



**HAL**  
open science

## Bacterial community structure in soil microaggregates and on particulate organic matter fractions located outside or inside soil macroaggregates

A. Blaud, T. Chevallier, Tiphaine Chevallier, I. Virto, A.L. Pablo, C. Chenu,  
Alain A. Brauman

### ► To cite this version:

A. Blaud, T. Chevallier, Tiphaine Chevallier, I. Virto, A.L. Pablo, et al.. Bacterial community structure in soil microaggregates and on particulate organic matter fractions located outside or inside soil macroaggregates. *Pedobiologia*, 2014, 57 (3), pp.191-194. 10.1016/j.pedobi.2014.03.005 . hal-01557711

**HAL Id: hal-01557711**

**<https://hal.science/hal-01557711>**

Submitted on 3 Jan 2024

**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.

1 **Bacterial community structure in soil microaggregates and on**  
2 **particulate organic matter fractions located outside or inside soil**  
3 **macroaggregates**

4

5 Aimeric Blaud<sup>1†\*</sup>, Tiphaine Chevallier<sup>1</sup>, Iñigo Virto<sup>2,3</sup>, Anne-Laure Pablo<sup>1</sup>, Claire

6 Chenu<sup>2</sup>, Alain Brauman<sup>1</sup>

7 <sup>1</sup> IRD, UMR 210 Eco&Sols, Place Viala (Bt. 12), 34060 Montpellier Cedex 01, France

8 <sup>2</sup> AgroParisTech, UMR Bioemco (UMR 7618), bâtiment EGER, 78850 Thiverval

9 Grignon

10 <sup>3</sup> Universidad Pública de Navarra-Escuela Técnica Superior de Ingenieros Agrónomos-

11 Campus Arrosadia s/n 31006 Pamplona, Spain

12 \* Corresponding author: [a.blaud@sheffield.ac.uk](mailto:a.blaud@sheffield.ac.uk); Tel: +44(0)114 2225785

13 † Present address: Department of Civil and Structural Engineering, Kroto Research

14 Institute, University of Sheffield, Sheffield S3 7HQ, UK

15

16 **Abstract**

17 Soil aggregates and particulate organic matter (POM) are thought to represent distinct soil

18 microhabitats for microbial communities. This study investigated whether organo-mineral

19 (0-20, 20-50 and 50-200 µm) and POM (two sizes: > 200 and < 200 µm) soil fractions

20 represent distinct microbial habitats. Microbial habitats were characterised by the amount

21 and quality of organic matter, the genetic structure of the bacterial community, and their

22 location outside or inside macroaggregates (> 200 µm). The denaturing gradient gel

23 electrophoresis (DGGE) profiles revealed that bacterial communities structure of organo-

24 mineral soil fractions were significantly different in comparison to the unfractionated soil.  
25 Conversely, there were little differences in C concentrations, C:N ratios and no differences  
26 in DGGE profiles between organo-mineral fractions. Bacterial communities between soil  
27 fractions located inside or outside macroaggregates were not significantly different.  
28 However, the bacterial communities on POM fractions were significantly different in  
29 comparison to organo-mineral soil fractions and unfractionated soil, and also between the  
30 2 sizes of POM. Thus in the studied soil, only POM fractions represented distinct  
31 microhabitats for bacterial community, which likely vary with the state of decomposition  
32 of the POM.

33

34 *Keywords:* microhabitats; coarse POM; fine POM; organo-mineral soil fraction; DGGE

35

36 Soil can be considered a benchmark heterogeneous environment for microbial  
37 ecologists, as it is typically a complex environment comprised of a huge diversity of  
38 microhabitats. A number of studies examining this complexity have defined soil aggregates  
39 as specific soil compartments (Mummey et al., 2006; Bland et al., 2012; Davinic et al.,  
40 2012). Several studies have shown that the different sizes of soil aggregates and locations  
41 within soil aggregates can select for different bacterial communities (Ranjard et al., 2000;  
42 Chotte et al., 2002; Fall et al., 2004; Mummey et al., 2006; Bland et al., 2012; Davinic et  
43 al., 2012). Soil aggregates are formed by mineral associations with particulate organic  
44 matter (POM) via binding agents (e.g. fungal hyphae, plant roots, polysaccharides) (Six et  
45 al., 2000, 2004). Microaggregates (size < 200  $\mu\text{m}$ ) are formed within macroaggregates (size  
46 > 200  $\mu\text{m}$ ) and can be released from fragmented macroaggregates. Therefore, organic

47 resources differ quantitatively and qualitatively between sizes and locations of aggregates  
48 (Six et al., 2000). Moreover, POM has been shown to influence microbial community  
49 structure within the soil surrounding it, called the “detritosphere” (Gaillard et al., 1999;  
50 Nicolardot et al., 2007). A study by Blackwood and Paul (Blackwood and Paul, 2003)  
51 showed that rhizosphere and shoot residues are distinct bacterial habitats compared to other  
52 soil fractions including mineral particles and humified organic matter. However, there is  
53 still an intense debate about the potential role of soil aggregates in structuring microbial  
54 communities, and within these microhabitats little is known about the impact of POM  
55 quality and localisation on microbial community. Therefore, the aims of this study were to  
56 i) to determine whether organo-mineral (0-20  $\mu\text{m}$ , 20-50  $\mu\text{m}$ , 50-200  $\mu\text{m}$ ) and POM (coarse  
57 POM: > 200  $\mu\text{m}$  and fine POM < 200  $\mu\text{m}$ ) soil fractions can represent distinct microbial  
58 habitats, and ii) to determine whether microaggregates and POM location, outside or inside  
59 macroaggregates (> 200  $\mu\text{m}$ ), can influence the bacterial community structure of these  
60 microhabitats. Henceforth, the term “organo-mineral soil fraction” is preferred to “soil  
61 aggregates” because this study did not separate soil aggregates from mineral particles.

