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Soil particle size fractions harbour distinct microbial communities and differ in potential for microbial mineralisation of organic pollutants

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ABSTRACT

Due to differences in mineralogical composition and organic matter density, soil particle size fractions (PSF) provide different surface properties and micro-environments, which may affect the adsorption of chemicals and select for distinct microbial communities. Using soils from a long-term fertilisation experiment, we examined the structural diversity of microbial communities associated with different PSF and their potential to mineralise two organic pollutants (phenol and 2,4-dichlorophenol (DCP)). The soils were taken from 0 to 18 cm depth of arable field plots that have been kept unfertilised (UNF), mineral fertilised (NPK), or treated with animal manure (AM) for 117 years. Sand, including particulate organic matter (POM), coarse silt, fine silt, and clay were isolated by gentle ultra-sonication, wet-sieving and centrifugation. Animal manuring increased the abundance of the three microbial domains *Bacteria*, *Archaea* and *Fungi*, with coarse silt being most responsive. The impact of manuring declined with decreasing particle size. Genetic profiling indicated that the composition of the bacterial communities was primarily shaped by soil particle size classes, while archaea responded predominantly to fertilisation. Particle size as well as fertilisation was equally important in structuring the fungal communities. All PSF showed capacity for phenol mineralisation with rates correlating negatively with particle size (except for the sand-POM fraction), and long-term fertilisation enhanced the mineralisation potential. In contrast, DCP mineralisation was associated with the clay fraction only and was highest for soil treated with mineral fertiliser. This study demonstrates that PSF harbour structurally distinct microbial communities with different functional potentials for mineralising organic pollutants.

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1. Introduction

Soil microorganisms typically live attached to or in close vicinity of particle surfaces (Mills, 2003) as these provide a favourable micro-environment in terms of accessible water, nutrients and organic substrates. The differently sized primary organo-mineral complexes (sand, 63–2000 μm; coarse silt, 20–63 μm; fine silt, 2–20 μm; clay, <2 μm) and particulate organic matter (POM) constitute the building blocks of a highly heterogeneous hierarchical system of micro- and macro-aggregates present in intact soil (Oades and Waters, 1991). The primary organo-mineral complexes or particle size fractions (PSF) differ in mineralogical composition (Acosta et al., 2011) and in specific sorption capacity, soil organic matter density and composition (Christensen, 1992), thereby generating diverse surface properties. Microbial biomass has been shown to increase with decreasing particle size corresponding to increasing surface area (Jocteur Monrozier et al., 1991; Lensi et al., 1995; Stemmer et al., 1998; Neumann et al., 2013). However, the presence of POM may support microbial biomass in coarser fractions (Kanazawa and Filip, 1986). Differences between PSF also relate to potential activities of various enzymes (Stemmer et al., 1998; Kandeler et al., 2000; Marx et al., 2005), and to microbiological processes like ammonification (Nacro et al., 1996), denitrification (Lensi et al., 1995), methanogenesis (Zhang et al., 2007), and the formation of non-extractable residues from organic pollutants (Botterweck et al., 2014). Carbon and nutrient mineralisation potentials have been shown to increase with decreasing particle size (Christensen, 1992). However, sand sized fractions enriched in POM may also show high carbon mineralisation rates.

PSF have been found to be associated with differently structured microbial communities in studies where analyses for phospholipid

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fatty acids (PLFA) or genetic fingerprinting were applied (Kandeler et al., 2000; Sessitsch et al., 2001; Poll et al., 2003; Hemkemeyer et al., 2014). However, the link between PSF-specific microbial communities and their *in situ* metabolic activity has yet to be detailed. Exploring the structure and function of microbial communities associated with differently sized PSF should allow clarifying microhabitat-specific interactions between abiotic and biotic soil components. This may help delineating functional entities and ultimately explain differences in metabolic activities and redundancies found in soils under different land use and management.

Based on soil retrieved from three differently fertilised arable field variants (unfertilised, mineral fertilised, animal manured), our objective was to reveal whether soil PSF are associated with distinct microbial communities and how long-term fertilisation affects the particle-associated microbial community structures and their functional potential for mineralising organic pollutants. Two structurally similar ^{14}C-labelled compounds (phenol and 2,4-dichlorophenol (DCP)) with assumingly different microbiological degradation potentials (Neumann et al., 2014) were selected as model compounds. Their mineralisation was studied in bulk soil and in model soils consisting of individual PSF mixed with sterile quartz sand. We attempt to reveal potential links between the structure and function of microbial communities associated with different PSF under the influence of long-term fertilisation.

