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Soil aggregate isolation method affects measures of intra-aggregate extracellular enzyme activity

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ABSTRACT

Within soil aggregates, binding of organic matter is known to occlude it from microbial attack. Within aggregate fractions of different sizes, microbial communities and activities have also been shown to differ. As a result the soil physical structure, organic inputs and microbial activity together impact the rate at which organic matter is decomposed and stored within soil. However, methods developed for isolating soil aggregates may affect subsequent biological assays. In this study, we sought to understand how enzyme activity within soil aggregates is influenced by aggregate isolation methodology, including wet, dry, and 'optimal moisture' sieving procedures within two contrasting ecosystems (a corn agroecosystem and a 2-yr old, diverse planted tallgrass prairie). Mass distribution of aggregates from wet-sieving was skewed toward small macroaggregates (250–1000 μm) and microaggregates (<250 μm), but the distribution of dry and optimal moisture aggregates was highly skewed toward large macroaggregates (>2000 μm). Wet-sieved macroaggregates (>1000 μm) had greater aggregate potential enzyme activity (nmol substrate h^{-1} g^{-1} dry aggregate) than smaller aggregate fractions and whole soil, particularly for C-cycling enzymes cellobiohydrolase and β-glucosidase. Also, wet-sieved aggregates from corn systems had higher potential cellobiohydrolase and β-glucosidase activity than aggregates isolated from prairie. Neither of these relationships was observed in dry and optimal moisture aggregates, suggesting that elevated activities are characteristic of water-stable aggregates and possibly stimulated by soil rewetting. The proportional contribution to total enzyme activity observed in water-stable microaggregates accounted for 46–62% of whole soil activity; although water-stable large macroaggregates (>2000 μm) had greater aggregate enzyme activity, they contributed a minority of overall soil activity. In contrast, the proportional contribution of large macroaggregates comprised 70–78% of whole soil activity when dry sieved and 38–66% under optimal moisture sieving. Wet-sieving soil aggregates is most useful to examine long-term changes in soil organic matter and microbial activity between soil types. Optimal moisture and dry sieved aggregates may be useful alternatives to more closely capture short-term *in situ* measures of seasonal and intra-annual soil microbial activity.

1. Introduction

Soil microbial ecologists face the difficult task of defining and quantifying microscopic habitats and relating ecological interactions in those micro-habitats to ecosystem level processes (Schimel and Schaeffer, 2012). Quantification of constraints on microbial activity within soil microhabitats is needed to better understand processing and stabilization of soil organic matter that result in storage of carbon (C) and nitrogen (N) in soils or respiration of greenhouse gases (Schmidt et al., 2011). Soil physical structure, specifically soil aggregation, is emerging as a critical factor influencing soil microbial communities and activities e.g. (Petersen et al., 1997; Schutter and Dick, 2002; Allison and Jastrow, 2006; Ndour et al., 2008; Briar et al., 2011; Davinic et al., 2012; Bailey et al., 2013). Soil aggregates, and more specifically intra-aggregate pore spaces, are the habitats in which microbes live and the physical constraints on microbial colonization, substrate availability, water and gas movement through these pores determines the environment in which microbes perform biogeochemical reactions that drive ecosystem functioning.

The most widely used traditional approach for soil aggregate isolation is wet-sieving. Wet-sieving aggregates involves submerging air dried soils in deionized water (slaking) and sieving by hand or machine through a series of sieves (Yoder, 1936; Elliott, 1986; Fig. 1). Slaking soils generates a pressure gradient in which entrapped air causes disintegration of all but the most structurally

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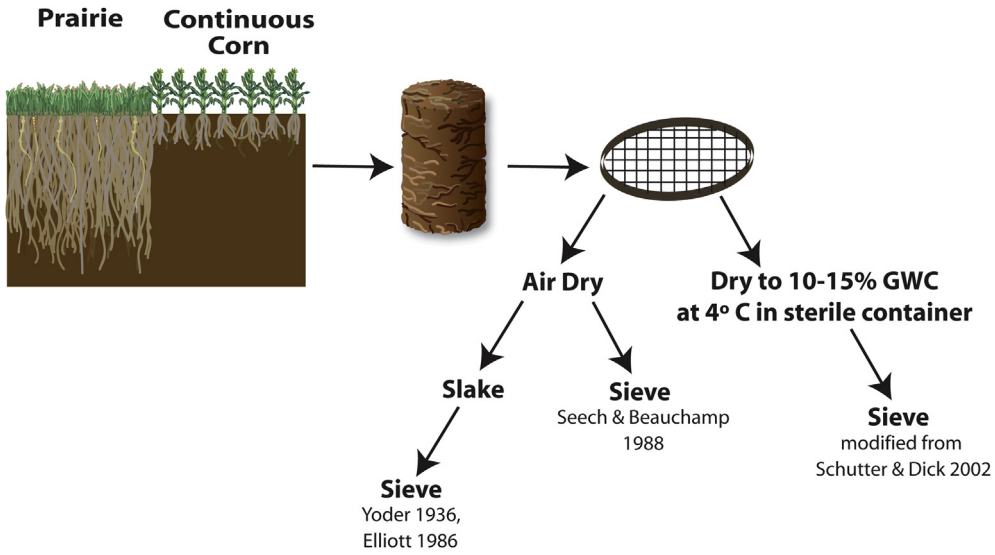

