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The influence of aggregate size fraction and horizon position on microbial community composition

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ABSTRACT

The influence of horizon position and aggregate size on bacterial and fungal community composition was determined. From nine sites, soils were collected from the top three horizon positions (H1, H2 and H3). Physical fractionation separated samples into large macroaggregate (LM, > 2000 μm), macroaggregate (MAC, > 250 μm), microaggregate (MIC, < 250 μm), and silt and clay (SC, 53 μm) fractions. In all samples, the structure of the bacterial and fungal community composition was assessed via restriction fragment length polymorphism (T-RFLP), and for the four aggregate sizes from the top two horizons positions an in-depth analysis of the bacterial community was conducted using next generation sequencing (NGS). Bacterial and fungal communities both differed between aggregate-sizes. Changes in the composition of the bacterial and fungal communities also occurred among horizon positions, with a significant interaction between aggregate size and horizon position evident for the bacterial community. Using NGS, it was shown that aggregate-size had a significant effect on the bacterial community in both horizon positions at both the phyla and family taxonomic levels. MAC and MIC significantly differed in the % relative abundance of bacterial groups, potentially indicating differing predation pressures. These results indicate that both horizon position and aggregate size support distinct microbial communities. Understanding these parameters is critical in our comprehension of the patterns of microbial diversity in soil.

1. Introduction

The majority of our understanding of soil microbial ecology has concentrated on bulk soil samples. While these studies have provided profound insights into the diversity and functioning of soil ecosystems (Bowles et al., 2014; Thomson et al., 2015; Wakelin et al., 2008), they have not accounted for the inherent heterogeneity of microbial diversity seen over small spatial scales (Mummey et al., 2006; Vos et al., 2013). Investigations of soil microbiology, at the appropriate spatial scale, are considered to be important in providing a deeper understanding into the functioning of soil ecosystems (O'Brien et al., 2016; Raynaud and Nunan, 2014; Vos et al., 2013).

The structure of soil is made up of an arrangement of tortuous physical networks which determine the flow of substrates and solutes in space and over time. This provides a diverse range of physicochemical niches characterized by variation in nutrient quantity and quality, redox conditions, variation in water filled pore space, and pore size classes available for microbial habitation (Mummey and Stahl, 2004). Much of the variation in these properties can be partitioned among the distinct aggregate size fractions present in soil, as these vary in physical, chemical, and structural characteristics (Ranjard et al., 2000). Aggregates are secondary structures formed through the interactions of mineral particles as well as organic and inorganic substances (Bronick and Lal, 2005; Tisdall and Oades, 1982). They are grouped according to size, with large macroaggregates being considered > 2 mm in size macroaggregates generally being considered over 250 μm in size while microaggregates are less than 250 μm in size (Bronick and Lal, 2005). Additionally, the stability, distribution, and microarchitecture within and between soil aggregates is linked to the composition and function of the microbiome (Mikha and Rice, 2004).

The two largest aggregate size fractions are formed through temporary associations of minerals, particulate organic matter, and large-

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or micro-aggregates. This binding is largely mediated through the enmeshment of fungal hyphae and plant roots (Mummey and Stahl, 2004; Rillig and Mummey, 2006). Microaggregates (MIC) are formed through the binding of bacterial polysaccharides with clay particles, organo-mineral complexes, and polyvalent metals (Six et al., 2004), and are largely formed within macroaggregate structures. These aggregate size fractions represent distinct microhabitats for microbial colonization and substrate utilization. The large macroaggregate (LM) and macro-aggregate (MAC) fractions are enriched with labile carbon (C) and nitrogen (N), predominantly of plant origin and fungal origin (Marx et al., 2005). Microaggregates (MIC) are characterized by having lower concentrations of labile C and increased amounts of biochemically recalcitrant C (i.e. compounds with a higher C:N ratio), and physically-protected C, i.e., C that is associated with soil organic matter and not free particular organic matter and which microbial decomposers and their enzymes have a greater difficulty gaining access to (Elliott, 1986; Kravchenko et al., 2015). In addition, the interior of microaggregates have been described as inherently oligotrophic, with low nutrient and O_2 availability resulting in reduced microbial activity (Mummey and Stahl, 2004). The silt and clay fraction (SC) has relatively stable C and N and high levels of microbial biomass have been reported within them (Elliott, 1986; Sessitsch et al., 2001; vanGestel et al., 1996). Microbial acquisition of substrate is compounded by the sorption of extracellular enzymes to clay particles in the SC, with this process likely being responsible for the distinct microbial activities and community structures reported (Allison and Jastrow, 2006; Mueller, 2015; Sessitsch et al., 2001).

Understanding the interplay between aggregate size and the soil microbiome is an important consideration for achieving sustainability in our agroecosystems, particularly the retention of soil organic carbon (SOC). Occlusion within aggregates is a well-known mechanism of SOC preservation, and the patterns of microbial community structure and activity within aggregates may be key to understanding this function (Six et al., 1998; Torres-Sallan et al., 2017). It will also have important implications for the preservation of soil biodiversity and the management of microbial communities for bio-control and plant disease suppression (Grundmann, 2004).

