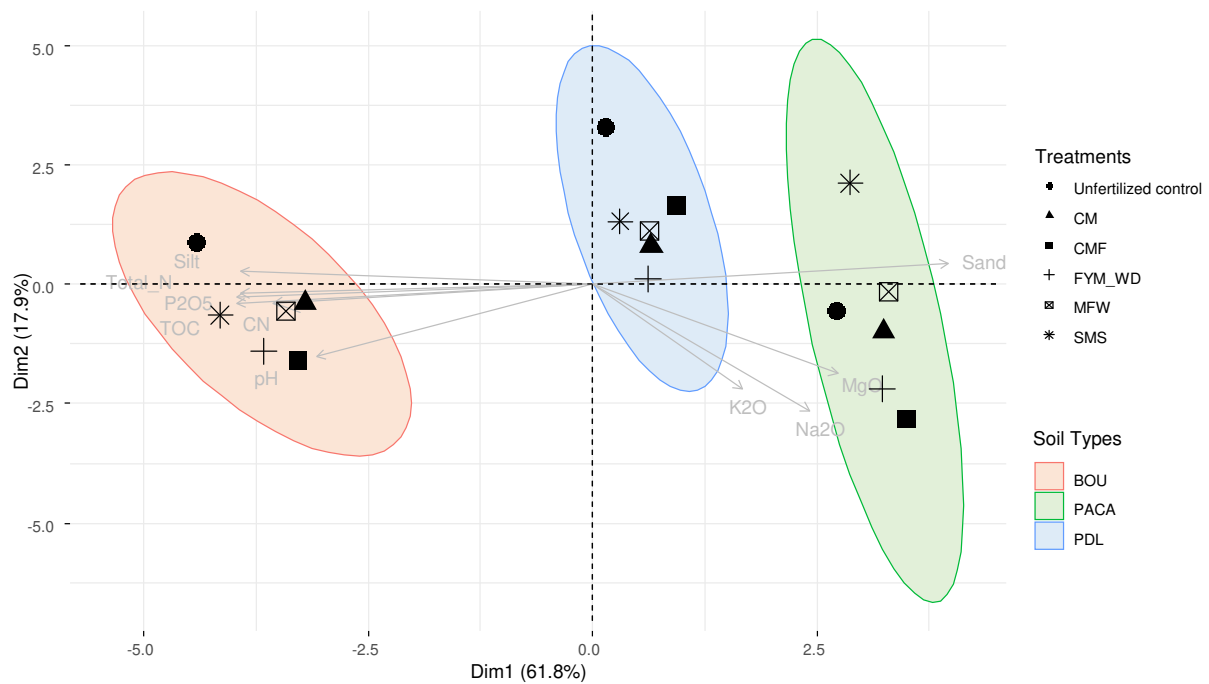


Figure 1. Principal Component analysis (PCA) performed on the soil physicochemical characteristics 42 days after organic materials application (CM; CMF; FYM; MFW; SMS) or sterile water (Unfertilized control) for the three soils (BOU; PACA and PDL).

Abbreviations: BOU, Bourgogne; PACA, Provence-Alpes-Côte d'Azur; PDL, Pays de la Loire; CM, cattle manure; CMF, cattle manure + fat; FYM, farmyard manure; MFW, manure + food waste; SMS, silage maize and sorghum; Total N, total nitrogen; TOC, total organic carbon; C/N, carbon/nitrogen ratio; pH, potential of hydrogen; P₂O₅, phosphorus pentoxide; K₂O, potassium oxide; MgO, magnesium oxide; Na₂O, sodium oxide.

