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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-β-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 tallgrass 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 (β-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-β-D-glucosaminidase, NAG, EC 3.2.1.30). The bG hydrolyzes β-d-glucopyranosides in the degradation of cellulose. The NAG cleaves the amino sugar N-acetyl-β-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

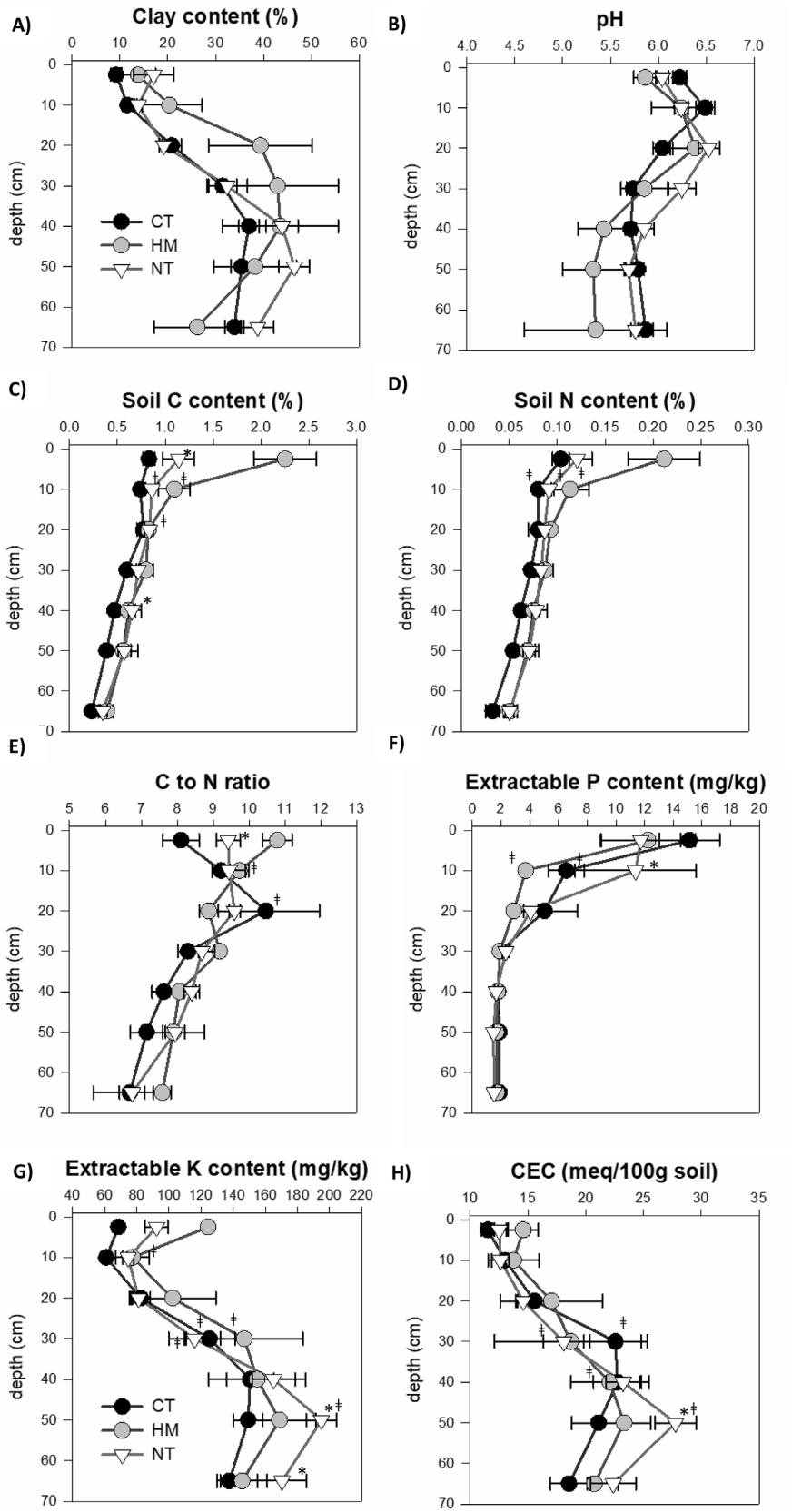

Fig. 1. Change of selected environmental variables with depth for different management practices (mean ± standard error of the mean (SE), nCT = 6, nNT = 8, nHM = 3). * significant difference between NT and CT soils at the same depth at the 90% confidence level. ǂ significant difference with upward depth intervals at the 90% confidence level.

acetate buffer for 1 h for bG, 2 h for AP, 4 h for NAG, or 18–20 h for POX and PER. Buffer blank, soil blank, negative control, MUB reference standard, and quench control were measured for each sample. Fluorescent absorbance was determined by a Multi-Mode Microplate Reader (FilterMax F5, Molecular Devices, Sunnyvale, California) with 365/450 nm excitation/emission for hydrolases and 450 nm absorbance for oxidases. Potential enzyme activities were reported as nanomoles activity per gram of dry soil per hour. Enzyme activities were normalized relative to total PLFA microbial biomass as a proxy for specific activity.

