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

4

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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; Blaud 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; Blaud 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 μm) are formed within macroaggregates (size
46 > 200 μm) 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 μm , 20-50 μm , 50-200 μm) and POM (coarse
57 POM: > 200 μm and fine POM < 200 μm) soil fractions can represent distinct microbial
58 habitats, and ii) to determine whether microaggregates and POM location, outside or inside
59 macroaggregates (> 200 μm), 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-Epoisse 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 g kg^{-1} ,
66 C:N ratio 12.4, pH (water) 7.8, CaCO_3 3.2 g kg^{-1} and CEC 25.1 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⁻¹ 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⁻¹.

93 Regular water flow through the 200 μm mesh screen ensured that the microaggregates (<
94 200 μm) 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 μm , **i**20-50 μm , **i**0-20 μm ,
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 μm) 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 μm
121 (55%) and 20-50 μm (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 μm 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

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194

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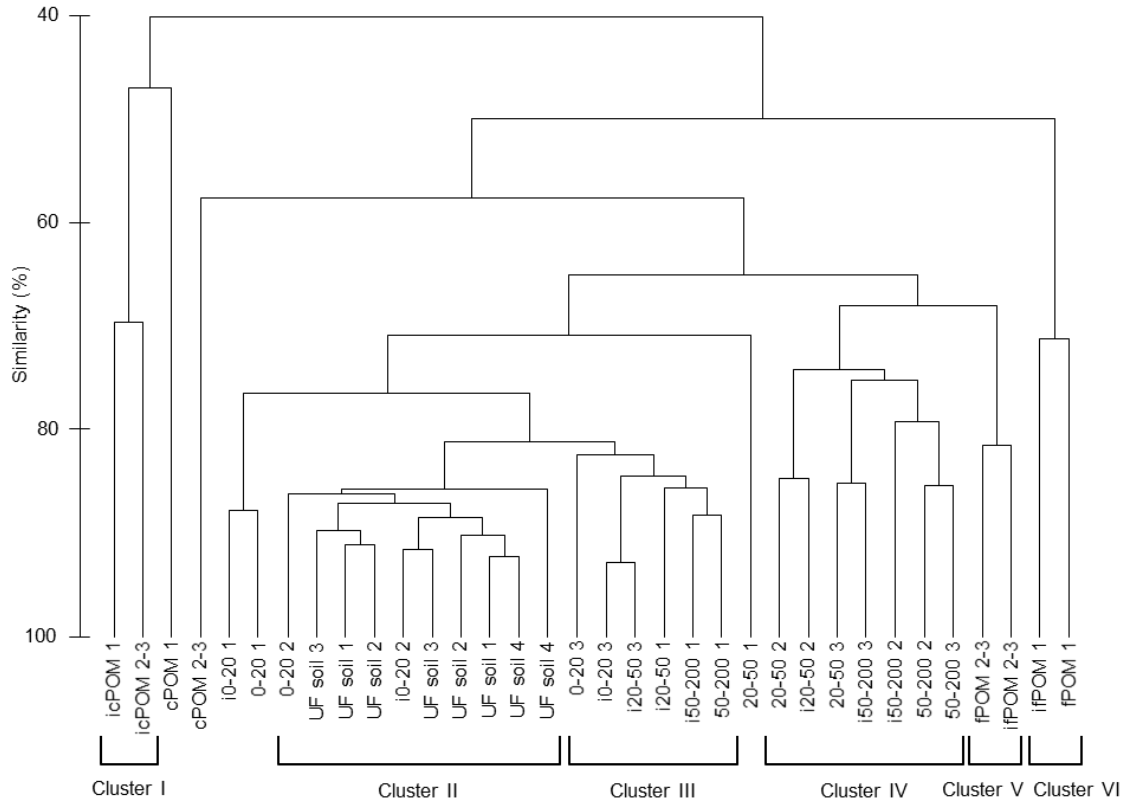
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250 **Figure 1.** Dendrogram of DGGE profiles of bacterial 16S rRNA genes amplified from
 251 unfractionated soil (UF soil), organo-mineral (50-200, 20-50 and 0-20 μm) and, coarse (>
 252 200 μm) and fine (< 200 μm) particulate organic matter (POM) 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 μm . fPOM: fine
 256 POM < 200 μm .

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Supplementary material and methods

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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 μl 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 μl mixtures using puReTaqTM Ready-To-GoTM PCR beads (Amersham-
299 Biosciences, Orsay, France) with 5 ng of template DNA and 1.25 μM 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 ρ were obtained. The ρ values close to 1 indicate a strong correlation,
339 while ρ 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 μm fractions (Table S3). Compared to other soil
356 fractions, 50-200 μm 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 μm soil
358 fractions (Table S2), the main soil OC reservoir was in the 0-20 μm fractions; *i.e.* 43% of
359 the total OC was located in 0-20 μm fractions, 12% outside and 31% inside
360 macroaggregates.