62 A clayey Eutric Cambisol was sampled at the INRA-Epouisse experimental farm in  
63 Burgundy (France). The experimental field plots have been cultivated and tilled for 10  
64 years with a rotation of wheat, rape, and barley. The soil texture was comprised of 11.2 %  
65 of sand, 41.8 % of silt and 47.0 % of clay. The organic C concentration was 26.8  $\text{g kg}^{-1}$ ,  
66 C:N ratio 12.4, pH (water) 7.8,  $\text{CaCO}_3$  3.2  $\text{g kg}^{-1}$  and CEC 25.1  $\text{C mol kg}^{-1}$ . Three soil cores  
67 (diameter, 7 cm) were randomly collected down to a depth of 30 cm, which represented the  
68 tilled layer of the soil (tilled annually), where the soil aggregates and POM are  
69 homogenised and fragmented. These soil samples were pooled to reduce any spatial

70 variability, fragmented by hand and were passed through a 10 mm sieve. Finally, soil was  
71 stored at 4 °C without drying until wet physical fractionation. All analyses were performed  
72 in triplicate.

73 The methods used for soil fractionation were adapted from Yoder (Yoder, 1936) for  
74 the isolation of soil fractions located outside macroaggregates, and from Virto et al. (Virto  
75 et al., 2008) for the isolation of soil fractions located inside macroaggregates. Soil samples  
76 (10 g) were placed on top of a 200 µm sieve inside a tank filled with approximately 2 l of  
77 milli-Q cold water (4 °C), and were immersed into the water for 5 min before sieving. Wet  
78 sieving was an up and down movement over a total distance of 32 mm with a frequency of  
79 30 cycles min<sup>-1</sup> for 10 min. After wet-sieving, materials retained on the 200 µm-sieve, *i.e.*  
80 water-stable macroaggregates (hereafter, macroaggregates), sand and POM were collected.  
81 The POM fraction was isolated by flotation in water and referred to as coarse POM (cPOM:  
82 > 200 µm). Coarse sands were removed by forceps from macroaggregates; the  
83 macroaggregates were then kept for a second soil fractionation to isolate the soil fractions  
84 held inside macroaggregates (see below). The remaining suspension (< 200 µm) was sieved  
85 at 50 µm and 20 µm to obtain the 50-200 µm and 20-50 µm soil fractions, respectively.  
86 Fine POM (fPOM: 50-200 µm) were isolated by flotation in water from the 50-200 µm soil  
87 fraction. The remaining suspension was centrifuged to obtain 0-20 µm fractions (2000 rpm  
88 for 10 min, 4 °C). These were the fractions located outside macroaggregates. To isolate the  
89 soil fractions held inside macroaggregates, water-stable macroaggregates were not dried  
90 after their isolation, but were directly immersed in 200 ml milli-Q water above a 200 µm  
91 mesh screen with fifty 6 mm glass beads (Virto et al., 2008). The macroaggregates and the  
92 beads were then agitated in an end-over-end shaker for 20 min at 45 rotations min<sup>-1</sup>.

93 Regular water flow through the 200  $\mu\text{m}$  mesh screen ensured that the microaggregates (<  
94 200  $\mu\text{m}$ ) passed through the mesh screen immediately after being released from  
95 macroaggregates, without further disruption by the beads (Six et al., 2000; Virto et al.,  
96 2008). After all the macroaggregates had been broken up (20 min, determined after  
97 preliminary experiments), the water and soil were sieved as described above. The resultant  
98 organo-mineral and POM soil fractions were named: **i**50-200  $\mu\text{m}$ , **i**20-50  $\mu\text{m}$ , **i**0-20  $\mu\text{m}$ ,  
99 **ic**POM and **if**POM, where **i** indicate soil fractions from inside macroaggregates. The  
100 isolated fractions (organo-mineral and POM soil fractions) and unfractionated soil were  
101 either stored at -20 °C for microbial community structure analysis or oven-dried at 40 °C  
102 and ground (< 200  $\mu\text{m}$ ) for C and N analyses with a CHN analyser (NA 2000 N-  
103 PROTEINE) (see Supplementary material).

104 Nucleic acids were extracted from 0.5 g (wet weight) of unfractionated soil and  
105 each fraction described above. Bacterial 16S rRNA genes were amplified with the bacterial  
106 primers 338f-GC and 518r and the amplicons were resolved by denaturing gradient gel  
107 electrophoresis (DGGE). The full details of the DNA extraction, PCR amplification and  
108 DGGE analysis are provided in the Supplementary material. To analyse the matrix obtained  
109 from DGGE band profiles, the total band intensity was normalised for each sample (i.e.  
110 each band intensity was divided by the total band intensity of each sample). The relative  
111 abundance data from the DGGE matrix was then square root transformed and a similarity  
112 matrix from DGGE profiles was generated using the Bray-Curtis method. A dendrogram  
113 was produced from the similarity matrix using the group average linking method  
114 implemented in the software PRIMER v6 (PRIMER-E Ltd, Plymouth, UK). To test for  
115 significant differences between bacterial communities of the different soil fractions, and to

116 correlate variation in bacterial communities to OC concentration and C:N ratio, ANOSIM  
117 and RELATE tests from PRIMER v6 software were performed, respectively (see  
118 Supplementary materials).