2.5. Phospholipid fatty acid (PLFA) analysis

The PLFA procedure was modified from the White and Ringelberg method (White and Ringelberg, 1998; Zeglin et al., 2013). Total lipids were extracted using 10 mL of methanol, 5 mL of chloroform, and 4 mL of phosphate buffer (pH 7.4) on 5 g freeze-dried soil. Water and chloroform were added 3 h after extraction to separate the mixture into polar and nonpolar fractions, while total lipids remained in the nonpolar phase. Phospholipids were separated from neutral lipids and glycolipids using silicic acid chromatography columns (Disposable BAKERBOND® SPE Columns, J.T. Baker®) and eluted with methanol. The phospholipids were then saponified by KOH, methylated to fatty acid methyl esters (FAME), and analyzed with a Thermo Scientific Trace GC-ISQ mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts) equipped with a DB5-MS column (30 m × 250 μm in diameter x 0.25 μm film thickness; Agilent Technologies, Santa Clara, California). Helium was used as the carrier gas; FAME peaks were recognized by retention time in comparison with the bacterial acid methyl esters mix (BAME; Matreya 1114; Matreya LLC, Pleasant Gap, Pennsylvania). Internal standards 19:0 FAME were used to determine concentrations. A total of 36 biomarkers were identified from all soil samples. Microbial groups were assigned based on characteristics of biomarkers: iso and ante-iso branched lipids often belong to Gram-positive bacteria; monosaturated and cyclopropyl lipids often belong to Gram-negative bacteria; actinobacteria have more methyl branched fatty acids; methyl linoleate (18:2ω9,12c) is typically found in fungi. Phospholipid fatty acid abundance was reported as nmol per gram of dry soil. The fungal:bacterial ratios were calculated by dividing the sum of fungal biomarkers by the sum of Gram-positive bacteria, Gram-negative bacteria, and actinomycetes. Microbial biomass was estimated as the sum of all PLFA biomarkers.

2.6. Data analysis

For analyzing depth and management practice effects on soil physio-chemical and microbial properties, a randomized, repeated-measures design with uneven replications was used that included management practice as a fixed factor, depth as a random factor with repeat measurements, and the variance of depth as covariance. Two-way analysis of variance (ANOVA) in SAS (University edition, SAS® Institute Inc., Cary, NC, USA) was used. All errors are reported as standard error. Means separation was performed using LSMEANS statement and PDIFF option test at $P \leq 0.1$ for unbalanced designs. Pairwise comparisons were made between management practices and depth intervals for each soil property. The relation between the measured soil parameters and depth or management practice factors was summarized using principal component analysis (PCA) in R (R Development Core Team, 2013). The k-means clustering algorithm was incorporated with PCA to partition out observations into three clusters with the nearest mean. The composition of the soil microbial community was summarized using PCA on the relative abundance of PLFAs in each sample. Pearson's correlation coefficient (R^2) was used to determine the degree of association between the measured parameters and the depth or management factors.

3. Results

3.1. Soil physical properties

The depth to the clay layer and the percent clay content varied by location in the field (Fig. 1A; Fig. S1). The clay content in the top 5 cm of soils was 10–20% and increased with depth to more than 50% at 20–40 cm depth. In general, the HM plots had the shallowest depth to the claypan.

The gravimetric soil water content was lowest in the top 5 cm of the soil and gradually increased with depth (data not shown). The gravimetric moisture content was significantly positively correlated with soil clay content with Pearson's R^2 of 0.81 (**$P < 0.01$; Fig. S2A).

3.2. Soil chemical properties

Soils were moderately acidic (pH 6) in the top 5 cm, with soil pH in CT (6.3) slightly higher than NT (6.0), followed by HM (5.8) (Fig. 1B; **$P = 0.0029$). Soil pH increased slightly to a maximum near 20 cm and then declined to an average of 5.5 for all management practices.

Soil organic C content decreased rapidly with depth (Fig. 1C; ***$P < 0.001$). In the top 5 cm, the HM had more than 2% of SOC content on average but the cropped soils had only 1% SOC; carbon in the NT soil was greater than in the CT soil (*$P = 0.0027$). Below 30 cm, the SOC level decreased to less than 0.8% for all management systems, with HM and NT soils significant higher than CT only at 40 and 50 cm ($P_{HM} = 0.0763$, $P_{NT} = 0.0681$). The SOC was positively correlated to the change in clay content with Pearson's R^2 of 0.70 within the claypan layer (soils below 35 cm; Fig. S2B).

Soil total N content showed a similar decline as C with depth (Fig. 1D; ***$P < 0.001$), with nearly a two-fold greater soil N level at the surface of HM than in either of the cropping systems. A level of 0.1–0.15% total N is considered low for agricultural soils in Kansas (Leikam et al., 2003). Soil total N was greatest in HM soils in the top 15 cm, followed by NT and then CT soils, though the difference was only significant for the HM. Below 30 cm, total N decreased to less than 0.9%, then decreased steadily to 0.05% at 65 cm depth.

Soil C:N ratio decreased with depth (Fig. 1E; ***$P < 0.001$). The C:N ratios in both HM and NT were greater than in CT in the top 5 cm (*$P_{HM} = 0.0034$, $P_{NT} = 0.0557$), while the difference between HM and NT was non-significant. No differences in C:N ratios were measured for either hay meadow or cropland soils below 5 cm.

