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Short communication

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.

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 (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 (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

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"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 (1936) for the isolation of soil fractions located outside macroaggregates, and from 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 (cPOM >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 (fPOM: 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: i50–200 μm, i20–50 μm, i0–20 μm, icPOM and ifPOM, 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 correlate variation in bacterial communities to OC concentration and C:N ratio, ANOSIM and RELATE tests from PRIMER v6 software were performed, respectively (see Supplementary materials).

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

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