119 Macroaggregates ( $> 200 \mu\text{m}$ ) and fractions  $< 50 \mu\text{m}$  constituted 75% and 20% of  
120 the soil, respectively (Table S1). Macroaggregates were mainly composed of 0-20  $\mu\text{m}$   
121 (55%) and 20-50  $\mu\text{m}$  (28%) soil fractions. All POM fractions represented about 1% of the  
122 soil. The proportions of the soil fractions  $< 200 \mu\text{m}$  and fine POM were significantly higher  
123 inside macroaggregates than outside macroaggregates ( $P < 0.05$ , Table S1). The bacterial  
124 community structure, assessed by a fingerprinting technique (DGGE), was strongly  
125 correlated with OC concentrations ( $\rho = 0.73$ ,  $P = 0.001$ ), but only weakly correlated with  
126 C:N ratios ( $\rho = 0.32$ ,  $P = 0.002$ ). The bacterial community structure of POM fractions were  
127 strongly correlated to C:N ratios ( $\rho = 0.55$ ,  $P = 0.004$ ) but not to OC concentrations ( $\rho =$   
128  $0.20$ ,  $P = 0.13$ ). The cluster analysis of the microbial structure revealed that POM  
129 communities formed separate clusters (cluster I, V and VI) from unfractionated soil and  
130 organo-mineral soil communities (cluster II, III, IV), which was confirmed by significant  
131  $P$  values and high R values of the ANOSIM (Fig. 1; Table S2). Moreover, coarse and fine  
132 POM communities were also significantly different from each other. All of the organo-  
133 mineral fractions (cluster III and IV) were significantly different from the unfractionated  
134 soil ( $P \leq 0.003$ ), which all grouped together (cluster II, Fig. 1, Table S2). These results  
135 confirmed that fractioning soil can reveal specific soil bacterial communities which are  
136 hidden in unfractionated soil (Ranjard et al., 2000; Chotte et al., 2002; Bland et al., 2012;  
137 Davinic et al., 2012). However, none of the communities associated with organo-mineral  
138 soil fractions were significantly different from each other ( $P > 0.05$ , Table S2). Finally, the

139 dendrogram and ANOSIM analyses showed that organo-mineral soil fractions from inside  
140 and outside macroaggregates were not significantly different ( $P = 0.32$ , Fig.1).

141 POM fractions (coarse and fine POM) clearly differed in the structure of their  
142 bacterial communities compared to the other soil fractions and unfractionated soil, which  
143 was mainly explained by the higher OC concentration. The specific bacterial communities  
144 on POM fractions, which accounted only for 0.3% of the soil mass (Table S1), are located  
145 on specific microhabitats which could be considered “hot spots”, where biological  
146 activities are potentially extremely high relative to the surrounding matrix. Several studies  
147 have demonstrated that plant residues represent hot spots, where readily available carbon  
148 and energy resources are present. These resources influence the biomass, the activity, and  
149 the genetic structure of the soil microbial communities close to the plant residues (Gaillard  
150 et al., 1999; McMahon et al., 2005; Nicolardot et al., 2007). However, hot spots are still  
151 too few to influence the whole soil microbial communities. Only by separating POM  
152 fractions from organo-mineral soil fractions allows access to this hidden bacterial  
153 community, as has already been shown for other soil microhabitats (Chotte et al., 2002;  
154 Mummey et al., 2006). Moreover, the different sizes of POM isolated in this current study  
155 harboured different bacterial communities structure. The differences in C:N ratio (which  
156 can be used as a proxy for the state of decomposition of POM) between cPOM and fPOM  
157 (~1.5 times higher in cPOM than fPOM), and the different location of coarse and fine POM,  
158 were likely to directly influence the bacterial communities. Thus, coarse and fine POM  
159 represented distinct microhabitats for the bacterial community in soil and are likely to  
160 represent two different hotspots of bacterial activity.

161 High levels of community structure similarities were found between organo-



162 mineral fractions (Fig. 1, Table S2). This result is not surprising, considering that bacterial  
163 community structure was strongly correlated with chemical environment, such as OC  
164 concentration and C:N ratio, which were relatively similar among organo-mineral soil  
165 fractions in the soil studied (Table S3). However, despite the higher OC concentrations and  
166 C:N ratios, the 50-200  $\mu\text{m}$  fractions did not have a different bacterial community structure  
167 from other organo-mineral soil fractions (Fig. 1, Table S2). The differences in OC  
168 resources were possibly not high enough to differentiate the bacterial community between  
169 fractions. In addition, as the OC quantity were likely distributed among soil fractions by  
170 fast macroaggregates turnover due to soil tillage (Six et al., 2000), the differentiation of  
171 specific microbial communities in such fractions could also be hindered. In a same way, as  
172 the organic concentration and quality was not different between soil fractions located inside  
173 or outside macroaggregates, bacterial community structure was not affected by location  
174 inside or outside macroaggregates. The potential fast turnover of macroaggregates due to  
175 soil tillage might also increase the turnover of microaggregates and finer fractions from  
176 inside to outside macroaggregates (Six et al., 2000), reducing any potential differences  
177 between microhabitats and subsequently the bacterial community of these soil fractions.  
178 However, the lack of differences in bacterial community structure between organo-mineral  
179 soil fractions could be also due to the low resolution of DGGE, which target only the most  
180 dominant bacterial taxa; it may be that higher resolution techniques (such as next  
181 generation sequencing) would be required to identify significant differences (Davinic et  
182 al., 2012). Nevertheless, DGGE indicated that the most dominant bacterial taxa did not  
183 differ between organo-mineral fractions located outside or inside macroaggregates.

184 This study clearly has shown that POM represent distinct bacterial microhabitats in

185 soil, and that the state of decomposition of this microhabitats (i.e. coarse POM vs. fine  
186 POM) might select bacterial community, highlighting the fact that soil microhabitats are  
187 dynamic within the soil, which directly influence bacterial community.