Soil extractable P decreased sharply with depth (Fig. 1F; ***$P < 0.001$). The measured level of 10–20 mg kg^{-1} extractable P in the top 5 cm of soil is considered low for agriculture fields in Kansas (Leikam et al., 2003). Extractable P content in NT was significantly greater than in both HM and CT at 10 cm (*$P_{CT} = 0.0426$). Below 30 cm, extractable P content stabilized around 1.5 mg kg^{-1}, with virtually no detectable differences between management practices. Soil organic C and total N contents were closely correlated, and extractable P was moderately correlated to both SOC and total N contents (Fig. S2A).

In contrast to changes in C, N and P with depth, soil extractable K content decreased slightly and then increased with depth to a maximum near 180 mg kg^{-1} at 50 cm (Fig. 1G; ***$P < 0.001$). The extractable K content was significantly correlated to the change in the clay content (Pearson's $R^2 = 078$; ***$P < 0.001$). Soil extractable K in the top 5 cm was greater in HM, followed by NT and CT, though the difference was only significant for the HM. The soil extractable K was the highest around 40–50 cm depth in all management systems.

The CEC was correlated to soil clay content (Pearson's $R^2 = 0.73$; ***$P < 0.001$). The maximum CEC was measured at 30 cm depth in CT, and at 50 cm in both NT and HM.

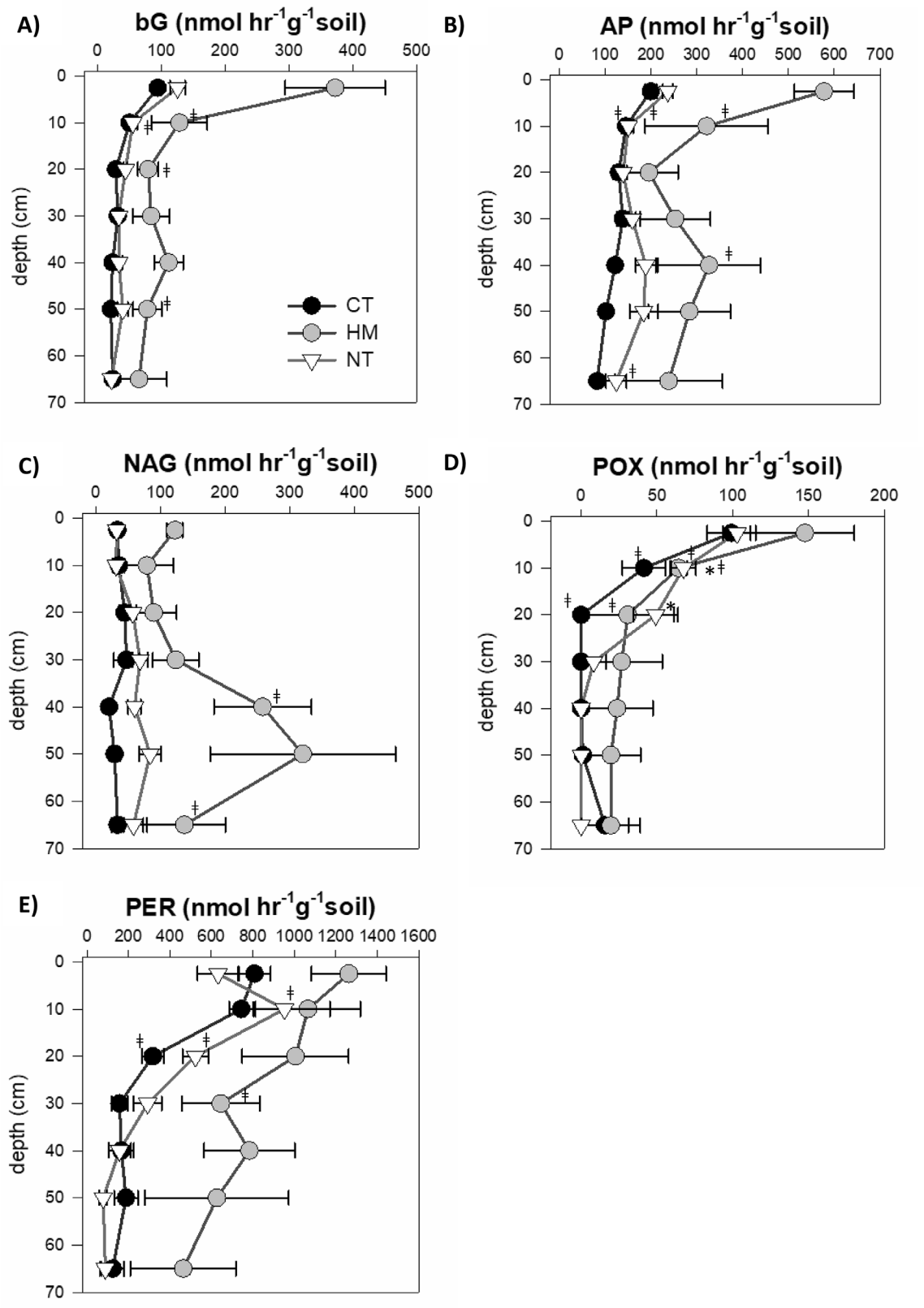

Fig. 2. Change in soil extracellular enzyme activities with depth for different management practices (mean ± 1 SE, nCT = 6, nNT = 8, nHM = 3). bG: β-glucosidase, C-acquiring enzyme; AP: acid phosphatase, C & P-acquiring enzyme; NAG: N-acetyl glucosidase, C & N-acquiring enzyme; POX: phenol oxidase; PER: peroxidase. Both POX and PER are lignin degradation enzymes. The bG, AP, NAG are hydrolases, and POX, PER are oxidases. * significant difference between NT and CT soils at the same depth at the 90% confidence level. ǂ significant difference with upward depth intervals at the 90% confidence level.

