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The role of macro-aggregation in regulating enzymatic depolymerization of soil organic nitrogen

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ABSTRACT

Extracellular enzymatic depolymerization of polymeric organic nitrogen (PON) is a rate-limiting step in N mineralization. However, enzymatic accessibility to PON might be regulated by physical occlusion of the PON resulting from the architectural packing of soil minerals during aggregate formation. To examine the extent to which enzymatic accessibility to PON is regulated by soil aggregation, we put forward a new approach involving the comparison of relationships between potential N depolymerase activity (protease and β-glucosaminidase; as an estimate of the *potential* to produce depolymerized products) and net N mineralization (as a bioassay for *actual* low molecular weight dissolved ON production) in aggregated and corresponding disaggregated soil. Soils were sampled from grassland (GL) and arable land (AL), separated by dry sieving into fractions (4.75–2, 2–0.25 and 0.25–0.063 mm) and fractions mixed (4:4:1 by mass, respectively) to obtain constructed aggregated soils. Corresponding disaggregated soils were prepared using a mortar and pestle. This procedure mainly disrupted the 4.75–2 mm (large macro-aggregate) fraction. Disaggregation significantly promoted ($p < 0.05$) net N mineralization rates by 1.3 times and 1.5 times in GL and AL soil, respectively. When net N mineralization - potential N depolymerase relationships for GL were examined, a greater slope parameter for disaggregated compared to aggregated soil ($p = 0.001$; ANCOVA) quantified the extent to which this promoted N mineralization could be attributed to disruption of macroaggregate-increased enzymatic accessibility to PON. For AL, which had low protease and β-glucosaminidase activity, promoted N mineralization rate could not be attributed to increased protease + β-glucosaminidase accessibility to PON reflecting a possible role for other N depolymerases and/or osmolyte/lysate effects. By proposing how differences between mineralization-depolymerase relationships for soils differing in aggregation status might, with assumptions, be interpreted to identify the role of physical occlusion in protection of PON, we give new insight on the regulation of enzymatic depolymerization by physical protection through macro-aggregation for soils from contrasting land use.

1. Introduction

Nitrogen (N) availability is the most important factor for ecosystem productivity, and soil organic matter (OM) is a sink and source of nitrogen for plants (Schulten and Schnitzer, 1997). In surface soil, up to 90% of nitrogen is stored as organic N in soil OM (Olk, 2008). The transformation of polymeric organic N (PON) to plant available forms depends initially on depolymerization mediated by extracellular enzymes (Geisseler et al., 2010) to yield monomeric/lower molecular weight dissolved organic N (LMW DON) which already may be plant-available (Schimel and Bennett, 2004; Jones et al., 2005) and also readily mineralizable to inorganic N (Schimel and Bennett, 2004). These extracellular enzymes may be of microbial, plant and animal origin (Vranova et al., 2013) and the depolymerization process appears to be the rate-limiting step in N mineralization (Schimel and Bennett, 2004; Jan et al., 2009).

However, depolymerization of PON could be regulated not only by the biochemical reactions described above but also by physical and chemical factors that alter the accessibility of PON substrates to

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the extracellular enzymes that act on them. While representing a chemical continuum of structures derived from the progressive decomposition of organic macromolecules, soil OM (with constituent N) has been conceptualized as belonging to discrete pools differing in their susceptibility to decomposition and the mechanisms by which the OM is stabilized, namely: (i) physical inaccessibility through occlusion within soil mineral or aggregate architecture; (ii) chemical interaction between OM and inorganic constituents (e.g., sorption, organo-metal cheletion) (Sollins et al., 1996). Polymeric OM could also be biochemically inaccessible to enzymatic attack through inherent or acquired recalcitrance of chemical structure (Six et al., 2002) but the importance of biochemical stabilization through molecular recalcitrance of soil OM has been questioned quite recently and greater importance given to the influences of physical occlusion and chemical interaction (Six et al., 2004; Schmidt et al., 2011; Dungait et al., 2012; Lehmann and Kleber, 2015). Much of the discussion of the mechanisms of persistence of soil OM has been focused on organic carbon, however, the accessibility of soil PON to enzymatic depolymerization might also be viewed within the same framework (Olk and Gregorich, 2006; Brzostek and Finzi, 2011). It is well established that soils contain significant potential activity of depolymerases that are involved in the breakdown of the proteinaceous and chitinaceous OM (Allison and Jastrow, 2006; Geisseler et al., 2010; Vranova et al., 2013) that represents a significant proportion of soil PON (Geisseler et al., 2010). However, the extent to which physical occlusion and mineral associations prevents this activity from being realized with respect to N mineralization has not been explicitly examined (Benbi and Richter, 2002).

