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Physiological and molecular characterisation of microbial communities associated with different water-stable aggregate size classes

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Abstract

We determined if the structure and function of microbial communities associated with different aggregate size classes was influenced when the aggregate formation occurred under either nitrogen (N) limitation (straw only incubation treatment) or carbon (C) limitation (straw + N incubation treatment). Using a combination of community-level physiological (BD Oxygen Biosensor assay) and molecular (terminal restriction fragment length polymorphism; T-RFLP) profiling methods, we found differences in both microbial community composition and the physiological response of these communities between different aggregate size classes. The response of fungal and bacterial communities to 'straw only' and 'straw + N' treatments differed in that bacterial community composition was affected by the treatments, whereas fungal community composition was not. The magnitude of change in the bacterial community response increased with decreasing aggregate size. However, there were no significant differences in the mean bacterial community richness (number of different terminal restriction fragments; TRFs) between different aggregate size classes for the two treatments. In general, microbial communities associated with larger aggregate size fractions (large and small macroaggregates) were found to have a significantly faster respiratory response than the communities associated with microaggregates. Application of the fungal inhibitor cycloheximide resulted in a significant reduction in the utilization of cellulose, chitin, mannose, xylan, and xylose by the microbial communities associated with all aggregate size classes, indicating that fungi are significant contributors to the utilization of these compounds. Our results demonstrate that the BD Oxygen Biosensor assay offers a valuable new tool for community level physiological profiling. When used in combination with census-based methods such as T-RFLP, a greater level of resolution can be achieved.

Keywords: Soil organic matter; Physical fractionation; Aggregation; Microbial communities; Fungi; Bacteria; CLPP; T-RFLP; Nitrogen; BD oxygen biosensor

1. Introduction

Agricultural management practices affect the structure and functioning of the soil microbial community (Grayston et al., 2001; Buckley and Schmidt, 2003). Increased tillage intensity is often associated with a reduction in fungal:bacterial biomass ratios (Beare et al., 1997; Frey et al., 1999), and mineral N additions have been shown to decrease aggregate formation and fungal biomass, causing a shift in community composition towards a more bacterial-dominated community (Bardgett et al., 1996, 1999a; Bardgett and McAlister, 1999; Bossuyt et al., 2001; Grayston et al., 2001).

While changes in soil organic matter (SOM) dynamics, aggregation, and soil microbial community structure and function are likely to be correlated, there have been relatively few studies that have assessed the microbial community within different aggregate size classes. Kanazawa and Filip (1986) observed that numbers of microorganisms generally decreased with decreasing size of organic and mineral soil particles. Additionally, a decrease in enzyme activity with a decrease in particle size was found, suggesting a drop in substrate concentrations with decreasing particle size (Kanazawa and Filip, 1986). Richaume et al. (1993) found, using direct (acridine orange

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direct count) and indirect (plate counting) enumeration methods, that bacterial numbers varied across soil aggregate size fractions and that the within aggregate size class counts varied depending on the method used. This was hypothesized to be due to inherent differences in the size and physiology of bacterial communities associated with different aggregate size classes (Richaume et al., 1993).

Gupta and Germida (1988) found that cultivation decreased microbial biomass and activity in all aggregate size classes, with the greatest effect observed for macroaggregates. They also observed a decrease in organic C content, microbial biomass C, fungal biomass and respiratory activity with decreasing aggregate size. Guggenberger et al. (1999) reported a higher C content and faster rate of substrate mineralisation in macroaggregates than in microaggregates. Fungal biomass dominated the microbial community in macroaggregates, while in microaggregates bacterial biomass dominated (Guggenberger et al., 1999).

To our knowledge, no studies have investigated the function and community composition of the microorganisms associated with different soil aggregate size classes. Sessitsch et al. (2001) applied molecular methods (terminal restriction fragment length polymorphism or T-RFLP) to examine the structure of bacterial populations associated with different particle sizes, ranging from fine sand, silt and clay, in soils receiving contrasting organic amendments. Microbial community composition was mainly affected by the particle size fraction and to a lesser extent by the organic amendments, and diversity increased with decreasing particle size (Sessitsch et al., 2001). Primary particles (sand, silt and clay) are important in that they, together with uncomplexed SOM, are the building blocks of soil aggregates (Christensen, 2000). However, whereas the particle size distribution of a soil is a static property, the aggregate size distribution is influenced by various biotic and abiotic factors. Therefore, it would be useful to investigate the influence of management practices on the structure and functioning of the microbial communities associated with different aggregate size classes.

T-RFLP analysis is a relatively new tool for microbial community profiling. It has been used in investigations of bacterial community structure and more recently also for studying the structure and diversity of fungal communities (Dunbar et al., 1999; Sessitsch et al., 2001; Klamer et al., 2002; Dickie et al., 2002). The technique relies on the heterogeneity in the position of restriction sites among PCR amplified sequences and on the determination of the length of fluorescently labelled terminal restriction fragments (TRFs) by automated DNA sequencers (Dunbar et al., 2001). It has been shown to be a broadly applicable and sensitive tool for studying relative richness and composition of soil microbial communities.

Community-level physiological profiling (CLPP) methods are commonly used to investigate functional characteristics and diversity of microbial communities (Campbell et al., 1997). Out of all CLPP methods available, Biolog MicroPlate assays are the most widely used. Over 120 scientific papers have been published on the use of different variations of the Biolog MicroPlate assay for characterising bacterial communities, though only a few for fungal communities (Preston-Mafham et al., 2002). However, Biolog results may be biased due to methodological constraints such as inoculum density, indirect measurement of respiratory activity using redox sensitive dyes, or the use of C sources that may not be ecologically relevant. A recent advance in this area is the development of a fluorescence-based microplate platform for assessing dissolved oxygen using the BD™ Oxygen Biosensor System (Garland et al., 2003; BD Biosciences, Bedford, MA). This approach consists of a 96-well microtiter plate containing an oxygen-sensitive fluorophore embedded in a gas-permeable silicone matrix. The fluorescence is quenched by the presence of oxygen, and as oxygen is depleted from the well as a result of microbial respiration, the decreasing concentration of O_2 in the matrix results in a quantitative increase in fluorescence.

Similarly to Biolog, inoculum density also affects the rate of response in the BD-Oxy assay (Garland et al., 2003). Therefore, depending on whether the question being asked concerns actual community function, when the inoculum size is not as important as in if it concerns functional potential, when one would need to ensure that one is actually comparing similar sized units. However, the BD-Oxy approach offers several improvements over Biolog methods, including: (1) lower substrate concentrations (10–100-fold) and shorter incubation times resulting in less potential for selective enrichment, (2) direct measurement of respiration via O_2 consumption compared to indirect measurement of redox dye resulting in a more complete assessment of community activity (including fungi), and (3) full definition and manipulation of chemical factors such as N levels that may influence substrate utilization (Garland et al., 2003). While the Biolog technology offered a rapid means of assessing shifts in bacterial community composition with little to no information on functional changes (Garland et al., 1997), the BD-Oxy approach can be viewed as a multivariate functional assay of increased ecological relevance.

Our objective was to determine how microbial community composition and function in different aggregate size fractions is influenced when the aggregates are formed under C or N limitation. We use ‘straw only’ (N limitation) vs. ‘straw + N’ (C limitation) to designate the treatments. We used both structural (T-RFLP) and functional (BD™ Oxygen Biosensor System) methods for characterising the microbial community. We anticipated that macroaggregates would be most affected by the two treatments in terms of both community physiological response and composition since they are generally considered to represent the most dynamic aggregate fraction.