3.3. Extracellular enzyme activities profiles

Soil bG activity decreased with depth in the soil profile (Fig. 2A; ***P < 0.001). The bG activity dropped sharply from an average of 375 nmol h^{-1} g^{-1} soil in the top 5 cm to an average of 125 nmol h^{-1} g^{-1} soil at 10 cm in the HM soil profile. The bG activity in the cropped soils also decreased between the upper two soil layers, and the overall activity was much less than that in the HM soils. There was a significant increase in bG activity at 40 cm in HM soils (P = 0.068). The difference between NT and CT soils was not significant.

Soil AP activity also decreased with depth in the soil profile but showed greater variability than for bG (Fig. 2B; ***P < 0.001). The AP activity in HM soils was significantly greater than cropland soils in the top 15 cm and at 40 cm. As with bG activity, AP activity dropped quickly from the maximum at the upper soil layer (5 cm) in all management systems. An increase in AP activity at depths of 40 and 50 cm was seen in the HM (P = 0.0871) and NT (P = 0.0938) systems. The AP activity in NT was not significantly greater than in the CT soils throughout the soil profile.

Changes in soil NAG activity with depth were more complex than bG or AP (Fig. 2C; ***P = 0.0008). The NAG activity in the HM soil decreased slightly from 125 nmol h^{-1} g^{-1} soil at the surface (5 cm) and then increased more than 3-fold to a maximum at 40–50 cm, then decreased at the greatest depth (65 cm) to an activity similar to that in the topsoil. The NAG activity in the cropping systems remained nearly constant throughout the soil profile, showing only a slight increase with depth in the NT system. The NAG activities in HM soils were significantly greater than in the cropped soils in the top 5 cm and below 35 cm. The difference in NAG between NT and CT was not significant throughout the soil profile.

Soil POX activity was greatest in the top 5 cm of the soil profile, declining rapidly with depth (Fig. 2D; ***P < 0.001). Some locations in the cropland systems showed no detectable POX activity below 20 cm. There was no significant change in POX activity in subsoils for any of the management systems, and the difference between CT and NT was only significant at 10 and 20 cm (P = 0.0647, 0.0007).

Soil PER activity decreased with depth in all management systems (Fig. 2E; ***P < 0.001). Although the enzyme activity levels were much greater than for the other enzymes measured, there was also greater variability, obscuring clear trends. The PER activities in HM soils were significantly greater than cropland soils throughout the soil profile except at 10 cm. The PER activities in NT soils were not significantly greater than CT soils throughout the soil profile.

Most soil extracellular enzyme activities decreased with depth in the soil profile. However, bG, AP, and especially NAG activity increased at around 40 cm depth. This increase was particularly evident in the HM soils. The bG, AP, POX, and PER activities were significantly positively correlated with soil C with Pearson's R^2 of 0.87, 0.71, 0.71, and 0.59, respectively (Fig. S2A; ***P < 0.001). Conversely, NAG activity was not correlated to either soil C or N in the entire soil profile. The NAG activity was only weakly correlated to clay content, soil moisture, and negatively correlated to pH (0.44, 0.37, and −0.56, respectively; Fig. S2A; *P < 0.05). Within the claypan soils, all hydrolases had a positive correlation with clay content with R^2 of 0.57, 0.74, and 0.43 for bG, AP, and NAG, respectively (Fig. S2B; *P < 0.05).

3.4. Specific enzyme activity and potential C:N:P acquisition ability

In contrast to the decline in hydrolase and oxidase activities with depth in the soil profile, the specific hydrolase (sum of bG, AP, and NAG) and oxidase (sum of POX and PER) activities per microbial biomass increased with depth in the soil profile (Fig. 3). Soil specific hydrolase activity increased at 30 cm in NT and CT soils and 40 cm in HM soils. There were no significant differences in specific hydrolase activities based on management system. The specific oxidase activity showed much greater variability than the specific hydrolase activity, and no difference with depth or management systems was observed except at 50 and 65 cm in CT.

Ratios of C-acquiring to P-acquiring enzyme activities (ln(bG):ln(AP)) were less than 0.959 and decreased with depth (Fig. 4A). Conversely, ratios of C-acquiring to N-acquiring enzyme activities (ln(bG):ln(NAG)) were greater than one in surface soils but decreased rapidly to 0.8 with depth in the HM and NT systems (Fig. 4b). They increased to just above 1.0 in the CT system below 30 cm. Ratios of P-acquiring to N-acquiring enzyme activities (ln(AP):ln(NAG)) were always greater than one in all management systems and at all depths (Fig. 4C).