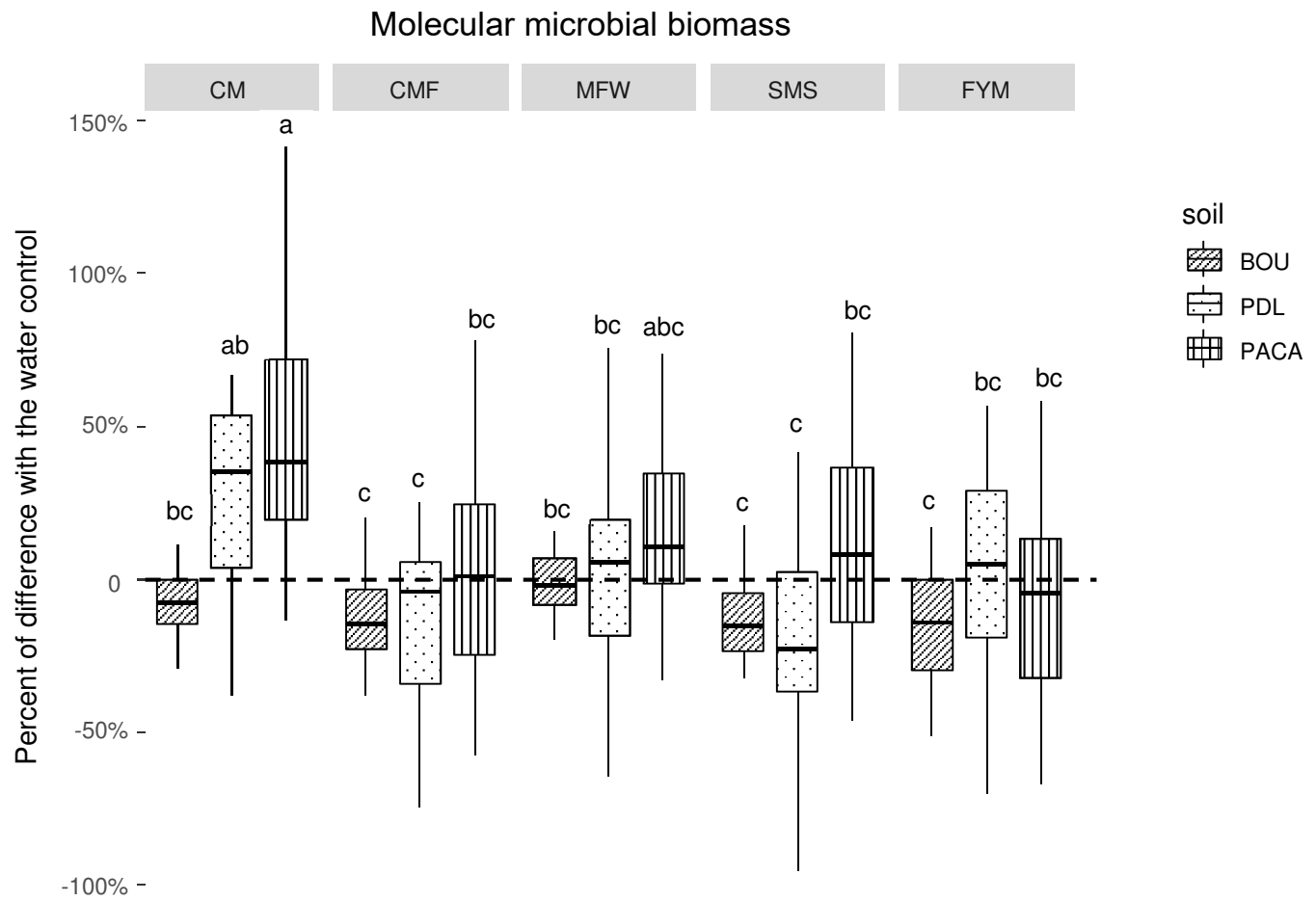


Figure 2. Effect of different organic materials (digestate and cattle manure) on soil microbial biomass from three soils, 42 days after application.

In order to take into account the natural evolution of soil microbial biomass throughout the incubation, results at the end of the experiment (42 days) from un-fertilized control were subtracted from those of the treated samples; results were expressed as percent of differences. Crossed effect soil x digestate, significant at the 0.05 probability level.

Abbreviations: BOU, Bourgogne; PACA, Provence-Alpes-Côte d'Azur; PDL, Pays de la Loire; CM, cattle manure; CMF, cattle manure + fat; FYM, farmyard manure; MFW, manure + food waste; SMS, silage maize and sorghum.

