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The abundance of nitrogen cycle genes and potential greenhouse gas fluxes depends on land use type and little on soil aggregate size

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ABSTRACT

Soil structure is known to influence microbial communities in soil and soil aggregates are the fundamental ecological unit of organisation that support soil functions. However, still little is known about the distribution of microbial communities and functions between soil aggregate size fractions in relation to land use. Thus, the objective of this study was to determine the gene abundance of microbial communities related to the nitrogen cycle and potential greenhouse gas (GHG) fluxes in six soil aggregate sizes (0–0.25, 0.25–0.5, 0.5–1.0, 1–2, 2–5, 5–10 mm) in four land uses (i.e. grassland, cropland, forest, young forest). Quantitative-PCR (Q-PCR) was used to investigate the abundance of bacteria, archaea and fungi, and functional guilds involved in N-fixation (*nifH* gene), nitrification (bacterial and archaeal *amoA* genes) and denitrification (*narG*, *nirS*, and *nosZ* genes). Land use leads to significantly different abundances for all genes analysed, with the cropland site showing the lowest abundance for all genes except *amoA* bacteria and archaea. In contrast, not a single land use consistently showed the highest gene abundance for all the genes investigated. Variation in gene abundance between aggregate size classes was also found, but the patterns were gene specific and without common trends across land uses. However, aggregates within the size class of 0.5–1.0 mm showed high bacterial 16S, *nifH*, *amoA* bacteria, *narG*, *nirS* and *nosZ* gene abundance for the two forest sites but not for fungal ITS and archaeal 16S. The potential GHG fluxes were affected by land use but the effects were far less pronounced than for microbial gene abundance, inconsistent across land use and soil aggregates. However, few differences in GHG fluxes were found between soil aggregate sizes. From this study, land use emerges as the dominant factor that explains the distribution of N functional communities and potential GHG fluxes in soils, with less pronounced and less generalized effects of aggregate size.

1. Introduction

Soil is a complex and heterogeneous matrix made up of an intricate organisation of pores filled with water and gas, mineral particles, and organic matter influencing the microorganisms that live within. Soil aggregates are essential for soil fertility (Amézketa, 1999; Bronick and Lal, 2005) and some fertile soils have been described as soils dominated by 0.25–10 mm soil crumbs (Shein, 2005). The vast variation in the size of aggregates, as well as their physico-chemical properties provides a huge diversity of microhabitats for microorganisms influencing carbon and nutrients dynamics within the soil. This study starts from the premise that soil aggregates are a fundamental ecological unit of organisation that support soil functions. These soil functions include biomass production, soil water retention and transmission, nutrient transformation, contaminant attenuation, C and N, P, K sequestration, and a major terrestrial pool of genetic diversity. The microbial community

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has been found to vary with the size of soil aggregates, and to be linked to the specific environmental conditions in the different sizes of aggregates. Previous studies showed differences in microbial community structure, diversity and abundance/biomass between soil aggregates of different size, which was correlated to the quality of organic matter available (Blaud et al., 2012; Davinic et al., 2012), the size of the pores (Kravchenko et al., 2014) or tillage (Helgason et al., 2010).

Although the distribution of microbial communities in soil aggregates has been studied, much less is known about the distribution of the microbial functional guilds among soil aggregates and how their sizes influence microbial functions. The size of soil aggregates in relation to their porosity (i.e. size and number of pores) was found to affect the GHG fluxes, with CO_2 emissions found to be higher in microaggregates (< 0.25 mm) than in macroaggregates (> 0.25 mm) in cropland sandy loam soil (Sey et al., 2008; Mangalassery et al., 2013). Similar results were found for CH_4 in cropland sandy loam and clay loam soil (Mangalassery et al., 2013), but the contrary was found in paddy rice soil (Ramakrishnan et al., 2000). Only a few studies have investigated specific microbial functional guilds such as N fixation (Mendes and Bottomley, 1998; Poly et al., 2001; Chotte et al., 2002; Izquierdo and Nüsslein, 2006) and denitrifiers (Beauchamp and Seech, 1990; Lensi et al., 1995) in soil aggregates. The biomass and composition of diazotrophs varies with the size of soil aggregates which was correlated with total C and N, and soil texture (Poly et al., 2001; Izquierdo and Nüsslein, 2006). Aggregates within size classes 0.6–2.0 mm and < 0.075 mm (from tundra, pasture and forest) were found to have the highest diazotroph richness (Izquierdo and Nüsslein, 2006) and microaggregates (< 0.25 mm) to host between 30% and 90% of the diazotrophic population (Mendes and Bottomley, 1998; Chotte et al., 2002). In contrast, denitrifiers were found to occur mainly in microaggregates, where nearly 90% of the potential denitrification activity can occur (Lensi et al., 1995). Hence, the diazotroph and denitrifier communities seem to exploit specific and different anaerobic niches within different soil aggregate size classes, although the drivers of these communities in different soil aggregate sizes remains unclear.