3.5. Phospholipid fatty acid (PLFA) profiles

Microbial biomass decreased rapidly with depth (***P < 0.001) in all systems, but changes were only significant in the HM soil (Fig. 5A). Unlike hydrolase activity profiles, there was no increase at 40 cm in HM soils. Microbial biomass in the NT and CT soils were not significantly different throughout the profile, though levels were elevated in the surface soils. The microbial biomass was much higher at the surface in the HM system, and remained above the biomass levels measured in CT and NT throughout the profile. The microbial biomass was significantly correlated to SOC with Pearson's R^2 of 0.8 (Fig. S2A; **P < 0.01).

Microbial community composition did not change significantly with management practices or depth (Figs. S3A and B). The fungal to bacterial ratios were similar for both cropping systems and decreased with depth in the soil profile (Fig. 5B). Compared to the cropping systems, HM supported greater fungal populations except in the top 5 cm of the soil. In contrast to the cropping systems, HM had an increase in fungal to bacterial ratio from 5 to 10 cm in the soil, followed by a gradual decline to levels similar to those observed in the cropping systems.

The textural changes with depth led to changes in soil properties. Clay content, oxidase activity, CEC, and soil C explained 46% of the variation in measured soil parameters in PCA axis 1 (Fig. 6). The PCA axis 2 explained an additional 24.2% of the variability, mainly from hydrolase activity, soil N, and microbial biomass. Soils at lower depths in the profile and those in the claypan fell to the left of PCA axis 1, and included clay content, CEC, K, and soil water. Soils above the claypan layer fell to the right of PCA axis 1 because of greater soil C, hydrolase activity, and microbial biomass (Fig. 6A). Results from the k-means clustering algorithm suggested clay content, CEC, extractable K, and soil water had a similar PCA distribution, while hydrolase, microbial biomass, soil C and N were grouped in a second distribution (Fig. 6B).

4. Discussion

The textural changes with depth in claypan soils impact the physical and chemical characteristics of the soil profile. The presence of the clay layer was notable as an increase in clay content beginning at around 20 cm in the soil profiles (Fig. S1). As in other studies (Jobbágy and Jackson, 2001; Stone et al., 2014), SOC, total N, and extractable P contents decreased rapidly from the highest levels measured at the soil surface to lowest levels within the profile. Conversely, extractable K increased with depth in parallel with the increase in clay content (Fig. 1; Fig. S2A). This is expected in a claypan soil (Myers et al., 2007) and highlights the impact of the claypan on the nutrient profile.

Claypan soils have been found to restrict root development. Myers et al. (2007) found soybean root length density declined to a minimum in the soil layer above the claypan, and then increased to a secondary maximum below the clay layer as the increasing soil pH increased nutrient availability for plant roots. Although claypan soils restrict root development in general, Clark et al. (1998) found Eastern gamagrass roots can penetrate the claypan layer to obtain moisture and nutrients. However, no significant increase in SOC with depth was measured in any of the management practices in our study (Fig. 1C). The lack of a strong eluvial horizon above the claypan and a thicker claypan layer