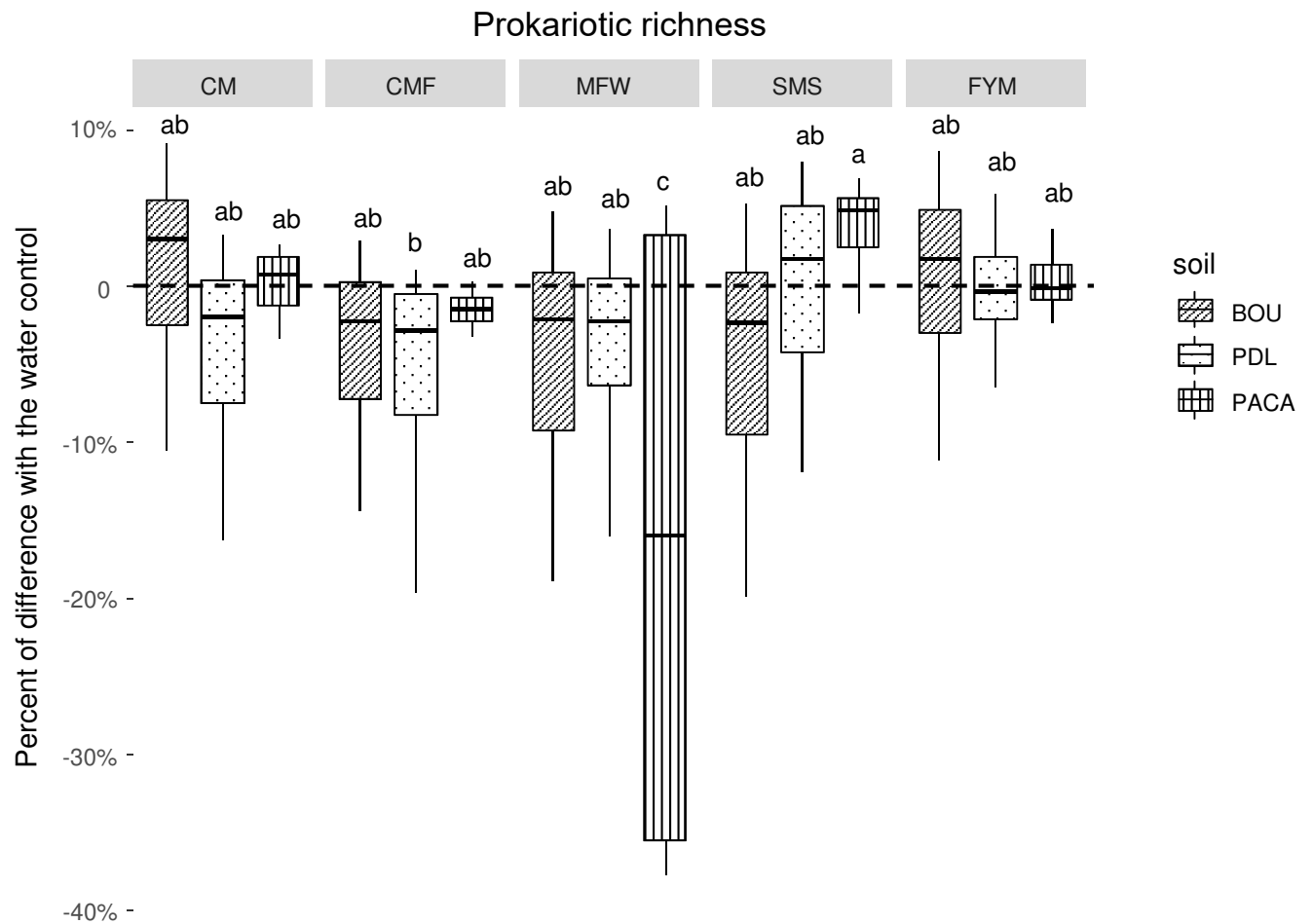


Figure 3. Effect of different organic materials (digestate and cattle manure) on the prokaryotic richness from three soils, 42 days after application.

In order to take into account the natural evolution of soil prokaryotic richness throughout the incubation, results at the end of the experiment (42 days) from un-fertilized control were subtracted from those of the treated samples; results were expressed as percent of differences.

Crossed effect soil x digestate, significant at the 0.05 probability level.

Abbreviations: BOU, Bourgogne; PACA, Provence-Alpes-Côte d'Azur; PDL, Pays de la Loire; CM, cattle manure; CMF, cattle manure + fat; FYM, farmyard manure; MFW, manure + food waste; SMS, silage maize and sorghum.