A significant mechanism for the physical occlusion of OM results from the architectural packing of soil minerals during aggregate formation (Golchin et al., 1994), which traps OM within pores created. Previous studies have reported that disaggregating soil structure, either through soil tillage or by soil physical treatments imposed in the laboratory, promotes N mineralization (Cabrera and Kissel, 1988; Balesdent et al., 2000). This disaggregation-promoted N mineralization might be consistent with the suggested role that physical occlusion within aggregates plays in limiting the accessibility of PON for decomposition. However, this promotion might also occur due to the physiological release of mineralizable osmolytes by microbial cells in response to disaggregation, for example, on exposure of cells that were previously inside aggregates to dehydration and rewetting (Navarro-García et al., 2012; Borken and Matzner, 2009; Halverson et al., 2000; Fierer and Schimel, 2002) or as a result of the rupture of macroaggregate-binding fungal hyphae (Jastrow et al., 2007; Hobbie and Hobbie, 2012). Quantifying the contribution of the release of PON from physical constraints to depolymerization to the promotion of N mineralization on disaggregation, to our knowledge, has not previously been attempted, potentially due to lack of approaches to untangle this contribution from that of the mineralization of osmolytes/lysates produced as a result of disaggregation.

Accordingly, our overall aim is to better understand the extent to which the promotion of N mineralization following the disruption of soil aggregates can be explained by release of PON from physical constraints to depolymerization rather than by osmolyte/lysate release. To do this, we put forward an approach involving the comparison of relationships between potential N depolymerase activity (as an estimate of the *potential* to produce depolymerized products) and net N mineralization (as a bioassay for *actual* LMW DON production) in aggregated and corresponding disaggregated soil. We apply this analysis to grassland and arable soil with the additional aim of understanding how the contribution of PON release to the flush in N mineralization on disaggregation varies with land use.

2. Materials and methods

2.1. Soil sampling and construction of "aggregated" and "disaggregated" soils

Soil samples (0–~20 cm depth) were taken from random locations within grassland (GL; N = 6) and arable (AL; N = 5) fields from the University of Reading farm (Sonning, Berkshire, U.K.; NGR: SU765765) on 15/05/2015. Following air-drying, "constructed aggregated" soils were prepared by sieving to obtain 4.75–2 mm, 2–0.25 mm and 0.25–0.063 mm size fractions and then by mixing these fractions, on a mass basis, in the following respective proportions: 4:4:1 (to approximately represent the proportions initially present in GL soil, Supplementary Fig. 1). The size classes were chosen to represent macro-aggregate (2–0.25 mm) and micro-aggregate (0.25–0.063 mm) fractions (Six et al., 2000) and large macro-aggregates (4.75–2 mm) and the same proportions of these size classes were used for both soils so that we could examine land use effects on the nature of the protection provided by aggregates with the same initial size distribution. Corresponding "constructed disaggregated" soils were prepared by disruption of a subsample of the constructed aggregated soil by grinding using a pestle and mortar until no further disaggregation could be achieved, as judged by eye. Selected properties of the constructed soils are shown in Table 1. Fig. 1 shows the percentage, on a mass basis, of the four different fractions (4.75-2 mm, 2–0.25 mm, 0.25–0.063 mm and <0.063 mm) in the constructed soils prior to and after disaggregation. The constructed soils were kept in the air-dried state at room temperature until sub-sampled for use in N mineralization and enzyme assays. Sub-samples for enzyme assays were processed within 14 days of the commencement of the net N mineralization assay.

2.2. Net anaerobic N mineralization

Constructed aggregated and disaggregated soils (54 g) were put into 100 mL flasks and the water content adjusted to 100% of water filled pore space (WFPS) as calculated using the bulk density and a soil particle density of 2.6 g cm^{-3}. After the flasks were flushed with N_2 gas for 2 min, the flasks were sealed with rubber stoppers and incubated at 26°*C* for 10 days. At the end of the incubation, inorganic N was extracted with 1 M KCl (200 ml, 30 min). The net N mineralization rate was determined by subtracting NH_4^+ measured at the beginning of the incubation (Day 0; Table 1) from NH_4^+ concentration measured on Day 10 (Hart et al., 1994) and expressed as mmol N kg^{-1} OD-$soil^{-1}$ 240 h^{-1}.

2.3. Potential N-acquiring enzyme activity assays

Protease activity was determined by measuring the concentration of tyrosine produced through depolymerization of Na-caseinate as described by Ladd and Butler (1972) and Geisseler and Horwath (2008). Briefly, aggregated or disaggregated soils (1 g air-dried basis) in autoclaved glass vials were amended with Tris buffer (2.5 mL, pH 8.0 modified with 1 M HCl) and Na-caseinate (2.5 mL, 2%) and incubated at 50°*C* for 2 h. Trichloroacetic acid (TCA, 5 mL, 10%) was then added to stop the reaction and a 1.5 mL aliquot centrifuged (16000×*g*, 2 min). Na_2CO_3 (0.9 mL, 1.4 M) and diluted Folin-Ciocalteu reagent (0.3 mL, water: Folin-Ciocalteu = 3:1; Sigma-Aldrich) were added to an aliquot (0.6 mL) of the resulting supernatant and the absorbance at 680 nm determined after 5 min using a spectrophotometer. Blank incubations followed the above procedure except Na-caseinate was added to the samples after the incubation and addition of TCA. Blank readings provided an estimate of concentrations of tyrosine and other