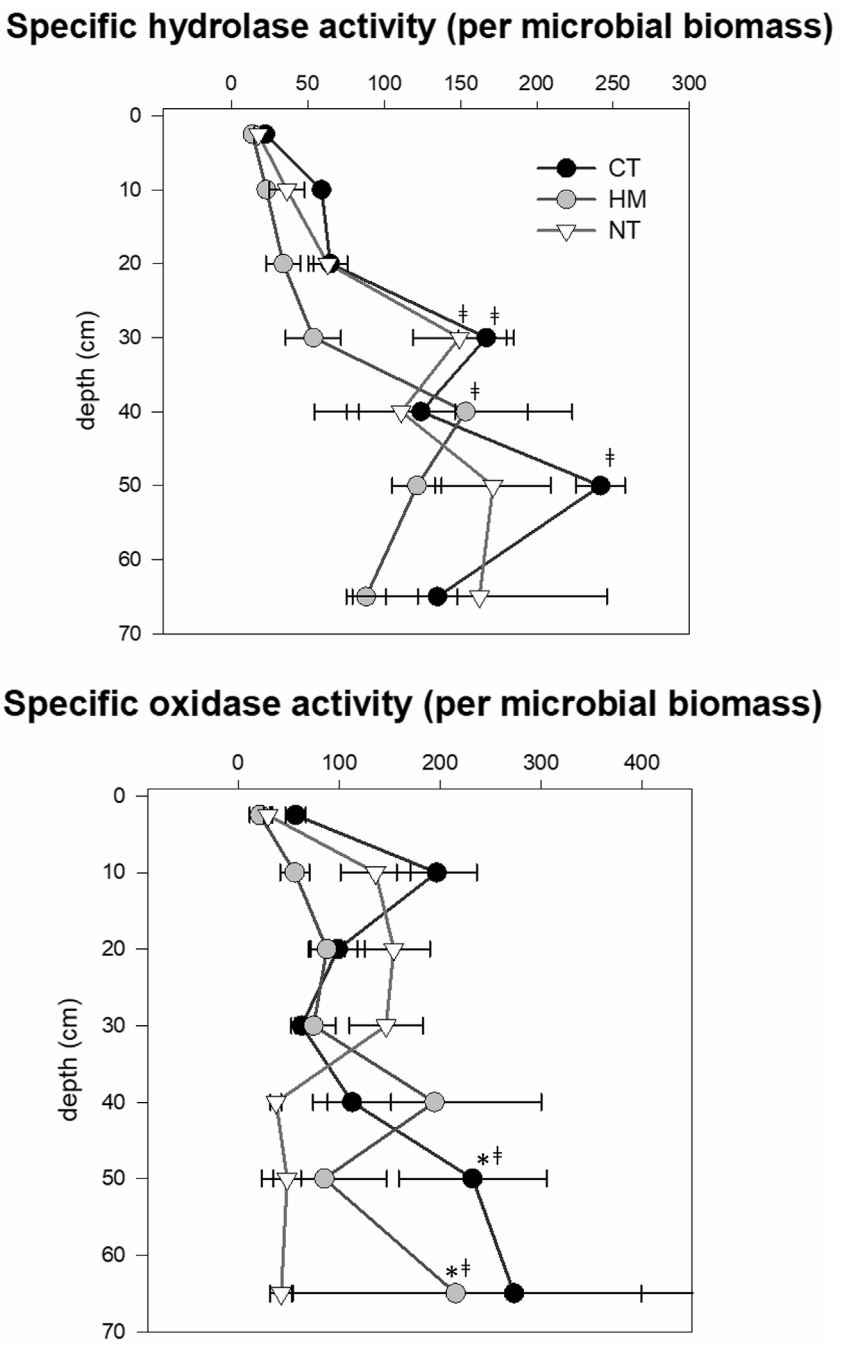

Fig. 3. Change in soil specific hydrolase and oxidase activities per unit microbial biomass with depth for different management practices (mean ± 1 SE, nCT = 6, nNT = 8, nHM = 3). Hydrolase = bG + AP + NAG; Oxidase = POX + PER. * significant difference between NT and CT soils at the same depth at the 90% confidence level. ǂ significant difference with upward depth intervals at the 90% confidence level.

may explain the insignificant SOC change in subsoils in our study, even in the long-term HM. Although other studies indicated rooting activity in and below the clay layer, the high clay content soil did not appear to accumulate SOC in our study.

The textural changes with depth led to a vertical stratification of soil properties (Fig. 6A): topsoil from 0 to 15 cm, intermediate from 15 to 35 cm, and claypan layer below 35 cm. Soil properties in the top layer were primarily determined by SOC, total N, microbial biomass, and hydrolase activity. Soil properties in the claypan layer were affected by clay content and its correlated parameters including CEC, extractable K, and soil moisture content (Fig. 6B). A stratification of hydrolase activity was also found (Fig. 3; Fig. S3C). The measured soil enzymes were closely correlated to SOC in the topsoil, which was affected by management practices (Fig. 1C). Within the claypan layer, however, the hydrolases and PER activity increased substantially and were correlated to clay content (Fig. 2; Fig. S2B). The impact of the clay content may account for the differences in soil enzyme profiles measured in the subsoils. Although the impact of clay content on enzyme activities was not consistent, all enzymes measured in this study except NAG were reported to have greater potential activities in the clay-enzyme complex forms (Sarkar et al., 1989; Allison and Jastrow, 2006). Conversely, although changes in actual microbial biomass concentration were observed with depth and production system (Fig. 5), no changes in microbial community assessed by PLFA relative abundance were observed with either depth or management practice (Figs. S3A and B). The dissimilar profiles of potential enzyme activity and microbial PLFAs within