188

### 189 **Acknowledgement**

190 We are grateful for the assistance and access to the soil samples provided by Fabrice Martin  
191 (INRA, Epoisse). We would also like to thank Joele Toucet for her help in laboratory work,  
192 Dr. Eric Blanchart for his useful advice on the article, and Dr Susan Johnston for proof-  
193 reading the article.

194

### 195 **References**

196 Blackwood, C.B., Paul, E.A., 2003. Eubacterial community structure and population size  
197 within the soil light fraction, rhizosphere, and heavy fraction of several agricultural  
198 systems. *Soil Biol. Biochem.* 35, 1245–1255.

199 Blaud, A., Lerch, T.Z., Chevallier, T., Nunan, N., Chenu, C., Brauman, A., 2012. Dynamics  
200 of bacterial communities in relation to soil aggregate formation during the  
201 decomposition of <sup>13</sup>C-labelled rice straw. *Appl. Soil Ecol.* 53, 1–9.

202 Chotte, J.L., Schwartzmann, A., Bally, R., Jocteur Monrozier, L., 2002. Changes in  
203 bacterial communities and *Azospirillum* diversity in soil fractions of a tropical soil  
204 under 3 or 19 years of natural fallow. *Soil Biol. Biochem.* 34, 1083–1092.

205 Davinic, M., Fultz, L.M., Acosta-Martinez, V., Calderón, F.J., Cox, S.B., Dowd, S.E.,  
206 Allen, V.G., Zak, J.C., Moore-Kucera, J., 2012. Pyrosequencing and mid-infrared  
207 spectroscopy reveal distinct aggregate stratification of soil bacterial communities

208 and organic matter composition. *Soil Biol. Biochem.* 46, 63–72.

209 Fall, S., Nazaret, S., Chotte, J.L., Brauman, A., 2004. Bacterial density and community  
210 structure associated with aggregate size fractions of soil-feeding termite mounds.  
211 *Microbial Ecol.* 48, 191–199.

212 Gaillard, V., Chenu, C., Recous, S., Richard, G., 1999. Carbon, nitrogen and microbial  
213 gradients induced by plant residues decomposing in soil. *Eur. J. Soil Sci.* 50, 567–  
214 578.

215 McMahon, S.K., Williams, M.A., Bottomley, P.J., Myrold, D.D., 2005. Dynamics of  
216 microbial communities during decomposition of carbon-13 labeled ryegrass  
217 fractions in soil. *Soil Sci. Soc. Am. J.* 69, 1238–1247.

218 Mummey, D., Holben, W., Six, J., Stahl, P., 2006. Spatial stratification of soil bacterial  
219 populations in aggregates of diverse soils. *Microbial Ecol.* 51, 404–411.

220 Nicolardot, B., Bouziri, L., Bastian, F., Ranjard, L., 2007. A microcosm experiment to  
221 evaluate the influence of location and quality of plant residues on residue  
222 decomposition and genetic structure of soil microbial communities. *Soil Biol.*  
223 *Biochem.* 39, 1631–1644.

224 Ranjard, L., Poly, F., Combrisson, J., Richaume, A., Gourbière, F., Thioulouse, J., Nazaret,  
225 S., 2000. Heterogeneous cell density and genetic structure of bacterial pools  
226 associated with various Soil microenvironments as determined by enumeration and  
227 DNA fingerprinting approach (RISA). *Microbial Ecol.* 39, 263–272.

228 Six, J., Bossuyt, H., Degryze, S., Denef, K., 2004. A history of research on the link between  
229 (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Till. Res.* 79,  
230 7–31.

231 Six, J., Elliott, E.T., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate  
232 formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biol.*  
233 *Biochem.* 32, 2099–2103.

234 Virto, I., Barré, P., Chenu, C., 2008. Microaggregation and organic matter storage at the  
235 silt-size scale. *Geoderma* 146, 326–335.

236 Yoder, R.E., 1936. A direct method of aggregate analysis of soils and a study of the  
237 physical nature of erosion losses. *J. Am. Soc. Agron.* 28, 337–351.