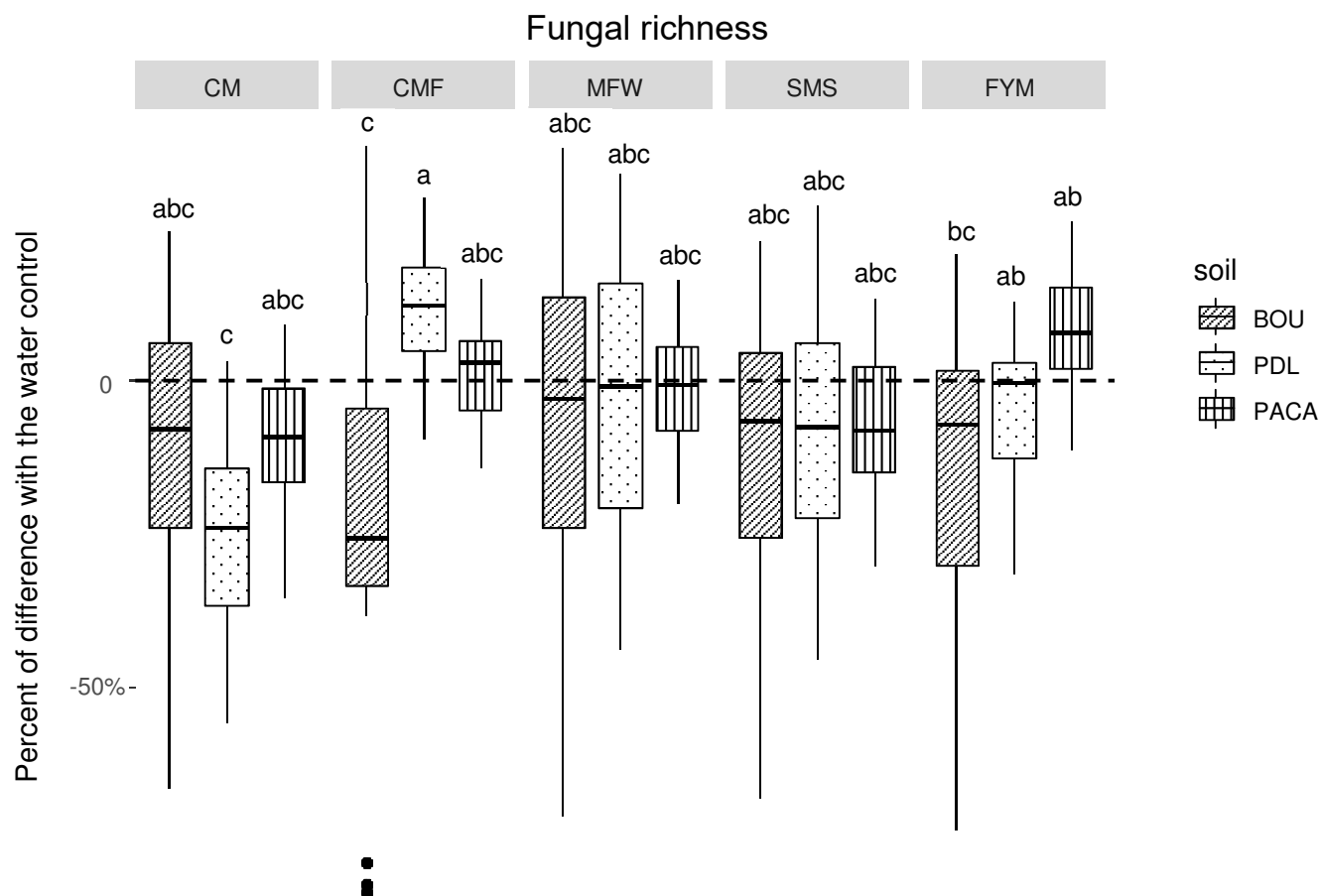


Figure 4. Effect of different organic materials (digestate and cattle manure) on the fungal richness from three soils, 42 days after application.

In order to take into account this natural evolution of soil fungal richness throughout the incubation, results at the end of the experiment (42 days) from un-fertilized control were subtracted from those of the treated samples; results were expressed as percent of differences. Crossed effect soil x digestate, significant at the 0.05 probability level.

Abbreviations: BOU, Bourgogne; PACA, Provence-Alpes-Côte d'Azur; PDL, Pays de la Loire; CM, cattle manure; CMF, cattle manure + fat; FYM, farmyard manure; MFW, manure + food waste; SMS, silage maize and sorghum.