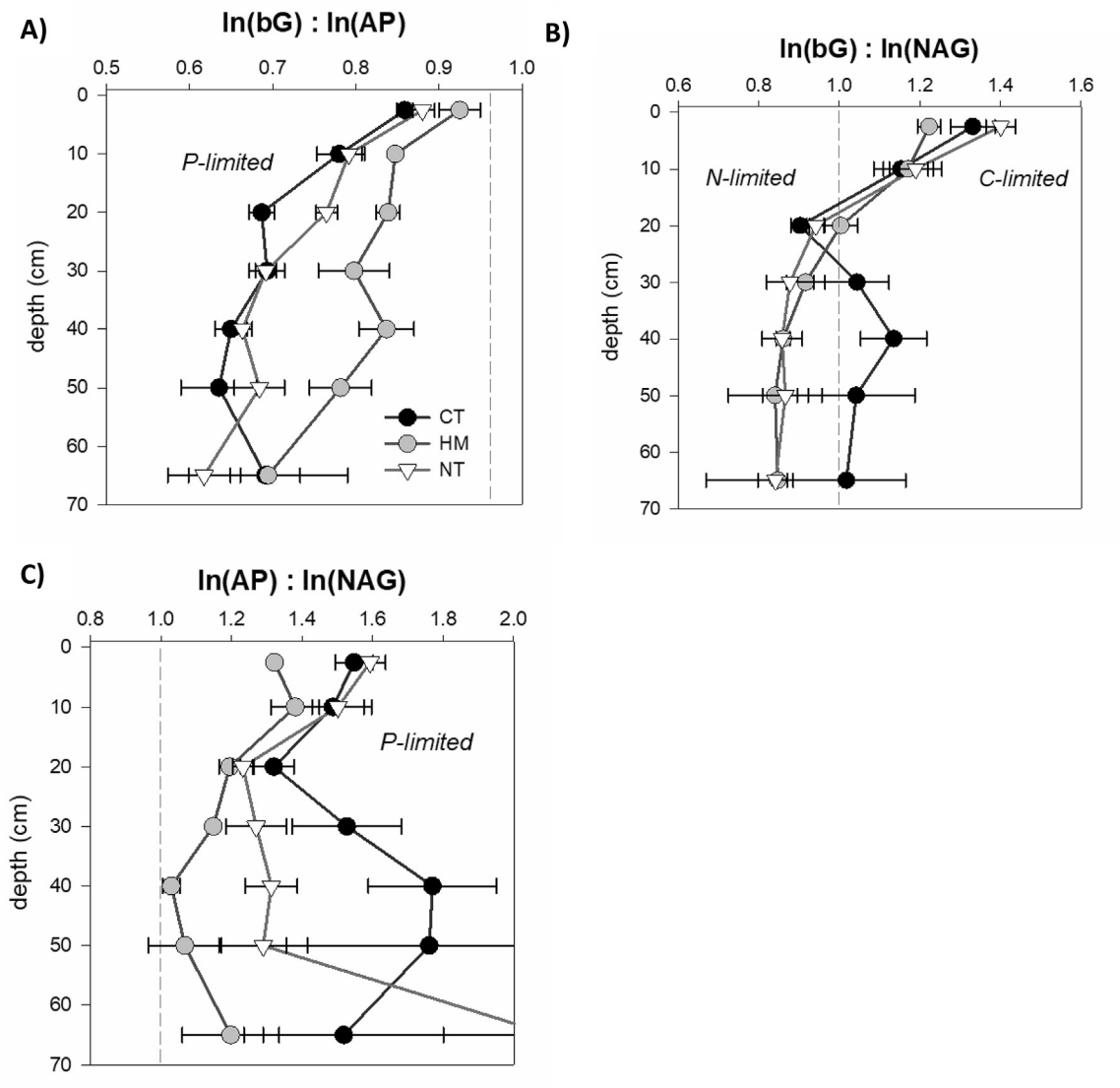

Fig. 4. Change in enzyme activity ratios with depth for different management practices (mean ± 1 SE, nCT = 6, nNT = 8, nHM = 3). A) C-acquiring to P- acquiring enzyme activities. A ratio below 0.959 indicates a P-limited environment; B) C- acquiring to N- acquiring enzyme activities. A ratio greater than one indicates a C-limited environment; C) P- acquiring to N-acquiring enzyme activities. A ratio greater than one indicates a P-limited environment. * significant difference between NT and CT soils at the same depth at the 90% confidence level. ǂ significant difference with upward depth intervals at the 90% confidence level.

the claypan layer are consistent with the idea that the microbial community structure and function are uncoupled in response to environment (Purahong et al., 2014).

The production system impacted the enzyme activity profiles in both the topsoil and the claypan layer. Soil bG, AP, and oxidase (POX, PER) activities decreased with depth, yet hydrolases showed some increase in activity at 40 and 50 cm especially in NT and HM soils (Fig. 2; Fig. S2B). In contrast to annual cropping systems, a long-term perennial grass system (such as the HM) has plants that occupy the land continuously, allowing development of substantial rooting systems with the potential to penetrate the clay layer (Clark et al., 1998). The micropore networks and channels created by previous perennial grass roots can assist root growth and SOC accumulation in claypan soil (Jassogne et al., 2009). Although we observed no SOC accumulation in the claypan, the long-term establishment of persistent plant roots in the HM may account for the increase in soil enzyme activities observed in this study, especially in the HM (Fig. 2A, B, C, and somewhat E). This may be similar to the increased oxidase activity observed in taiga ecosystems (Schnecker et al., 2015). The substantial increase in NAG activity in the claypan layer of the HM system is intriguing. It may indicate the potential of grass systems to utilize more of the soil profile by establishing roots within the clay layer. Additional research is needed to fully delineate the impact of clay on NAG activity and the interactions between perennial roots and NAG activity.

The fungal to bacterial ratio was greater in CT than NT in the top 5 cm in our study (Fig. 5B), contradicting the notion that no-till agricultural systems lead to fungal dominance because the hyphae of fungi are relatively more affected by tillage (Hendrix et al., 1986). However, consistent evidence in support of the impacts of tillage on fungal:bacterial ratios have not been established (Strickland and Rousk, 2010). As other studies have found, changes from NT to CT led to an overall increase in both bacterial and fungal biomass. The abundance of fungi and bacteria has also been shown to be affected by soil pH, moisture, texture, and so on. Although fungi have been reported to be more acid-tolerant than bacteria, the fungal:bacterial ratio does not always increase with acidity (Rousk et al., 2010; Strickland and Rousk, 2010). However, soils with high clay content favor bacteria over fungi (Stotzky, 1966a, 1966b; Wei et al., 2014). Thus, it is possible that lower pH did not really affect the fungal:bacterial ratio, but instead the combination of higher clay content shifted the microbial community to

