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

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Abstract

Soil aggregates and particulate organic matter (POM) are thought to represent distinct soil microhabitats for microbial communities. This study investigated whether organo-mineral (0-20, 20-50 and 50-200 µm) and POM (two sizes: > 200 and < 200 µm) soil fractions represent distinct microbial habitats. Microbial habitats were characterised by the amount and quality of organic matter, the genetic structure of the bacterial community, and their location outside or inside macroaggregates (> 200 µm). The denaturing gradient gel electrophoresis (DGGE) profiles revealed that bacterial communities structure of organo-

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

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

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

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

A clayey Eutric Cambisol was sampled at the INRA-Epoisse experimental farm in Burgundy (France). The experimental field plots have been cultivated and tilled for 10 years with a rotation of wheat, rape, and barley. The soil texture was comprised of 11.2 % of sand, 41.8 % of silt and 47.0 % of clay. The organic C concentration was 26.8 g kg^{-1}, 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 (diameter, 7 cm) were randomly collected down to a depth of 30 cm, which represented the tilled layer of the soil (tilled annually), where the soil aggregates and POM are homogenised and fragmented. These soil samples were pooled to reduce any spatial

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

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

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

Nucleic acids were extracted from 0.5 g (wet weight) of unfractionated soil and each fraction described above. Bacterial 16S rRNA genes were amplified with the bacterial primers 338f-GC and 518r and the amplicons were resolved by denaturing gradient gel electrophoresis (DGGE). The full details of the DNA extraction, PCR amplification and DGGE analysis are provided in the Supplementary material. To analyse the matrix obtained from DGGE band profiles, the total band intensity was normalised for each sample (i.e. each band intensity was divided by the total band intensity of each sample). The relative abundance data from the DGGE matrix was then square root transformed and a similarity matrix from DGGE profiles was generated using the Bray-Curtis method. A dendrogram was produced from the similarity matrix using the group average linking method implemented in the software PRIMER v6 (PRIMER-E Ltd, Plymouth, UK). To test for 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 µm) and fractions < 50 µm 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 µm 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; Blaud 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-

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

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

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

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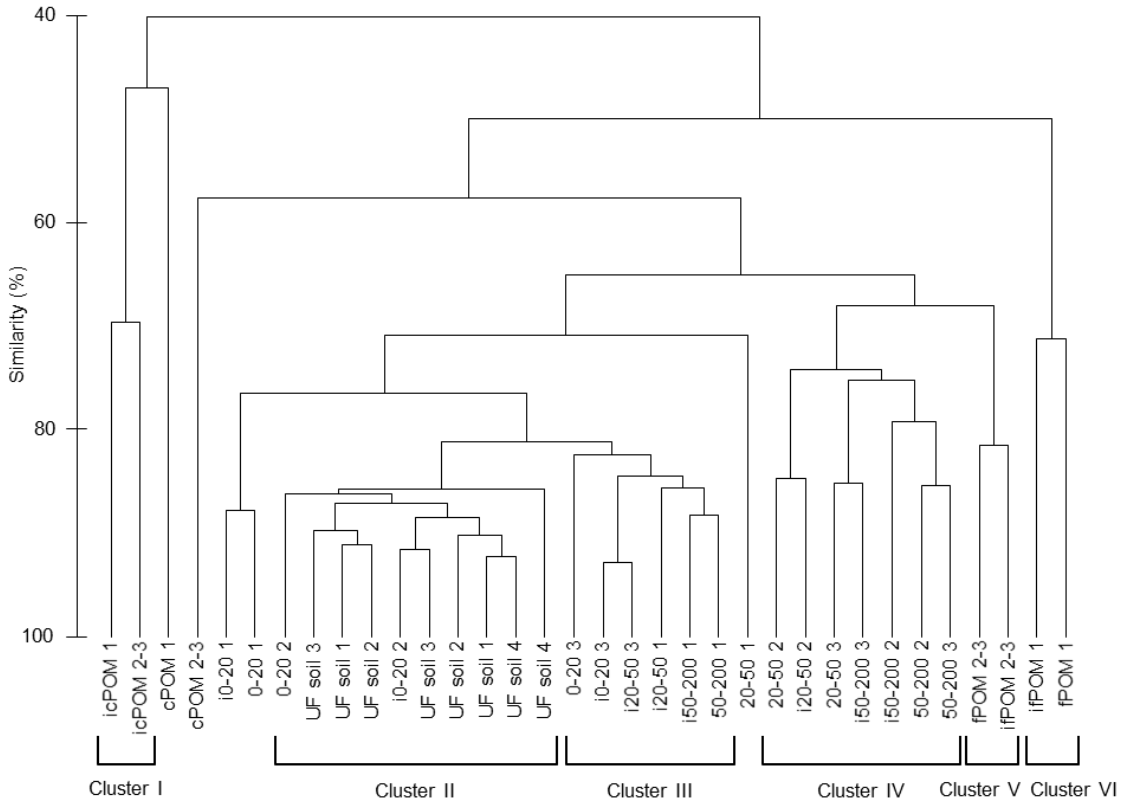

Figure 1. Dendrogram of DGGE profiles of bacterial 16S rRNA genes amplified from unfractionated soil (UF soil), organo-mineral (50-200, 20-50 and 0-20 µm) and, coarse (> 200 µm) and fine (< 200 µm) particulate organic matter (POM) soil fractions located outside and inside macroaggregates. The fractions located inside macroaggregates were prefixed by an **i,** as **i**nside. The different replicates are indicated by 1, 2 or 3. 2-3: replicates 2 and 3 were pooled for these soil fractions. cPOM: coarse POM > 200 µm. fPOM: fine POM < 200 µm.