The type of land use and management directly influences the physico-chemical properties of soil aggregates as well as the distribution of microbial communities, their functions and resulting nutrient transformations and GHG fluxes. For example, the soil aggregates turnover rate is increased by soil tillage (Six et al., 2004), which decreases the C storage within the aggregates (Bossuyt et al., 2002), but can also decrease N_2O fluxes (Ball, 2013). Furthermore, the type of vegetation and input of organic manure influence the aggregate size distribution and the contents of organic C and N within soil aggregates (Pinheiro et al., 2004; Six et al., 2004; An et al., 2010). Subsequently, bacterial and fungal community composition was found to differ between land use types (Lauber et al., 2008) and also microbial activity such as nitrification (Hayden et al., 2010).

The above leads to the overarching hypothesis that in conjunction with land use, different microbial functions are preferentially hosted or fostered by specific size classes of aggregates. The specific objectives of the current study were: i) to assess the difference in microbial genes abundance between different soil aggregate size classes and bulk soil from different land uses, ii) to assess the difference in greenhouse gases fluxes between soil aggregate sizes classes and bulk soil from different land uses, iii) to identify possible relationships between microbial gene abundances, potential GHG fluxes and the physico-chemical characteristics of the soil aggregates.

2. Material and methods

2.1. Study area

The study area is originated from the Critical Zone Observatory Marchfeld/Fuchsenbigl area (Banwart, 2011) located east of Vienna, Austria, in the National Park “Donau-Auen” on a floodplain of the Danube River (Fig. S1). The mean annual temperature in the area is ~9 °C and mean annual precipitation ~550 mm. The study sites are located along a chronosequence starting from a young river island (created < 70 years; average inundation frequency: 10 day $year^{-1}$) named “young forest”, and sites disconnected from the river through a flood control dike: forest, grassland and cropland. The young forest is impacted by flood events, and covered by “soft-wood” dominated by *Salicetum* albae, while the forest site is covered by “hard-wood” dominated by *Fraxino-Ulmetum*, respectively. The grassland site was converted from forest to grassland (presently *Onobrychido viciifoliae-Brometum*) between 1809 and 1859 and is currently cut twice a year. The cropland site was grassland before 1781 and was converted to intensive cropland in the first half of the 20th century. Cropland site was conventionally managed, with annual tillage and NPK mineral fertilisers. The field is under crop rotation (maize, sugar beet, barley and wheat), with summer wheat the year of the sampling which was shortly harvested before the soil sampling. According to Lair et al. (2009), the topsoil (0–10 cm) of the young forest was deposited after 1986, whereas a topsoil age of approx. 250–350 years on the forest, grassland, and cropland site can be estimated . The soils are classified as Epigleyic Fluvisol (young forest) and Mollic Fluvisols (forest, grassland and cropland; (IUSS Working Group WRB, 2014). The Epigleyic Fluvisol is at least one time of the year impacted by groundwater and is located close to the Danube River. In contrast, the Mollic Fluvisols have no impact of groundwater and are characterized by a fast OC accumulation in the topsoil. In our study area Mollic Fluvisols develop towards a Chernozem. The soil characteristics and soil aggregates size distribution of bulk soil are shown in Table 1.

2.2. Soil sampling and fractionation

The soil sampling was identical at all sites and was performed in September 2011 under dry soil moisture conditions (capillary potential pF 3.8–4.0). At each site, three sampling spots (70 × 70 cm) were randomly selected within a circle of about 30 m radius. The soil layer from 5 to 10 cm soil depth was sampled to avoid the main rooting zone in grassland and the litter layer in forest sites, focusing on the similar mineral soil layer across sites. The soil samples were manually dry sieved to obtain 6 soil aggregate size classes: < 0.25, 0.25–0.5, 0.5–1, 1–2, 2–5, and 5–10 mm. The soil fraction > 10 mm was not included in the study as it was composed of a wide range of aggregates and large clumps (100 − 500 g per clump). During dry sieving, visible roots were removed. Sieving continued with freshly excavated soil until ~200 g of soil aggregates was obtained for each aggregate size class. Additional bulk soil samples were collected at each site and sampling spot. Soil aggregate size fractions and bulk soil samples were stored at 4 °C and samples for DNA extraction at − 20 °C before subsequent analysis. Dry-sieving was chosen over wet-sieving to avoid any bias due to dry/wet cycles with wet-sieving that could have direct effect on GHG emissions (Kaiser et al., 2015). Despite knowing that the sieving method affects the gene abundance quantification, dry-sieving can nonetheless reveal differences in gene abundance between soil aggregate sizes (Blaud et al., 2017).

2.3. DNA extraction and quantitative-PCR

Total nucleic acids were extracted from 0.20 to 0.55 g of fresh soil aggregates from all size classes and from bulk soil samples with PowerSoil® DNA Isolation Kit (Mo-Bio laboratories, Carlsbad, CA, USA) according to manufacturer’s instruction, except for the final step where the nucleic acids were eluted in 100 µl of sterile nuclease free water instead of solution C6. Microbial abundance was investigated by Quantitative-PCR (Q-PCR) targeting specific genes or genetic regions. Bacterial and archaeal communities were targeted via the 16S rRNA genes, while the fungal community abundance was investigated by targeting the ITS region. The different communities involved in most