238

239

240

241

242

243

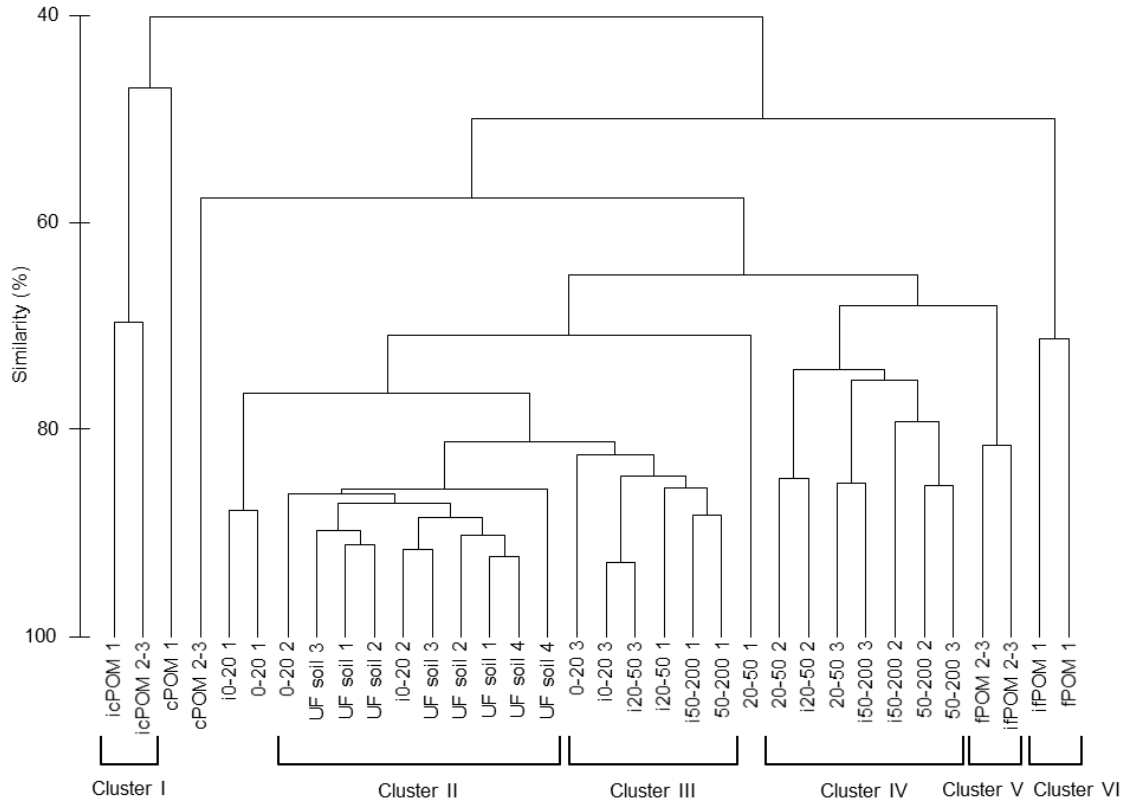
244

245

246

247

248



249

250 **Figure 1.** Dendrogram of DGGE profiles of bacterial 16S rRNA genes amplified from  
 251 unfractionated soil (UF soil), organo-mineral (50-200, 20-50 and 0-20  $\mu\text{m}$ ) and, coarse (>  
 252 200  $\mu\text{m}$ ) and fine (< 200  $\mu\text{m}$ ) particulate organic matter (POM) soil fractions located  
 253 outside and inside macroaggregates. The fractions located inside macroaggregates were  
 254 prefixed by an **i**, as **inside**. The different replicates are indicated by 1, 2 or 3. 2-3: replicates  
 255 2 and 3 were pooled for these soil fractions. cPOM: coarse POM > 200  $\mu\text{m}$ . fPOM: fine  
 256 POM < 200  $\mu\text{m}$ .

257

258

259

260

261

## Supplementary material and methods

262

263

### 264 **C and N analysis**

265           Unfractionated-soil and organo-mineral soil fraction samples were decarbonated  
266 prior to C and N determination. Ten ml of water was added to 1 g of soil or soil fraction  
267 and 0.5 M HCl was then dripped onto the sample until there was no more effervescence.  
268 Samples were then centrifuged for 5 min (270 x g), washed in water and centrifuged again  
269 until soil pH reached 7. All samples were then oven-dried for CHN analyses using a CHN  
270 analyser (NA 2000 N-PROTEINE) (Pansu and Gautheyrou, 2006).

271

### 272 **DNA extraction**

273

274 DNA was extracted from 0.5 g (wet weight) of unfractionated soil and each soil fraction  
275 studied. For the coarse and fine particulate organic matter (POM) samples, 2 replicates for  
276 each size of POM were pooled, as only a very small amount of POM was recovered in  
277 some individual replicates (< 0.3 g). In each fraction, the replicates with the smallest  
278 amount of POM were pooled, so that, for each POM fraction, DNA extractions were made  
279 of two samples: one composite POM sample and one single fractionation replicate. DNA  
280 extraction followed the protocol described by Griffiths et al. (2000). Briefly, 0.5 g glass  
281 beads (0.1 mm in diameter), 1 ml lysis buffer, 0.5 ml of hexadecyltrimethylammonium  
282 bromide (CTAB), and 0.5 ml phenol chloroform isoamyl alcohol (25:24:1) (pH 8.0) were  
283 added to each organo-mineral or POM soil fraction. The bacterial cells in organo-mineral  
284 and POM soil fractions were then lysed by bead beating twice for 30 s (Retsch MM200).

285 The aqueous phase containing nucleic acids was separated by centrifugation ( $16,000 \times g$ )  
286 at  $4\text{ }^{\circ}\text{C}$  for 5 min. The aqueous phase was then extracted, and phenol was removed by  
287 mixing with an equal volume of chloroform-isoamyl alcohol (24:1) followed by repeated  
288 centrifugation ( $16,000 \times g$ ) at  $4\text{ }^{\circ}\text{C}$  for 5 min. Total nucleic acids were subsequently  
289 precipitated from the extracted aqueous layer with 2 volumes of 30% (w/vol) polyethelene  
290 glycol 6000 (Fluka BioChemika) and 1.6 M NaCl for 2 hours at room temperature,  
291 followed by centrifugation ( $18,000 \times g$ ) at  $4\text{ }^{\circ}\text{C}$  for 10 min. The nucleic acid pellets were  
292 finally washed in ice cold 70% (vol/vol) ethanol and air dried prior to resuspension in 20  
293  $\mu\text{l}$  of sterile milli-Q water.

