



## Vertical changes of soil microbial properties in claypan soils

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### ABSTRACT

Microbial activity within the soil is critical for plant growth and development, and a major determinant of crop performance and yield. Claypan soils are characterized by a dense, impermeable subsoil that impedes root system development. Little is known about soil microbial properties in claypan soils or how microbial activity changes with depth in the soil profile. We explored how management practices mediate changes in soil microbial composition and potential enzyme activities with depth in a claypan soil. The soil microbial biomass and composition were examined through phospholipid fatty acid (PLFA) assay. We found that the soil organic carbon (SOC), microbial biomass, and oxidative enzyme activities declined with depth, while hydrolase activity increased in the upper layer of the claypan. Changes in soil management practices affected the degree of increase in hydrolase activity in subsoils, especially for N-acetyl- $\beta$ -D-glucosaminidase. No accumulation of SOC in the claypan layer was observed. Contrary to our expectation, soil microbes deeper within the soil profile were phosphorus- and nitrogen-limited rather than carbon-limited. Vertical stratification of measured soil properties was found with an upper layer from 0 to 15 cm, an intermediate layer between 15 cm and approximately 30 cm, and the lowest layer of soils in the claypan below 30 cm. The interaction between clay content and changes in soil factors with depth resulted in an increased potential activity but unaltered microbial composition in the claypan layer.

### 1. Introduction

Claypan soils cover approximately four million hectares in the central US, including portions of Illinois, Iowa, Kansas, Missouri, and Oklahoma (USDA-NRCS, 2006). Claypan soils are characterized by a dense, impermeable clay layer in the subsoil covered by silt loam soil at the surface. The soils can be productive, but the productive capacity is often limited by shallow topsoil depth. There is no clear delineation of clay amount, but a typical description is a sharp increase in clay over an abrupt boundary (Buckley et al., 2008). It is not known how the textural changes in claypan soils impact microbial activity and communities, or the potential impact of the soil microbial activity on plant production in claypan soils.

The clayey subsoils of the Cherokee Prairies ecoregion in the tall-grass prairie in southeast Kansas are classified as smectite dominant or smectite and kaolinite mixed mineralogy (Hartley et al., 2014). They were formed by clay translocation and loess deposition on top of clayey alluvium or residuum weathered mainly from Pennsylvanian shale and limestone (USDA-NRCS, 2006; Hartley et al., 2014). The claypan layer

stores water. In southeast Kansas, the volumetric moisture content in the claypan layer generally exceeds 25% even in the dry season, compared to around 10% in surface soils, although the plant-available water is low in the claypan layer due to the high water retention by the clay (Buckley et al., 2008; Hartley et al., 2014). The low hydraulic conductivity of the clayey layer creates saturated surface soils after rainfall events, impairing root growth and exacerbating soil erosion (Soil Survey Staff, 2012). Moreover, plant roots do not develop extensively in the clay layer (Myers et al., 2007). Soil responses to crop management practices including crop rotation, irrigation, and tillage may be different on claypan soils than on well-drained soils (Buckley et al., 2008).

Inherent soil properties, such as clay, silt or sand composition and parent material, impact microbial properties directly or indirectly through nutrient distribution and stabilization (Solly et al., 2015). Amino sugars and carbohydrates tend to concentrate in clays, while phenolic compounds and fatty acids are more abundant in silts (Paul, 2016). Soil C in the topsoil is mainly associated with macroaggregates as a mineralizable resource, and vegetation and root exudates strongly

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influence soil organic carbon (SOC) stability. Conversely, below 30 cm in the soil profile, soil C absorbed by clay or other minerals is more protected from mineralization and stabilized as a C sink. Aluminum complexes have been shown to contribute to C stability and microbial activity, and soil pH changes both the solubility of the metal-humus complex and microbial properties, as well as enzyme activities and community composition (Heckman et al., 2009; Paul, 2016). Previous studies reported that clay content and clay mineralogy influenced enzyme kinetics through a reduction in the substrate turnover (Kcat) but an increase in the half-life of enzymes; thus the impact of clay on soil enzymes was not consistent (Fuka et al., 2008; Burns, 2013; Burns et al., 2013; An et al., 2015). The clay content of soil modifies the microbial community structure by favoring bacteria over fungi (Wei et al., 2014).

Microbial properties are different in subsoils than in surface soils. Soil nutrients, microbial biomass, and hydrolase activities decreased exponentially with depth in several studies (Allison et al., 2007; Eilers et al., 2012; Stone et al., 2014), but oxidase activities in subsoils were reported to be stable or even higher than in topsoils in taiga ecosystems (Schnecker et al., 2015). The soil organic matter (SOM) chemistry and spatial separation, rather than SOM content, had a greater influence on enzymatic activities in the subsoil (Stone et al., 2014; Schnecker et al., 2015). Microbial community composition was also found to shift with depth, along with a decline in fungal:bacterial ratios, an increase in Gram-positive and sulfate-reducing bacteria, and a decrease in Gram-negative bacteria (Allison et al., 2007; Stone et al., 2014). Microbial communities in deep soils were relatively similar regardless of landscape position or cropping systems (Allison et al., 2007; Eilers et al., 2012). A laboratory soil incubation study reported that the microbes in the subsoil had higher utilization of amino acids, whereas the microbes in topsoil showed higher C mineralization (Tian et al., 2017).