361 The OC concentration of the 20-50 μm soil fraction located inside macroaggregates
362 significantly decreased by ~21% in comparison to outside ($P < 0.05$), while OC
363 concentration of the 0-20 μm fraction inside macroaggregates slightly increased by ~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 ~14% in C:N ratio was observed when cPOM were located
367 inside macroaggregates.

368

369 **Supplementary references**

370

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400 **Table S1.** Distribution of organo-mineral (0-20, 20-50 and 50-200 μm) 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 (μm)	Fractions outside macroaggregates		Fractions inside macroaggregates	
	(g 100 g ⁻¹ soil)	(g 100 g ⁻¹ soil)	(g 100 g ⁻¹ soil)	(g 100 g ⁻¹ 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⁻¹ soil) (* $P < 0.05$; ** $P < 0.01$); n.s.: non significant; n.d.: no determined.

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413 **Table S2:** One-way ANOSIM showing variability in the structure of bacteria between
 414 organo-mineral (0-20, 20-50 and 50-200 μm), 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 μm).

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

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423 **Table S3.** Organic C concentrations and C:N ratios of organo-mineral (0-20, 20-50 and 50-
 424 200 μm) 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 (μm)	Fractions outside macroaggregates			Fractions inside macroaggregates		
	C	C	C:N	C	C	C:N
	g C kg ⁻¹ fractions	g C 100g ⁻¹ C soil		g C kg ⁻¹ fractions	g C 100g ⁻¹ C soil	
UF soil	26.8 \pm 0.2		12.4 \pm 0.8			
> 200	23.9 \pm 1.3 ^A	66.6 \pm 6.9	10.7 \pm 0.7 ^A			
50-200	38.2 \pm 6.9 ^C	2.3 \pm 0.9	15.0 \pm 0.9 ^C	42.6 \pm 1.4 ^B	9.8 \pm 0.9	15.9 \pm 0.5 ^A
20-50	20.5 \pm 1.4 ^E	3.8 \pm 1.2	11.2 \pm 1.0 ^A	16.1 \pm 0.5 * ^D	10.8 \pm 0.1	10.5 \pm 1.3 ^{BC}
0-20	22.4 \pm 0.5 ^A	11.8 \pm 1.4	8.8 \pm 0.7 ^D	23.5 \pm 0.3 * ^E	31.1 \pm 0.6	9.2 \pm 0.5 ^C
cPOM	257.9 \pm 30.3 ^B	1.2 \pm 0.4	19.5 \pm 1.2 ^B	303.1 \pm 14.2 ^A	2.1 \pm 0.3	16.8 \pm 1.3 ^A
fPOM	147.8 \pm 4.4 ^D	1.2 \pm 0.6	12.3 \pm 0.9 ^A	125.6 \pm 13.6 ^C	3.4 \pm 0.6	11.2 \pm 0.8 ^B
Total		= 86.9 \pm 4.8			= 57.2 \pm 0.6	

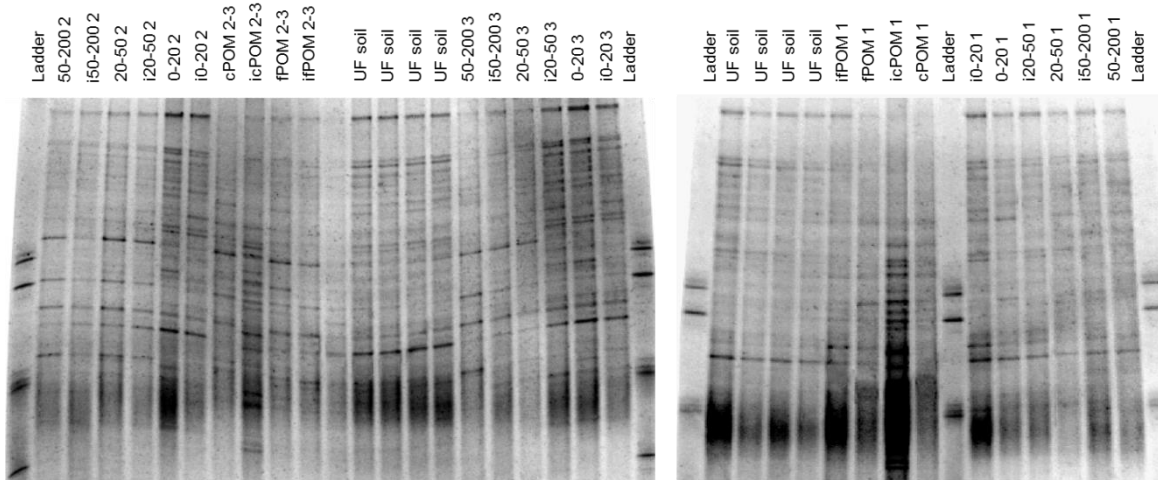
428 Values followed by different letters for the same columns are significantly different ($P <$
 429 0.05). * indicates significant differences between fractions outside and inside
 430 macroaggregates (* $P < 0.05$).

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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 μm) 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 μm . fPOM: fine POM < 200 μm .

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