Fig. 1. Schematic flow of aggregate sieving methods and pre-treatments. Field soil was collected as an intact core, gently passed through 8 mm sieve and either air-dried in preparation for dry and wet-sieving or partially dried at 4 °C in sterile containers for optimal moisture sieving.

stable aggregates (LeBissonnais, 1996; Saygin et al., 2012). Soil scientists adopted the practice of slaking to represent soil surface disruption by rain and quantify potential erosion (Yoder, 1936; Elliott, 1986). However, the extent to which soils experience slaking forces *in situ* is not well understood. Wet-sieved aggregates are widely reported in the literature to differentiate soil structure and long-term changes in C-cycling pools between soils (Cambardella and Elliott, 1994; Jastrow, 1996; Six et al., 2000a; Denef et al., 2004; Wilson et al., 2009; Chivenge et al., 2011). Soil ecologists are also looking to soil aggregates as a means to detect differences in microbial communities and activities at a microbial scale. Wet-sieving has been a natural tool for isolating aggregates for biological analyses including extracellular enzyme assays (Fansler et al., 2005; Allison and Jastrow, 2006; Ndour et al., 2008; Lagomarsino et al., 2012), T-RFLP (Mummey and Stahl, 2004), PLFA (FAME) (Petersen et al., 1997; Kong et al., 2011), and pyrosequencing (Davinic et al., 2012). These exciting and valuable studies have provided evidence that certain aggregate fractions support elevated microbial activity and potentially unique communities; however, more data is needed to relate observations at the aggregate scale to microbially-driven ecosystem processes.

Coupling measures of extracellular C-cycling enzyme activity within the same wet-sieved fractions can be an effective approach to investigating changes in microbial activity that drive long-term cycling of C (Fansler et al., 2005; Marx et al., 2005; Allison and Jastrow, 2006; Dorodnikov et al., 2009). However, enzyme turnover times have been estimated on the order of hours to days (Allison, 2006). Mineral-stabilized enzymes may persist for months to years (Tabatabai and Dick, 2002), but mineral-stabilization may also inhibit the ability for enzyme active sites to bind with substrate (Allison, 2006). Additionally, it is well established that there is a pulse of microbial activity, releasing CO_2 when dry soils are rewetted (Stark and Firestone, 1995; Halverson et al., 2000; Fierer and Schimel, 2003), termed the 'Birch effect' (Birch and Friend, 1956). This flush of CO_2 has been detected within minutes of wetting in both the laboratory and field experiments (Borken et al., 2003; Lee et al., 2004; Sponseller, 2007) and is attributed to rapid microbial mineralization of soluble organic C compounds, considered end products of extracellular enzyme reactions. Thus, it is unclear how to interpret extracellular enzyme activity within wet-sieved aggregates in a broader ecosystem context.

Air-drying soil prior to slaking is important for determination of water-stable aggregate distribution as it maximizes the pressure gradient from air entrapped in aggregates to the surrounding water (Chenu and Cosentino, 2011). However, air-drying soils prior to sieving can impact measures of soil microbial communities and activities (Sparling and Cheshire, 1979; Wollum, 1994; Boone et al., 1999). Extracellular enzyme activity and microbial biomass have been shown to decrease after air-drying soils, especially when field-moist soils are not in drought condition (Zornoza et al., 2006, 2007; Wallenius et al., 2010; Peoples and Koide, 2012). In some studies, field-moist soil is slaked directly, avoiding this additional complication (e.g. Allison and Jastrow, 2006; Ndour et al., 2008; Davinic et al., 2012). Field-moist soils with high moisture content will experience lower energy disruption during slaking because pores will already contain water, reducing the pressure gradient generated by slaking and leaving more macroaggregates intact. Aggregate-disrupting energy needs to be consistent to compare replicate samples within a study, and results across studies (Yoder, 1936; Panabokke and Quirk, 1957; LeBissonnais, 1996; LeBissonnais and Arrouays, 1997). Wet-sieving field moist samples can generate variable aggregate fraction distributions and result in heterogenous aggregate stability within isolated size fractions (Marquez et al., 2004), which could obfuscate ecological patterns and impede interpretation of intra-aggregate biology at an ecosystem scale.

Since it is often difficult to perform biological assays immediately following aggregate isolation, aggregates are commonly frozen prior to analysis. Freezing field-moist soils can affect extracellular enzyme activity (DeForest, 2009; Peoples and Koide, 2012), phospholipid fatty acids (Liu et al., 2009; Wu et al., 2009), and nucleic acids (Lauber et al., 2010; Rissanen et al., 2010). Although effects of freezing field-moist soils prior to biological measurements are well documented, the effects of freezing saturated soils, such as water-stable aggregates, are less understood. Flash-freezing saturated soils in liquid N has been shown to reduce denitrification enzyme activity (DEA) and nitrification enzyme activity (NEA) (Cooke, 1990). Freezing flooded paddy soils had no significant effect on total PLFA concentration, but did affect the distribution of PLFA types (Liu et al., 2009). Freezing of water-saturated aggregates is a