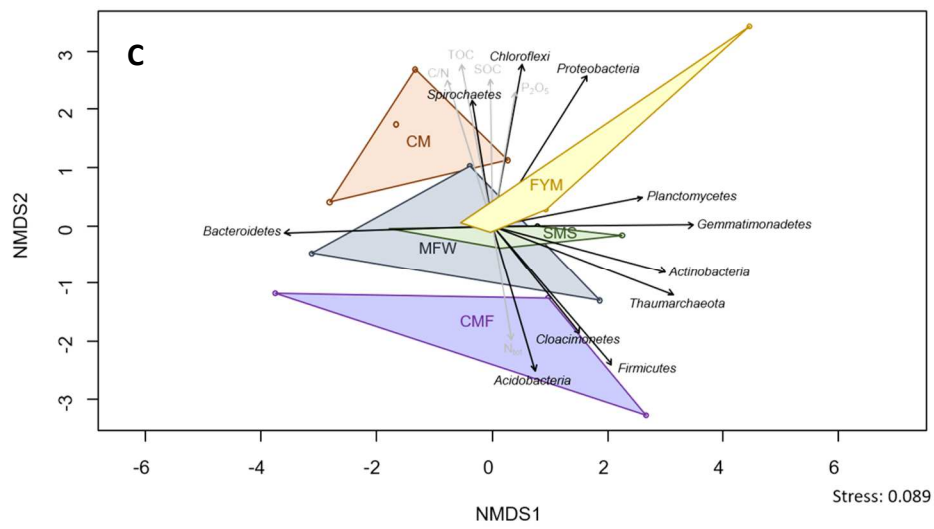
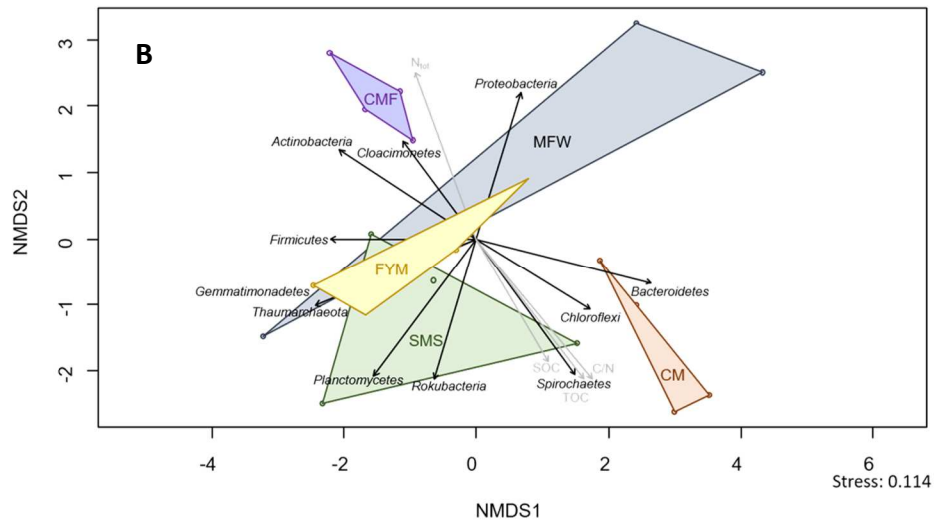
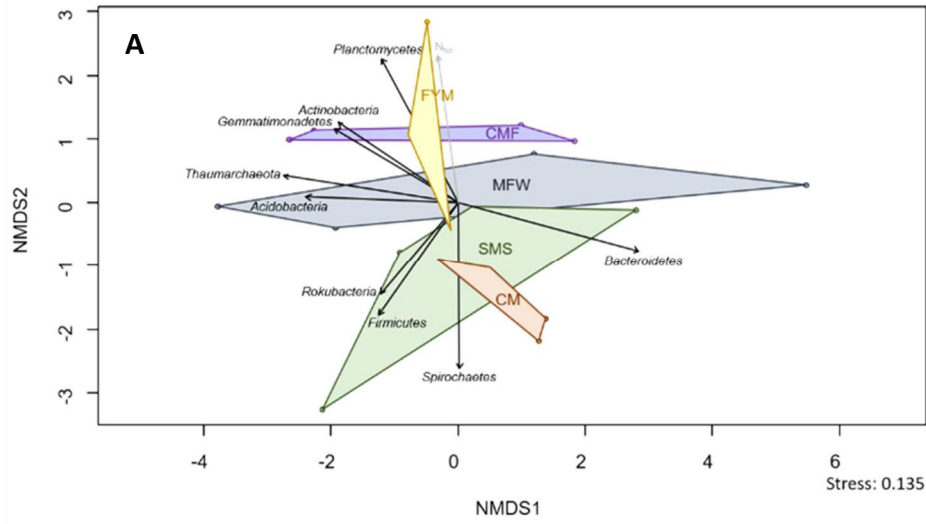


Figure 5. Non-metric multi-dimensional scaling (nMDS) ordination plot derived from the taxonomy table of the prokaryotic phyla (abundance > 1%) from three soils ((**A**) BOU, (**B**) PACA, and (**C**) PDL), 42 days post treatment.

Abbreviations: CM, cattle manure; CMF, cattle manure + fat; FYM, farmyard manure; MFW, manure + food waste; SMS, silage maize and sorghum. The most explanatory phyla and soil chemical parameters are fitted on the plot. TOC, total organic carbon; C/N, carbon/nitrogen ratio; pH, potential of hydrogen.

Colors are not mandatory for this figure in print.