294

#### 295 **PCR amplifications and Denaturing Gradient Gel Electrophoresis (DGGE) analyses**

296 The bacterial 16S rRNA gene sequences were amplified with the bacterial primers  
297 338f-GC (Olsson et al., 1996) and 518r (Muyzer et al., 1993). PCR amplifications were  
298 performed in 25  $\mu\text{l}$  mixtures using puReTaq<sup>TM</sup> Ready-To-Go<sup>TM</sup> PCR beads (Amersham-  
299 Biosciences, Orsay, France) with 5 ng of template DNA and 1.25  $\mu\text{M}$  of each primer, using  
300 a GeneAmp PCR System 9700 (Applied Biosystems, Courtabœuf, France). Bacterial 16S  
301 rRNA genes were amplified using an initial denaturation at  $94\text{ }^{\circ}\text{C}$  for 2 min, followed by  
302 20 cycles of  $94\text{ }^{\circ}\text{C}$  for 30 s (denaturing),  $65\text{ }^{\circ}\text{C}$  for 30 s (annealing), and  $72\text{ }^{\circ}\text{C}$  for 60 s  
303 (extension), with a  $0.5\text{ }^{\circ}\text{C}$  touchdown every cycle during the annealing stage; this was,  
304 followed by an additional 10 cycles with an annealing temperature of  $55\text{ }^{\circ}\text{C}$ , before a final  
305 extension at  $72\text{ }^{\circ}\text{C}$  for 10 min.

306 The amplicons were resolved by DGGE using 8% acrylamide gels (acrylamide-  
307 bisacrylamide 40%, 37.5:1) (Sigma-Aldrich, St. Quentin Fallavier, France) and a gradient

308 of 45-70% denaturant (Muyzer et al., 1993) in 1x TAE buffer with the Ingeny phorU system  
309 (Ingeny International, Goes, The Netherlands) at 60 °C and 50 mA-100 V for 17 h. Because  
310 of the number of samples, 2 DGGE gels were required for the analysis (Fig. S1). In order  
311 to compare gels and normalise gels differences, the same marker was used on both gels and  
312 PCR products of the unfractionated soil samples from four DNA extractions were used on  
313 both gels (Fig. S1). Hence, eight PCR products were obtained during the same PCR run  
314 from the unfractionated soil samples (i.e. 2 PCR per sample). Gels were stained with  
315 Ethidium Bromide and images were captured using Bio-capt software (Ets Vilbert  
316 Lourmat, France). The 16S rRNA gene DGGE band patterns were analysed using Totallab  
317 TLV120 software (Nonlinear dynamics, Newcastle, UK) to obtain matrices of band  
318 profiles with the intensity of each band.

319

## 320 **Statistical analyses**

321 The statistical analyses of the distribution of organo-mineral and POM soil  
322 fractions, C concentrations, and C:N ratios were performed using mean comparisons by  
323 paired student t-tests. The normality and homoscedasticity of data were checked prior to  
324 statistical analysis.

325 To test any significant differences between the bacterial communities of the  
326 different soil fractions or between communities from outside vs. inside macroaggregates,  
327 one-way ANOSIM (analysis of similarity: all possible permutations were done) were  
328 performed on the DGGE similarities matrix obtained using the Bray-Curtis method.  
329 ANOSIM give the significance levels, i.e. *P* value, and *R* value, i.e. the strength of the  
330 factors on samples. *R* values close to 1 indicate a high separation between groups (e.g.



331 between soil fractions), while R values close to 0 indicate a low group separation.

332 Correlations between the bacterial community structure and the OC concentration  
333 and C:N ratio were performed using the permutation-based test (rank correlation method:  
334 Spearman, 999 permutations) RELATE from the PRIMER software. The similarity matrix  
335 of the DGGE profiles obtained using Bray-Curtis method was correlated to the similarity  
336 matrices of OC concentration or C:N ratio obtained by Euclidean distance (Clarke and  
337 Ainsworth, 1993). Significance levels, i.e. *P* values, and correlation strengths, i.e.  
338 Spearman coefficient  $\rho$  were obtained. The  $\rho$  values close to 1 indicate a strong correlation,  
339 while  $\rho$  values close to 0 indicate a weak correlation.

340

## 341 **Supplementary results**

342

### 343 **Soil and POM fractions size distribution**

344 The soil fractionation procedure resulted in very small losses of material, as the  
345 mean mass recovery was about 96% of the original unfractionated soil and about 94% of  
346 macroaggregates (Table S1).

347

### 348 **Organic carbon (OC) concentrations and C:N ratios of the organo-mineral and POM** 349 **soil fractions**

350 The soil fractionation procedure resulted in slight losses of OC, as the mean carbon  
351 concentration recovery was 87% of the original unfractionated soil and 86% of the  
352 macroaggregates (Table S3). POM fractions had higher OC concentration and C:N ratio  
353 than other soil fractions. The OC concentrations and C:N ratios decreased with the POM

354 size (Table S3). All the organo-mineral soil fractions had close OC concentrations and C:N  
355 ratios with the exception of the 50-200  $\mu\text{m}$  fractions (Table S3). Compared to other soil  
356 fractions, 50-200  $\mu\text{m}$  fractions had significantly higher OC concentration (nearly two fold;  
357  $P < 0.05$ ) and higher C:N ratio. However, because of the weight of the 50-200  $\mu\text{m}$  soil  
358 fractions (Table S2), the main soil OC reservoir was in the 0-20  $\mu\text{m}$  fractions; *i.e.* 43% of  
359 the total OC was located in 0-20  $\mu\text{m}$  fractions, 12% outside and 31% inside  
360 macroaggregates.

361 The OC concentration of the 20-50  $\mu\text{m}$  soil fraction located inside macroaggregates  
362 significantly decreased by  $\sim 21\%$  in comparison to outside ( $P < 0.05$ ), while OC  
363 concentration of the 0-20  $\mu\text{m}$  fraction inside macroaggregates slightly increased by  $\sim 5\%$  in  
364 comparison to outside (Table S3). Location of the POM fractions inside or outside the  
365 macroaggregates did not significantly influence the OC concentration and the C:N ratio ( $P$   
366  $> 0.05$ ). However, a decrease of  $\sim 14\%$  in C:N ratio was observed when cPOM were located  
367 inside macroaggregates.