2. Materials and methods

2.1. Field experiment and soil sampling

Soil samples were retrieved from the Lermarken site of the Askov Long-Term Experiments on Animal Manure and Mineral Fertilizers, initiated in 1894 at Askov Experimental Station, Denmark (55°28′N, 09°07′E). Site characteristics and the experiments have been described in detail by Christensen et al. (2006). Mean annual precipitation and temperature is 862 mm and 7.7 °C, respectively. Lermarken is a loamy sand (Typic Hapludalf) with 11% clay (<2 μm) and 13% fine silt (2–20 μm) based on terminal morainic deposits from the Weichsel glaciation. The sand fraction is dominated by quartz and feldspars, while the dominant clay minerals are illite, smectite and kaolinite. The experiments grow a four-course rotation comprising spring sown cereals, grass-clover ley used for cutting, autumn sown cereals and row crops. Guided by soil analyses, soil pH is kept at 5.5 to 6.5 by occasional addition of magnesium-enriched lime (3–5 t ha^{-1} every 4–5 years). Soils for this study were sampled in 0–18 cm depth in the B2w-field using the treatments: unfertilised (UNF; plot 124), mineral fertilised (NPK; plot 125) and animal manured (AM; plot 116). Since 1973, the NPK soil has received a mean annual input of 100 kg total-N ha^{-1}, 19 kg P ha^{-1} and 87 kg K ha^{-1}; the corresponding inputs to the AM soil were 143 kg total-N, 30 kg P and 134 kg K. Animal manure is cattle slurry (37.5 t wet weight ha^{-1}) that has been stored anaerobically in slurry tanks for 4–9 month before application. To eliminate any direct effect of fertilisation, soil was sampled in August 2011 following the last cut of the grass-clover ley (18 months after fertilisation and before glyphosate treatment). Up to 25 soil cores were sampled across each plot and pooled. Samples were slightly air dried to be sieved with a mesh size of 2 mm. The coarse organic matter and any lime grains were removed by hand. The samples were stored in the dark at 4 °C at 50–55% water holding capacity. Soil sampling for the DCP mineralisation experiments took place in September 2012 between the stubbles of preceding winter wheat (7 months after fertilisation).

2.2. Particle size fractionations and separation of particulate organic matter (POM)

Bulk soil was fractionated into sand including POM (63–2000 μm), coarse silt (20–63 μm), fine silt (2–20 μm) and clay (<2 μm) using the protocol of Amelung et al. (Amelung et al., 1998) but with lower ultrasonication energies, as previously reported (Neumann et al., 2013). In brief, soil was suspended with distilled water at a ratio of 1:5. To disrupt the soil aggregates while keeping as many microorganisms as possible attached, the ultrasound energy was adjusted to 30 J ml^{-1} with the probe having an energy output of 70 W (Sonoplus HD 2200 homogeniser, Bandelin Electronic, Berlin, Germany). The tip of the Sonotrode (Model VS 70T) was immersed 20 mm into the soil suspension. During sonication the suspension was kept at room temperature by water cooling. The sand-sized fraction was isolated by wet-sieving through a 63 μm mesh (Retsch Technology GmbH, Haan, Germany). The silt fraction was isolated by stepwise centrifugation at 25 × *g* for 2 × 15 min, 2 × 13 min, 2 × 12 min, and 2 × 11 min. The individual supernatants containing the clay were bulked and the clay particles precipitated by adding a 1 M $MgCl_2$ solution to a final concentration of 3.3 mM and then stored at 4 °C overnight. The silt fraction was further separated by wet-sieving through a 20 μm mesh (Retsch). Wet-sieving and centrifugations were conducted at room temperature. Fine silt sedimentation took place over night at 4 °C. In contrast to the previous study (Neumann et al., 2013), the addition of $MgCl_2$ did not completely precipitate the clay of the present soils. Therefore the precipitate and the remaining suspension were centrifuged at 2450 × *g* for 10 min at room temperature and supernatant was decanted. To evaluate the efficiency of our low-sonication fractionation the yield of each PSF was compared to yields following higher ultrasonic energy inputs and/or the standardised sedimentation technique (see Results section).

For the adsorption studies, POM was isolated from the fraction 63–2000 μm by a modified version of the density fractionation protocol previously described (Neumann et al., 2013). The oven-dried fraction was suspended in 40 ml 2 g ml^{-1} sodium polytungstate (SPT; Sometu, Berlin, Germany) by manual shaking overhead for 15 s. After sedimentation for 2 h, the supernatant was separated by decantation and the procedure repeated with the sediment. Floating POM was collected on a 0.45-μm polyamide filter (Sartorius, Göttingen, Germany). The sediment was rinsed with SPT on a second filter. After vacuum filtration at room temperature, both filters were oven-dried overnight at 50 °C.

2.3. Measurement of chemical parameters

Soil pH was measured in duplicates of soil suspensions (one part of air-dried soil diluted in two parts of 0.01 M $CaCl_2$) using a Professional Meter PP-25 and the electrode PY-P21 (Sartorius). Total carbon (TC) and nitrogen (N_t) were determined using a TrueMac® CN (Leco Corp., St. Joseph, MI) with combustion at 1350 °C. Before analysis, bulk soil and PSF samples were oven-dried at 105 °C and then bulk soil and clay was ground in a mortar. Total inorganic carbon (TIC) was determined by CO_2 analysis (Martens, 1985; in der Wiesche et al., 1996). Total organic carbon (TOC) was determined by subtracting TIC from TC. For PSF, TOC was considered to equal TC due to dissolution and loss of carbonates during fractionation. All measurements were done in triplicates.

2.4. DNA extraction and quantification

DNA was extracted from 0.5 g fresh bulk soil, 0.5 g sand, 0.4 g silt and 0.1 g clay. PSF weights correspond to dry mass (see below). The sand-sized fraction was retrieved directly from the sieve while

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