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Greatest soil microbial diversity found in micro-habitats

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ABSTRACT

Microbial interactions occur in habitats much smaller than those generally captured in homogenized soil cores sampled across a plot or field. This study uses soil aggregates to examine soil microbial community composition and structure of both bacteria and fungi at a microbially-relevant scale. Aggregates were isolated from three land management systems in central Iowa, USA to test if aggregate-level microbial responses were sensitive to large-scale shifts in plant community and management practices. Bacteria and fungi exhibited similar patterns of community structure and diversity among soil aggregates, regardless of land management. Microaggregates supported more diverse microbial communities, and Fimbriimonadales, Acidimicrobiales, Actinomycetales, Alteromonodales, Burkholderiales, Gemmatimonadales, Rhodobacterales, Soligubrobacterales, Sphingobacteriales, Sphingomonodales, Spirobacillaes, Onygenales, Chaetosphaeriales, and Trichosporanales were indicator taxa for microaggregate communities. Large macroaggregates contained greater abundance of Pedosphaerales, Planctomycetales, Syntrophobacterales, and Glomeromycota (arbuscular mycorrhizal fungi). To demonstrate the potential for additional insights into soil microbial diversity, we calculated of a weighted proportional whole soil diversity, which accounted for microbes found in aggregate fractions and resulted in 65% greater bacterial richness and 100% greater fungal richness over independently sampled whole soil (i.e. bulk soil). Our results show microaggregates support highly diverse microbial communities, including several unidentified genera. Isolating aggregates with a microbially sensitive approach provides new opportunities to explore soil microbial communities and the factors shaping them at relevant spatial scales.

1. Introduction

Microbial access to soil organic matter is a key constraint limiting soil organic matter decomposition, affecting cycling and storage of carbon (C) and nitrogen (N) (Schimel and Schaeffer, 2012; Lehmann and Kleber, 2015). Organic matter can be enmeshed into soil aggregates, ranging in size from more than 2 mm to less than 0.25 mm (Tisdall and Oades, 1982). Physical separation, therefore, can limit microbial access to organic matter within aggregates. When microbes are established within an aggregate, microbial community structure and metabolic capacity can also play an important role in processing and protecting soil organic matter.

Soil aggregates and the pores within and around them create microhabitats that support different microbial communities (Ruamps et al., 2011; Rabbi et al., 2016). Within these microhabitats, differences in the quantity and chemistry of organic substrates are likely major drivers of these microbial community differences (Gupta and Germida, 1988; Hattori, 1988; Davinic et al., 2012; Lagomarsino et al., 2012; Smith et al., 2014). Large macroaggregates contain more organic carbon and greater concentrations of less chemically complex and new organic matter inputs (Hofmockel et al., 2011; Davinic et al., 2012). In addition to differences in organic matter, environmental conditions within and between aggregates, such as oxygen concentration, also vary (Sexstone et al., 1985), resulting in diverse niches that harbor different guilds of microorganisms.

Previous work has investigated microbial communities within water-stable aggregates, isolated through a wet-sieving technique (Sessitsch et al., 2001; Mummey and Stahl, 2004; Davinic et al., 2012; Rabbi et al., 2016). Large macroaggregates (> 2 mm) contain more filamentous fungi, which contribute to macroaggregate formation and stabilization (Tisdall et al., 1997, 2012; Rillig and Mummey, 2006; Wilson et al., 2009; Rillig et al., 2015). Water-stable macroaggregates support greater relative abundance of *Actinobacteria*, which form filaments that can bridge and bind soil particles, and *α-Proteobacteria,* whose members perform diverse metabolic functions under wide-ranging oxidation potentials (Sessitsch et al., 2001; Mummey et al., 2006; Davinic et al., 2012). Microaggregates contained more *Rubrobacteriales*, a poorly understood order (Mummey and Stahl, 2004; Davinic et al., 2012).

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Wet-sieving, however, may not best reflect microbial communities and activities for testing hypotheses of *in situ* aggregate communities and functioning because it requires completely drying soil over weeks and rapidly re-wetting the soil. Weeks of drying, typically in non-sterile conditions, is sufficient time for microbial physiology and community structure to shift in response to these conditions (Schimel et al., 2007). Rapid re-wetting and sieving can elevate microbial activity (Bach and Hofmockel, 2014), evoke large releases of stored microbial osmolytes (Schimel et al., 2007), and dissolve and remove soluble organic compounds. An alternative approach partially dries soil in cold, sterile conditions, reducing microbial responses to the laboratory sieving (Schutter and Dick, 2002; Bach and Hofmockel, 2014). Consideration of soil micro-habitats represented by aggregates provides an under-appreciated approach to investigate the factors shaping soil microbial communities and biodiversity, as well as their contribution to ecosystem C and N cycling (Gupta and Germida, 1988; Hattori, 1988). A few previous studies have investigated microbial communities within aggregates using a microbially-sensitive approach. Phospholipid fatty-acid analysis of such aggregates showed that microaggregates had lower fungal:bacterial ratios than macroaggregates, indicating the increased importance of bacteria in these micro-habitats (Helgason et al., 2010; Tiemann et al., 2015). Across individual macroaggregates, bacterial communities were highly variable, indicating aggregate habitats play a key role in supporting the high diversity of bacteria observed in whole soil (Bailey et al., 2013). Our study is one of the first efforts to sequence soil microbial communities within soil aggregates isolated through a microbially-focused approach.