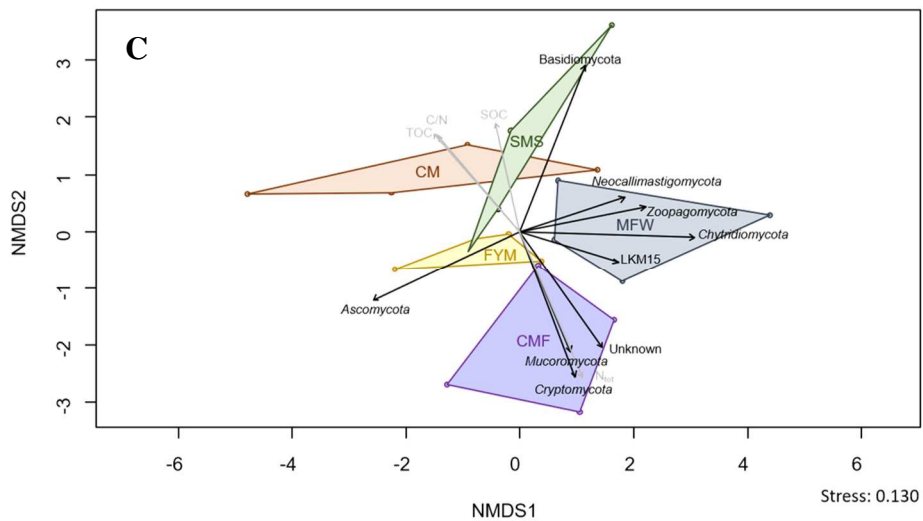
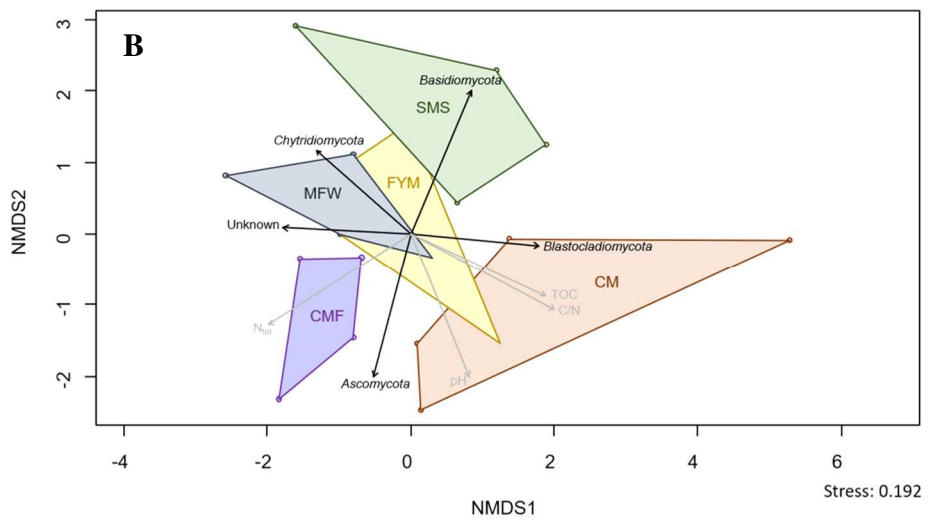
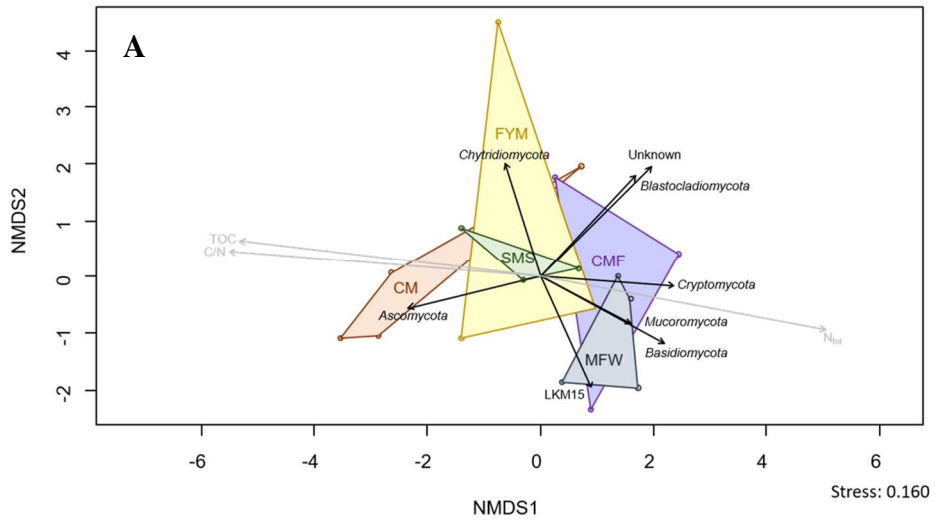


Figure 6. Non-metric multi-dimensional scaling (nMDS) ordination plot derived from the taxonomy table of the fungal *phyla* (abundance > 1%) from three soils ((A) BOU, (B) PACA, and (C) PDL), 42 days post treatment.