Soil management practices also drastically affect the soil environment. Tillage is known to have negative impacts on soil nutrients, pH, and biological properties (Roger-Estrade et al., 2010; Capelle et al., 2012; Mbuthia et al., 2015). Soil C and pH can further change soil microbial communities (Allison et al., 2007; Kaiser et al., 2016). Fungi were found to be more abundant than bacteria in no-till agricultural systems because of less disruption of fungal and plant communities (Hendrix et al., 1986). Studies have found that nutrient concentration is the predominant factor determining enzyme activity and microbial composition where climate conditions are not limiting (Margalef et al., 2017).

Crop production on claypan soils requires careful management to maintain productive capacity. Understanding the impact of management practices on soil microbial properties can be useful to sustain soil health in claypan soils. However, microbial properties within claypan soils are poorly characterized. The objective of this study was to assess how management practices mediate changes in soil microbial properties with depth in a claypan soil. We examined three production systems: conventionally tilled crop production, no-till crop production, and long-term grass (hay meadow). Soil extracellular enzyme activity was used as an indicator of microbial functional diversity, and phospholipid fatty acid (PLFA) profile was used as an indicator of the microbial community structure and living microbial biomass. Soil characteristics were measured with depth, including texture, pH, soil water content, and nutrient contents.

## 2. Materials and methods

### 2.1. Study sites and experimental design

A 3.8 ha long-term research field located in Cherokee County, Kansas (37.21 N, 94.87 W) was used in this study. The experiment was a complete randomized design with uneven replications. Seventeen test plots were used: six for long-term conventional tillage row crop production (CT; plot size 9.1 m × 21.3 m), eight for long-term no-till row crop production (NT; plot size 9.1 m × 36.6 m), and three for grass (hay

meadow, HM; plot size, 22.9 × 61 m). The CT practice included chisel plowing and disking prior to planting corn; and disk harrow after corn harvest prior to wheat planting. Soybean was planted by no-till after wheat harvest. All crops are grown each year in the long-term rotation study. The hay meadow was mowed twice yearly. Nitrogen, phosphorus, and potassium fertilizers and herbicides were applied according to standard agricultural practices for each production system.

The mean annual temperature for the area was 14.4 °C, with average annual precipitation of 1157.3 mm. The predominant soil type in the field is a Parsons silt loam (Fine, mixed, active, thermic Mollic Albaqualfs) with 0.2% slope. It is an Alfisol that has an abrupt textural change between the mollic epipedon and the argillic horizon, with low saturated hydraulic conductivity, rich ferrous iron, and aquic moisture conditions (Soil Survey Staff, 2014). Parsons silt loam soils are characterized as fertile surface soils with poorly drained subsoils that formed in clayey old alluvium or residuum weathered from sandstones, shales, and limestones of Permian, Pennsylvanian, and Mississippian age. This soil is common to the claypan region of the Midwest.

### 2.2. Soil sample processing

Soil samples were collected near the end of June 2015 from corn and soybean plots and the hay meadow fields using a tractor-mounted hydraulic press (Giddings, Windsor, CO). Within each plot, two 75-cm deep soil cores (diameter 7.6 cm) were collected at random locations and partitioned into seven depth intervals. Samples were refrigerated at 4 °C and transported to the Soil Microbial Ecology lab in Manhattan, KS. Cores from each plot were composited by depth, homogenized, and subsampled for subsequent analyses. Subsamples for physical and chemical properties were air-dried, ground, and sieved through 2 mm mesh. Subsamples for microbial properties were stored at –20 °C.

### 2.3. Soil physical and chemical properties analysis

Soil gravimetric moisture content was determined after oven-drying samples for 24 h at 105 °C. Soil particle size analysis was completed using the standard pipette method (Kilmer and Alexander, 1949). Soil pH was determined in a 1:10 soil:water slurry. Total C and total N concentrations of soils were determined by dry combustion analysis using a Carlo-Erba C and N analyzer (Thermo Finnegan Flash EA1112, Milan, Italy). Soil properties were measured using the standard Mehlich-3 method (Frank et al., 1998) for extractable P and the standard ammonium acetate method for both extractable K (Warncke and Brown, 1998) and cation exchange content (CEC; Chapman, 1965) at the Soil Testing Lab at Kansas State University, Manhattan, KS.

### 2.4. Extracellular enzyme activities

The potential activities of hydrolases were measured following a modified fluorometric method using fluorometric substrates 4-methylumbelliferone (MUB), and the potential activities of oxidases were measured using colorimetric substrate L-3,4-dihydroxyphenylalanine (L-DOPA) (Zeglin et al., 2013). Hydrolase assays included a C-acquiring enzyme ( $\beta$ -glucosidase, bG, EC 3.2.1.21), a phosphorus- (P) acquiring enzyme (acid phosphatase, AP, EC 3.1.3.2), and a nitrogen- (N) acquiring enzyme (N-acetyl- $\beta$ -D-glucosaminidase, NAG, EC 3.2.1.30). The bG hydrolyzes  $\beta$ -D-glucopyranosides in the degradation of cellulose. The NAG cleaves the amino sugar N-acetyl- $\beta$ -D-galactosamine from chitin in soils. Preliminary data indicated that leucine-aminopeptidase (LAP) activity was relatively low compared to NAG, which is common in acid-to-neutral soils such as those in this study. Therefore, we examined only NAG activity levels in the soils. Acid phosphatase releases inorganic P from soil organic matter into biologically available forms. Oxidase assays included two main categories of lignin degradation enzymes: phenol oxidase (POX, EC 1.10.3.2) and peroxidase (PER, EC 1.11.1.7). All assays were run at room temperature in 50 mM pH 5

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