368

## 369 **Supplementary references**

370

371 Clarke, K.R., Ainsworth, M., 1993. A method of linking multivariate community structure  
372 to environmental variables. Mar. Ecol. Prog. Ser. 92, 205–205.

373 Griffiths, R.I., Whiteley, A.S., O'Donnell, A.G., Bailey, M.J., 2000. Rapid method for  
374 coextraction of DNA and RNA from natural environments for analysis of ribosomal  
375 DNA- and rRNA-based microbial community composition. Appl. Environ.  
376 Microbiol. 66, 5488–5491.

377     Muyzer, G., de Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial  
378             populations by denaturing gradient gel electrophoresis analysis of polymerase chain  
379             reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* 59,  
380             695–700.

381     Olsson, P.A., Chalot, M., Bååth, E., Finlay, R.D., Söderström, B., 1996. Ectomycorrhizal  
382             mycelia reduce bacterial activity in a sandy soil. *FEMS Microbiol. Ecol.* 21, 77–  
383             86.

384     Pansu, M., Gautheyrou, J., 2006. Organic and total C, N (H, O, S) analysis, In: Pansu, M.,  
385             Gautheyrou J. (Eds), *Handbook of Soil Analysis: Mineralogical, Organic and*  
386             *Inorganic Methods.* Springer, Berlin Heidelberg, pp. 327–370.

387

388

389

390

391

392

393

394

395

396

397

398

399

400 **Table S1.** Distribution of organo-mineral (0-20, 20-50 and 50-200  $\mu\text{m}$ ) and coarse and fine  
 401 particulate organic matter (POM) soil fractions located outside and inside macroaggregates  
 402 ( $> 200 \mu\text{m}$ ) (mean  $\pm$  standard deviation,  $n = 3$ ). cPOM: coarse particulate organic matter  
 403 ( $> 200 \mu\text{m}$ ). fPOM: fine particulate organic matter ( $< 200 \mu\text{m}$ ).

Fractions ( $\mu\text{m}$ )	Fractions outside macroaggregates		Fractions inside macroaggregates	
	(g 100 g <sup>-1</sup> soil)	(g 100 g <sup>-1</sup> soil)	(g 100 g <sup>-1</sup> soil)	(g 100 g <sup>-1</sup> macroaggregates)
Sand	n.s.	10.1 $\pm$ 2.7	13.4 $\pm$ 2.8	
> 200	74.7 $\pm$ 6.0	n.d.	n.d.	
50-200	1.6 $\pm$ 0.5	6.2 $\pm$ 0.7 *	8.3 $\pm$ 0.4	
20-50	5.1 $\pm$ 1.9	18.0 $\pm$ 0.5 *	24.2 $\pm$ 1.3	
0-20	14.1 $\pm$ 1.8	35.5 $\pm$ 0.9 **	47.8 $\pm$ 5.2	
cPOM	0.1 $\pm$ 0.1	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	
fPOM	0.2 $\pm$ 0.1	0.7 $\pm$ 0.1 *	1.0 $\pm$ 0.1	
Total	95.9 $\pm$ 1.6	60.6 $\pm$ 0.4	94.9 $\pm$ 5.2	

404 \* Significant differences between soil fractions outside and inside macroaggregates (g 100  
 405 g<sup>-1</sup> soil) (\*  $P < 0.05$ ; \*\*  $P < 0.01$ ); n.s.: non significant; n.d.: no determined.

406  
 407  
 408  
 409  
 410  
 411  
 412

413 **Table S2:** One-way ANOSIM showing variability in the structure of bacteria between  
 414 organo-mineral (0-20, 20-50 and 50-200  $\mu\text{m}$ ), coarse and fine particulate organic matter  
 415 (POM) fractions and unfractionated soil. ANOSIM R values and *P* values are given.  
 416 Significant values at  $P < 0.05$  are shown in bold text. UF soil: unfractionated soil. cPOM:  
 417 coarse particulate organic matter ( $> 200 \mu\text{m}$ ). fPOM: fine particulate organic matter ( $< 200$   
 418  $\mu\text{m}$ ).

Factors compared	R value	<i>P</i> value
cPOM vs. fPOM	<b>0.42</b>	<b>0.029</b>
cPOM vs. 50-200	<b>0.74</b>	<b>0.005</b>
cPOM vs. 20-50	<b>0.73</b>	<b>0.005</b>
cPOM vs. 0-20	<b>0.80</b>	<b>0.005</b>
fPOM vs. 50-200	<b>0.52</b>	<b>0.014</b>
fPOM vs. 20-50	<b>0.52</b>	<b>0.01</b>
fPOM vs. 0-20	<b>0.78</b>	<b>0.005</b>
50-200 vs. 20-50	-0.05	0.522
50-200 vs. 0-20	0.28	0.063
0-20 vs. 20-50	0.12	0.123
UF Soil vs. cPOM	<b>0.87</b>	<b>0.002</b>
UF Soil vs. fPOM	<b>0.88</b>	<b>0.002</b>
UF Soil vs. 50-200	<b>0.62</b>	<b>0.0003</b>
UF Soil vs. 20-50	<b>0.55</b>	<b>0.0003</b>
UF Soil vs. 0-20	<b>0.41</b>	<b>0.003</b>