Supplementary material and methods

C and N analysis

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

DNA extraction

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

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

PCR amplifications and Denaturing Gradient Gel Electrophoresis (DGGE) analyses

The bacterial 16S rRNA gene sequences were amplified with the bacterial primers 338f-GC (Olsson et al., 1996) and 518r (Muyzer et al., 1993). PCR amplifications were performed in 25 µl mixtures using puReTaq™ Ready-To-Go™ PCR beads (Amersham-Biosciences, Orsay, France) with 5 ng of template DNA and 1.25 µM of each primer, using a GeneAmp PCR System 9700 (Applied Biosystems, Courtabœuf, France). Bacterial 16S rRNA genes were amplified using an initial denaturation at 94 ºC for 2 min, followed by 20 cycles of 94 ºC for 30 s (denaturing), 65 ºC for 30 s (annealing), and 72 ºC for 60 s (extension), with a 0.5 °C touchdown every cycle during the annealing stage; this was, followed by an additional 10 cycles with an annealing temperature of 55 °C, before a final extension at 72 °C for 10 min.

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

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

Statistical analyses

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

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

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

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

Supplementary results

Soil and POM fractions size distribution

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

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

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

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

The OC concentration of the 20-50 µm soil fraction located inside macroaggregates significantly decreased by ~21% in comparison to outside ($P < 0.05$), while OC concentration of the 0-20 µm fraction inside macroaggregates slightly increased by ~5% in comparison to outside (Table S3). Location of the POM fractions inside or outside the macroaggregates did not significantly influence the OC concentration and the C:N ratio ($P > 0.05$). However, a decrease of ~14% in C:N ratio was observed when cPOM were located inside macroaggregates.

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Table S1. Distribution of organo-mineral (0-20, 20-50 and 50-200 μm) and coarse and fine particulate organic matter (POM) soil fractions located outside and inside macroaggregates (> 200 μm) (mean ± standard deviation, n = 3). cPOM: coarse particulate organic matter (> 200 μm). fPOM: fine particulate organic matter (< 200 μm).

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

* Significant differences between soil fractions outside and inside macroaggregates (g 100 g^{-1} soil) (* $P < 0.05$; ** $P < 0.01$); n.s.: non significant; n.d.: no determined.

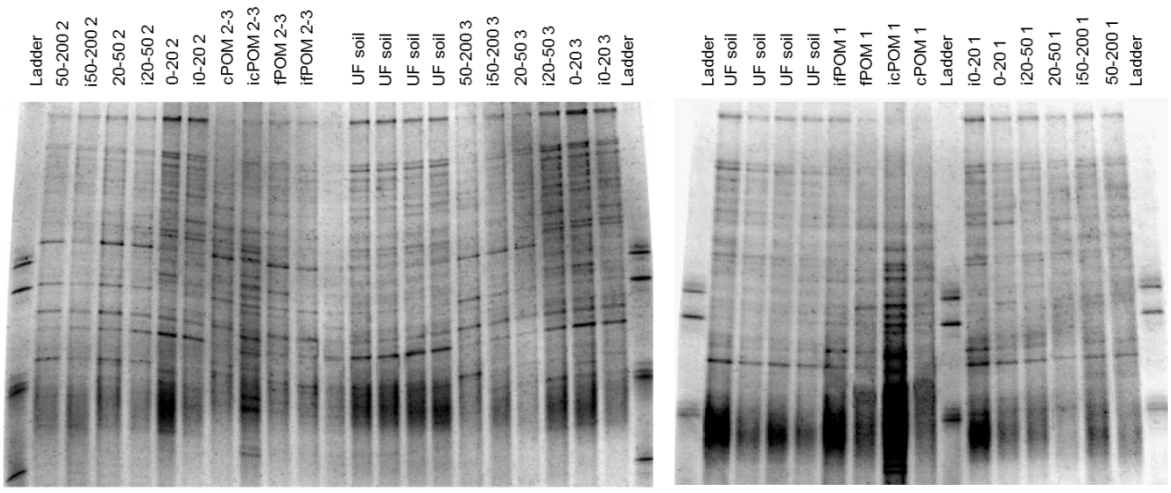
Table S2: One-way ANOSIM showing variability in the structure of bacteria between organo-mineral (0-20, 20-50 and 50-200 μm), coarse and fine particulate organic matter (POM) fractions and unfractionated soil. ANOSIM R values and *P* values are given. Significant values at $P < 0.05$ are shown in bold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

Factors compared	R value	*P* 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**

Table S3. Organic C concentrations and C:N ratios of organo-mineral (0-20, 20-50 and 50-200 μm) and coarse and fine particulate organic matter (POM) soil fractions located outside and inside macroaggregates (> 200 μm) (mean ± standard deviation, n = 3). UF soil: unfractionated soil. cPOM: coarse particulate organic matter (> 200 μm). fPOM: fine particulate organic matter (< 200 μm).

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

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



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 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 **i**nside. 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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