Land management can influence the distribution as well as C and N content of soil aggregates (Elliott, 1986; Six et al., 1998; Baer et al., 2010). Therefore, we investigated soil microbial community structure within aggregates from three land management systems: continuous maize rotation and two reconstructed tallgrass prairie ecosystems, one receiving inorganic N fertilizer input and one without fertilizer inputs. These management systems represent a gradient of C and N inputs. Carbon inputs were least in the maize system, which had 9 times less root biomass than the fertilized prairie and 11 times less root biomass than unfertilized prairie (Jarchow et al., 2015). In contrast, the nitrogen input gradient ranged from no inorganic N inputs in unfertilized prairie to 84 kg N ha^{-1} $year^{-1}$ in fertilized prairie and 200 kg N ha^{-1} $year^{-1}$ in the continuous maize system. Previous work from our research group showed these differences in management systems affected aggregate distribution, turnover, and soil C and N pools and fluxes, including extracellular enzyme activity (Bach and Hofmockel, 2015, 2016). This experimental set-up allowed us to:

1) Test whether bacterial and fungal communities differ at the aggregate-level, and if those differences are affected by land management within the same soil type;
2) Investigate whether microbial community composition at the aggregate-level could provide insight into soil C accrual and stability in these systems.

1.1. We specifically tested two hypotheses

H1. We expected large macroaggregates would support lower diversity as microbes that thrive on abundant and labile organic matter (copiotophs) would outcompete slower growing oligotrophs. Microaggregates would support greater diversity due to access to more chemically complex organic matter in micropores, which would increase niche space, favoring coexistence of many taxa.

H2. We expected increased root biomass in planted prairie systems to support larger and more diverse microbial communities, particularly in large macroaggregates.

2. Materials & methods

2.1. Study site

We collected soil from the Iowa State University Comparison of Biofuel Systems (COBS) experimental site located on the South Reynoldson Farm in Boone County, IA (41°55′14.42″N, 93°44′58.96″W). COBS is a randomized complete block design with four replicates containing 27 m × 61 m plots (Fig. 1a). The present study includes three of the experimental agroecosystem treatments: no-till continuous maize (*Zea mays*, corn), planted tallgrass prairie, and fertilized planted tallgrass prairie. Fertilization rates and application dates can be found in Table S1. Both prairie systems were planted in 2008 with the same seeding mixture of 31 native species. Previous land management for all plots was row-crop maize and soybean (*Glycine max*) rotations for at least 50 years prior to establishment. Details of site establishment including prairie plant species lists can be found in

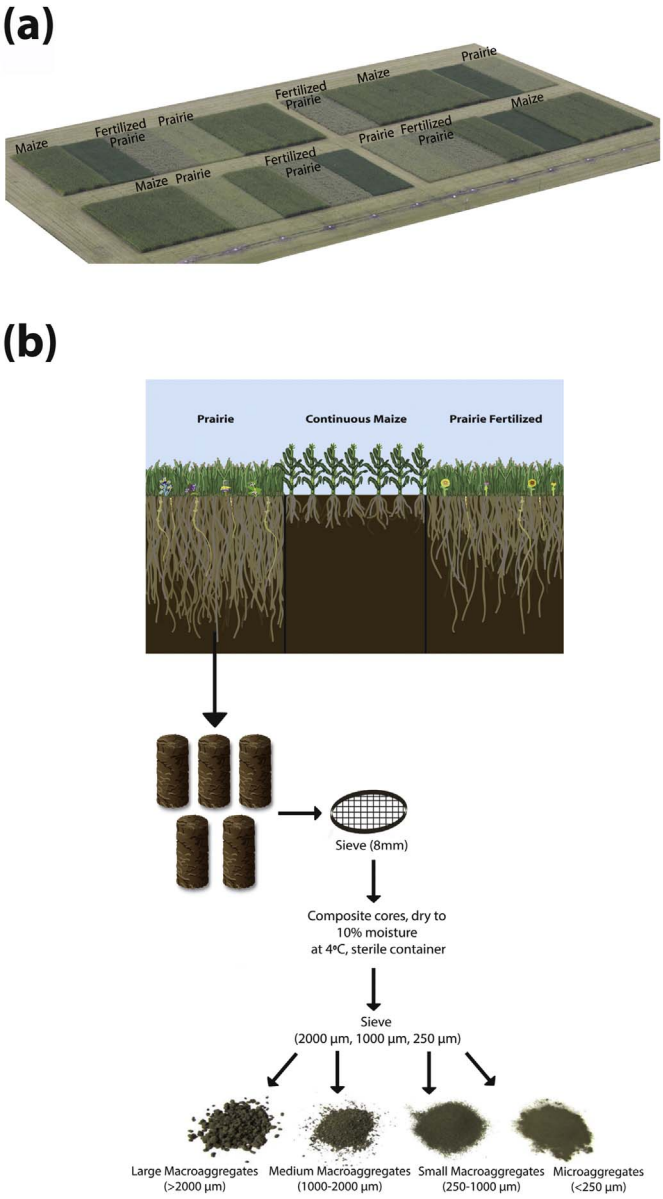

Fig. 1. Comparison of Biofuel Systems (COBS) experimental field site (a), Photo by Tom Schultz, image modified by Amy Sojka. Graphical summary of the optimal moisture soil aggregate isolation method (b), image by Amy Sojka.