419

420

421

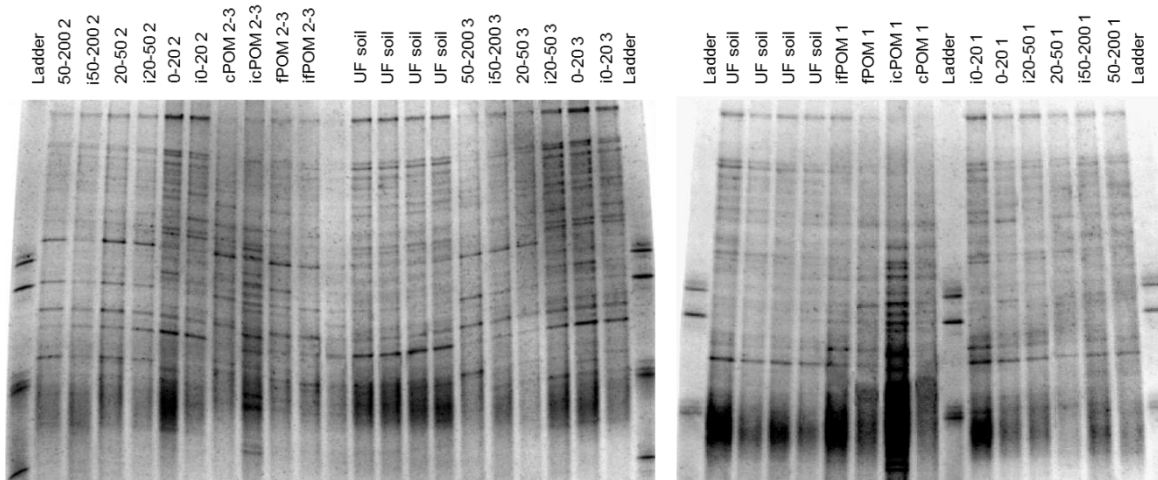
422

423 **Table S3.** Organic C concentrations and C:N ratios of organo-mineral (0-20, 20-50 and 50-  
 424 200  $\mu\text{m}$ ) and coarse and fine particulate organic matter (POM) soil fractions located outside  
 425 and inside macroaggregates ( $> 200 \mu\text{m}$ ) (mean  $\pm$  standard deviation,  $n = 3$ ). UF soil:  
 426 unfractionated soil. cPOM: coarse particulate organic matter ( $> 200 \mu\text{m}$ ). fPOM: fine  
 427 particulate organic matter ( $< 200 \mu\text{m}$ ).

Fractions ( $\mu\text{m}$ )	Fractions outside macroaggregates			Fractions inside macroaggregates		
	C	C	C:N	C	C	C:N
	g C $\text{kg}^{-1}$ fractions	g C $100\text{g}^{-1}$ C soil		g C $\text{kg}^{-1}$ fractions	g C $100\text{g}^{-1}$ C soil	
UF soil	26.8 $\pm$ 0.2		12.4 $\pm$ 0.8			
> 200	23.9 $\pm$ 1.3 <sup>A</sup>	66.6 $\pm$ 6.9	10.7 $\pm$ 0.7 <sup>A</sup>			
50-200	38.2 $\pm$ 6.9 <sup>C</sup>	2.3 $\pm$ 0.9	15.0 $\pm$ 0.9 <sup>C</sup>	42.6 $\pm$ 1.4 <sup>B</sup>	9.8 $\pm$ 0.9	15.9 $\pm$ 0.5 <sup>A</sup>
20-50	20.5 $\pm$ 1.4 <sup>E</sup>	3.8 $\pm$ 1.2	11.2 $\pm$ 1.0 <sup>A</sup>	16.1 $\pm$ 0.5 * <sup>D</sup>	10.8 $\pm$ 0.1	10.5 $\pm$ 1.3 <sup>BC</sup>
0-20	22.4 $\pm$ 0.5 <sup>A</sup>	11.8 $\pm$ 1.4	8.8 $\pm$ 0.7 <sup>D</sup>	23.5 $\pm$ 0.3 * <sup>E</sup>	31.1 $\pm$ 0.6	9.2 $\pm$ 0.5 <sup>C</sup>
cPOM	257.9 $\pm$ 30.3 <sup>B</sup>	1.2 $\pm$ 0.4	19.5 $\pm$ 1.2 <sup>B</sup>	303.1 $\pm$ 14.2 <sup>A</sup>	2.1 $\pm$ 0.3	16.8 $\pm$ 1.3 <sup>A</sup>
fPOM	147.8 $\pm$ 4.4 <sup>D</sup>	1.2 $\pm$ 0.6	12.3 $\pm$ 0.9 <sup>A</sup>	125.6 $\pm$ 13.6 <sup>C</sup>	3.4 $\pm$ 0.6	11.2 $\pm$ 0.8 <sup>B</sup>
Total		= 86.9 $\pm$ 4.8			= 57.2 $\pm$ 0.6	

428 Values followed by different letters for the same columns are significantly different ( $P <$   
 429 0.05). \* indicates significant differences between fractions outside and inside  
 430 macroaggregates (\*  $P < 0.05$ ).

431  
 432  
 433  
 434



435

436 **Figure S1:** DGGE gels of bacterial 16S rRNA genes amplified from unfractionated soil  
 437 (UF soil), organo-mineral (50-200, 20-50 and 0-20  $\mu\text{m}$ ) and, coarse and fine particulate  
 438 organic matter (POM) soil fractions located outside and inside macroaggregates. The  
 439 fractions located inside macroaggregates were prefixed by an **i**, as **inside**. The different  
 440 replicates are indicated by 1, 2 or 3. 2-3: replicates 2 and 3 were pooled for these soil  
 441 fractions. cPOM: coarse POM > 200  $\mu\text{m}$ . fPOM: fine POM < 200  $\mu\text{m}$ .

442