Abbreviations: CM, cattle manure; CMF, cattle manure + fat; FYM, farmyard manure; MFW, manure + food waste; SMS, silage maize and sorghum. The most explanatory *phyla* and soil chemical parameters are fitted on the plot. TOC, total organic carbon; C/N, carbon/nitrogen ratio; pH, potential of hydrogen.

Colors are not mandatory for this figure in print.

Table 1. Initial physicochemical characteristics of the three soils.

	BOU	PACA	PDL
Water-holding capacity (%)	33	23	26
Clay (g.kg ⁻¹ DM)	386	102	115
Silt (g.kg ⁻¹ DM)	530	232	392
Sand (g.kg ⁻¹ DM)	80	666	493
pH *	7.96 ± 0.013 a	6.68 ± 0.017 b	6.63 ± 0.005 c
CEC (cmol ⁺ .kg ⁻¹ DM) *	25.44 ± 0.192 a	7.10 ± 0.477 b	6.94 ± 0.527 b
TOC (g.kg ⁻¹ DM) *	19.81 ± 0.360 a	7.68 ± 0.748 c	9.65 ± 0.239 b
Total N (g.kg ⁻¹ DM) *	1.74 ± 0.011 a	0.77 ± 0.043 c	0.99 ± 0.019 b
NH ₄ ⁺ (mg.kg ⁻¹ DM) †	1.01 ± 0.118 a	1.15 ± 0.220 a	1.54 ± 0.564 a
NO ₃ ⁻ (mg.kg ⁻¹ DM) *	5.58 ± 0.193 b	9.53 ± 2.408 a	2.90 ± 0.359 b
C/N ratio *	11.4 ± 0.236 a	10.0 ± 0.503 b	9.7 ± 0.087 b
P ₂ O ₅ (g.kg ⁻¹ DM) *	0.070 ± 0.001 a	0.014 ± 0.002 c	0.040 ± 0.001 b
K ₂ O (g.kg ⁻¹ DM) *	0.17 ± 0.002 b	0.22 ± 0.478 a	0.18 ± 0.008 b
CaO (g.kg ⁻¹ DM) *	6.86 ± 0.032 a	1.41 ± 0.053 c	1.73 ± 0.025 b
MgO (g.kg ⁻¹ DM) *	0.12 ± 0.002 b	0.29 ± 0.018 a	0.09 ± 0.002 c
Na ₂ O (g.kg ⁻¹ DM) *	0.011 ± 0.001 b	0.021 ± 0.002 a	0.010 ± 0.001 b

*Significant at the 0.001 probability level; †Values with the same lower-case letters in a row are not significantly different at P < 0.05.