The experimental aim of this study was to elucidate the influence of both aggregate size fraction and horizon position on the patterns of both bacterial and fungal community structures. The working hypothesis being that the two largest aggregate sizes (LM and MAC) would significantly differ from the two smaller aggregate sizes (MIC and SC) due to changing physicochemical conditions (i.e., increased microporosity). The study also hypothesised that different horizon positions would also significantly impact microbial community compositions with the aggregates sizes, as soil physicochemical conditions will also vary by horizon. Firstly, bacterial and fungal community composition from the top three horizon positions was determined using community-level fingerprinting. Secondly, the study narrowed its focus to ascertain how specific bacterial groups were influenced by aggregate size in the top two horizons using next generation sequencing (NGS).

2. Materials and methods

2.1. Soil collection

Soil samples were collected from nine (predominantly grassland soils used in livestock production) sites in the North-West of the Republic of Ireland as part of the Irish Soil Information System (Irish SIS) soil survey (Creamer et al., 2014) (project soils collected were: RPM79br01, RPM68br01, RPM66br01, RPR49br01, RPM45br01, RPM85br01, RPG62Br02, RPG52BR01 and RPG63br01). At each sampling site a 1 m^2 × 1 m deep pit was dug. After the pit face was cleaned of loose soil, the profile was designated into distinct horizons following the UN food and agricultural organizations (FAO) guidelines for soil description (FAO, 2006) and classified using the World Reference Base system (WRB, 2006). The top three horizon positions were sampled (the top horizon position from each site is henceforth collectively referred to as H1, the second horizon position as H2 and the third horizon position as H3) to a depth of no more than 1 m, resulting in 26 samples. No samples could be gathered from the third horizon position of RPM66Br01 due to the large amount of stony material present. Details on soil type, drainage, horizon types and depths, textural class as well as sand, silt and clay proportions are provided in the supplementary materials (SM1 and SM2).

Approximately 300 g of soil was collected from each of the described horizons, across the nine soil profiles pits sampled. Samples were collected using aseptic technique as far as was practicable under field conditions i.e., use of 70% ethanol and sterilized water to sanitise equipment between samplings. Furthermore, samples were collected from the lowest horizon up to prevent soil from the upper horizons contaminating the lower horizons. Soil samples were collected into sterile twist-seal bags and kept at 4 °C in a cool box for transportation. Soils were not frozen at this stage to prevent damage to the constituent aggregate structures. Once back in the lab (within 48 h of collection), samples were homogenized and sieved (< 8 mm).

2.2. Soil aggregate isolation

The aggregate isolation procedure was based on a technique described previously (Six et al., 1998). Briefly, soil samples were dried at 40 °C for 1 week. Wet-sieving, with the retention of the material on the sieve as an 'operationally defined fraction' and re-sieving of the soil which passed through the sieve was conducted. This process was sequential through 2 mm, 250 µm, and 53 µm sieves which provided the large macroaggregate (LM), macroaggregate (MAC), and micro-aggregate (MIC) fractions. Material < 53 µm was deemed the silt and clay fraction (SC). The LM, MAC, and MIC fractions were collected into 100 ml containers. Any material, e.g., dead plant material, which floated in the water during the isolation of the LM aggregate size was removed by hand as this was not considered soil organic matter. The SC fraction was collected along with the excess water from the procedure into dual 500 ml containers. All material was dried at 50 °C for 1 week, after which time the samples were weighed and the % proportion of each aggregate size fraction was corrected relative to the bulk soil (Six et al., 1998). To test the replicability of the fractionation procedure, every 10th sample was repeated. Samples were homogenized via mixing and a subsample (approximately half of the material obtained during fractionation) was immediately stored at −80 °C for later molecular work. All laboratory materials pertaining to the fractionation procedure were thoroughly washed in ethanol (70% v/v) between each sample to disinfect equipment. A total number of 104 aggregate size fraction samples were obtained.

2.3. Aggregate course/fine sand determination

Stones > 2 mm were removed from LM and the associated mass subtracted. The amount of coarse and fine sand contained within the MAC and MIC fraction was also calculated as it may distort the true proportion of these aggregates within the bulk soil. Approximately 20 g of soil was placed into a 250 ml Erlenmeyer flask. To remove organic matter, 6% (v/v) hydrogen peroxide (H_2O_2) was added to cover the soil. The solution was boiled at 100 °C with additional H_2O_2 being added to replace that lost by evaporation. Organic matter was deemed to be completely removed when the bubbles formed during the process turned clear. Any excess H_2O_2 was decomposed through the addition of 25 ml of 10% ammonium hydroxide. Contents of the flasks were washed through a series of sieves (250 µm sieve on top and 53 µm sieve on the bottom) to collect the coarse (> 250 µm) and fine (> 53 µm) sands. Once dried, these were weighed and the proportion of coarse and fine sand removed from the MAC and MIC fractions, respectively (Massey et al., 2014).