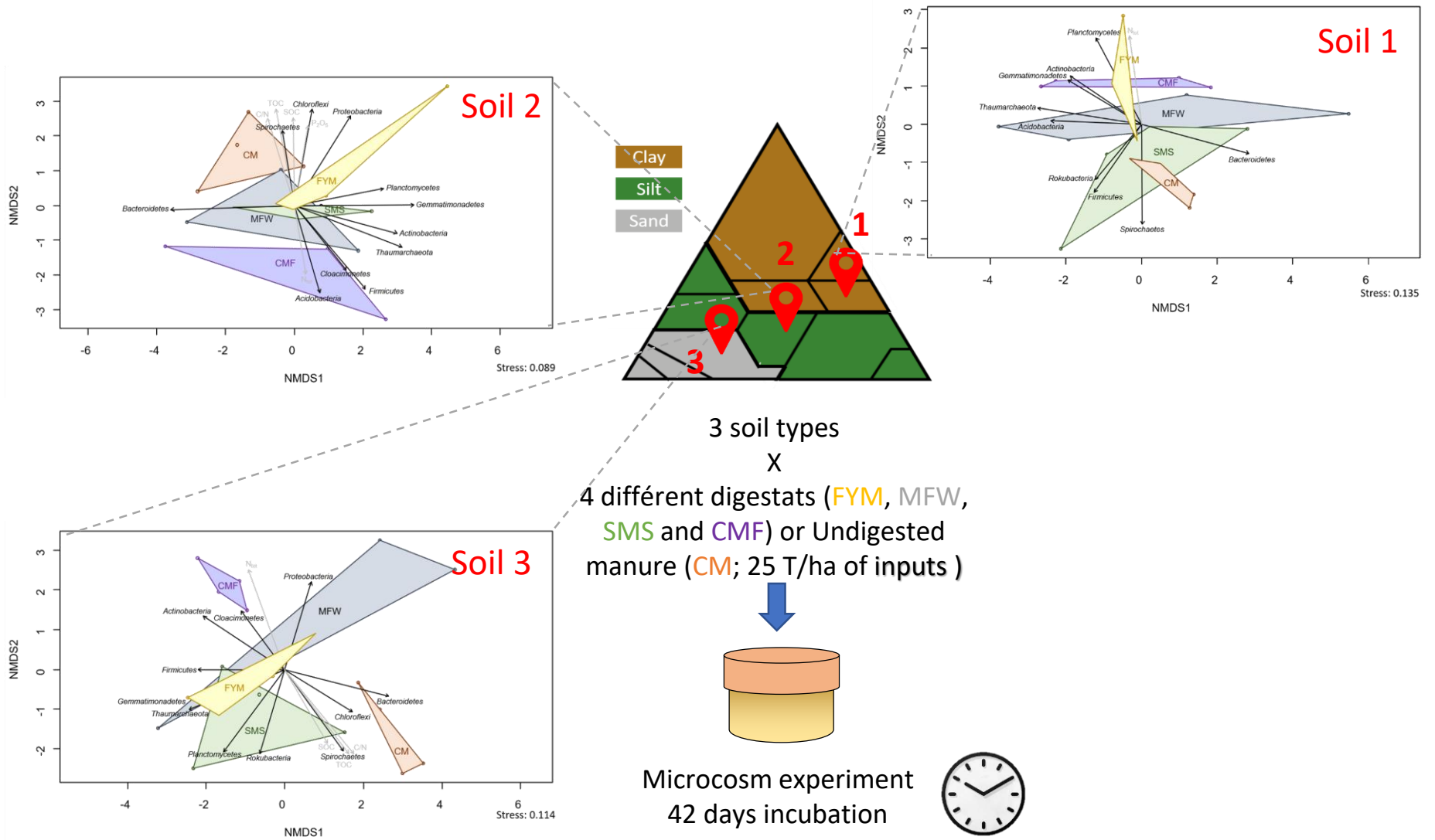
Abbreviations: BOU, Bourgogne; PACA, Provence-Alpes-Côte d'Azur; PDL, Pays de la Loire; DM, dry matter; CEC, cation exchange capacity; TOC, total organic carbon; NH₄⁺, ammonium nitrogen; NO₃⁻, nitric nitrogen; TOC, total organic carbon; C/N, carbon/nitrogen ratio; pH, potential of hydrogen; P₂O₅, phosphorus pentoxide; K₂O, potassium oxide; CaO, calcium oxide; MgO, magnesium oxide, Na₂O, sodium oxide.

Table 2. Elements brought with the digestates and the manure, expressed as the amount loaded in each microcosm.

Organic amendments	Total N	Organic N	NH ₄ ⁺	NO ₃ ⁻	TOC	C/N	pH	P ₂ O ₅	K ₂ O	CaO	MgO	Na ₂ O	SOC	Hemicellulosis	Cellulosis	Lignin cutin
	mg			µg	mg	-	-	mg								
FYM	3.75	2.39	1.33	2.75	26.60	6.30	8.80	1.84	5.59	2.46	0.89	1.02	10.29	4.57	6.54	5.16
MFW	4.23	2.30	1.92	2.70	14.32	2.80	8.30	1.09	4.84	2.11	0.34	0.82	9.62	1.76	1.27	1.65
SMS	2.52	1.70	0.81	2.73	13.30	4.50	8.00	0.82	3.27	1.02	0.34	0.14	8.07	2.25	1.25	1.73
CMF	4.64	1.43	3.11	2.70	3.82	0.80	8.40	0.34	1.43	0.20	0.07	1.09	3.81	0.00	0.00	0.01
CM	2.66	2.26	0.38	2.76	42.97	18.30	8.90	1.16	3.27	1.57	0.68	0.27	9.88	11.51	14.22	7.35

Weights are expressed as the amount loaded in each microcosm.

Abbreviations: NH₄⁺, ammonium nitrogen; NO₃⁻, nitric nitrogen; TOC, total organic carbon; C/N, carbon/nitrogen ratio; pH, potential of hydrogen; P₂O₅, phosphorus pentoxide; K₂O, potassium oxide; CaO, calcium oxide; MgO, magnesium oxide, Na₂O, sodium oxide; SOC, soluble organic carbon; FYM, farmyard manure; MFW, manure + food waste; SMS, silage maize and sorghum; CMF, cattle manure + fat; CM, cattle manure.



Different soils with distinct microbial community structures, responded differently to different digestates application. Response also digestate-dependent