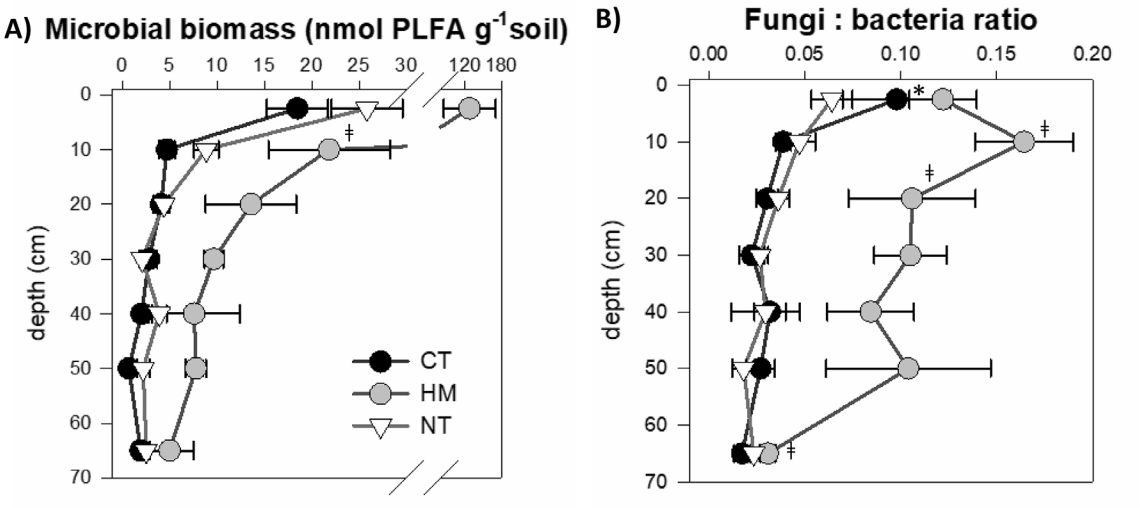

Fig. 5. Change in soil microbial community compositions with depth for different management practices (mean ± 1 SE, nCT = 6, nNT = 8, nHM = 3). * significant difference between NT and CT soils at the same depth at the 90% confidence level. ǂ significant difference with upward depth intervals at the 90% confidence level.

bacterial dominance with depth in our study.

Compared to soils from global surveys, ratios of enzymatic activities in these soils indicated greater investment toward P acquisition relative to C acquisition (Fig. 4A) and N acquisition (Fig. 4C), implying a primary microbial P limitation (Sinsabaugh et al., 2008). Both HM and cropping systems were P-limited throughout the soil profile, but the P limitation was not as restrictive in the HM soils. Ratios of ln(bG):ln (NAG) were greater than one in surface soils but decreased rapidly with depth, indicating a relatively N-rich condition in the surface soil but N-limiting conditions to soil microbes below 20 cm in the HM and NT systems (Fig. 4B). Overall, P and N became more limiting to microbes with increasing depth in all management systems. Nitrogen was limiting in subsoils, but P was more restricted than N throughout the soil profile. Our data indicated that P was the most limiting nutrient for microbes at lower levels, followed by N and C. Fertilizer applications may have contributed to N being limiting only in the lower soil layers. It is reasonable that higher N availability relative to P is required for organisms to begin investing in the production of phosphatase (Margalef et al., 2017), and it also suggests that C is not the predominant factor limiting microbial activity. This is consistent with research in remnant prairie soils in Illinois that showed that soil C was not the primary factor controlling soil microbial community composition in lower soil layers. Instead, P, exchangeable calcium, and soil water had a greater influence (Allison et al., 2007).

We acknowledge that clay mineral stabilization of enzymes should increase through the soil profile. Our interpretations are based on the assumptions that activities within all categories of hydrolytic enzyme are equally affected by changing soil abiotic conditions and that vertical translocation of enzymes is minimal. Also note that despite using extracellular enzyme activity as a convenient tool to represent the functional diversity of soil ecosystems, the assumption that the ratio of nutrient-acquiring enzyme activities is an indicator of nutrient dynamics in soil may over-simplify the many direct and indirect interactions between enzymes and other abiotic or biotic factors involved in soil microbial activity (Nannipieri et al., 2012).

The complex profiles in oxidases and specific oxidase activity are similar to the patterns observed by other researchers, and are likely due to control by both biological and chemical environmental factors (Allison and Jastrow, 2006; Sinsabaugh, 2010). The observed increase in specific hydrolase activity at 30–40 cm (Fig. 3) may arise because the clay minerals protect the extracellular enzymes from degradation. Alternatively, soil microorganisms may spend more energy on enzyme production in deep soils. This is consistent with the cellular economics research showing that microbes continuously secrete low levels of enzymes to maintain the capacity to rapidly respond to substrate availability changes even in a nutrient poor environment (Burns et al., 2013). The third possible explanation is that clay-stabilized AP could be released during Fe(III) reduction (Chacon et al., 2006). Therefore, the increased specific microbial activity could be due both to the clay-enzyme interaction and the greater amount of enzymatic production.

Phosphatase activity was correlated with SOC and total N content but not available P in our study (Fig. S2A). This finding is in agreement with other research demonstrating that organic P, rather than available P, is a better predictor of phosphatase activity (Margalef et al., 2017), because phosphatase activity is related to the potential capacity to release phosphate from the soil organic material. Soil with higher SOC content is considered to contain more organic matter, which can be a good proxy of organic P (Margalef et al., 2017).

5. Conclusions

Vertical stratification of soil properties was found in the claypan soils in southeast Kansas, with an upper layer from 0 to 15 cm, a lower layer in the claypan (below 35 cm), and an intermediate soil layer between 15 and 35 cm. The microbial biomass and extracellular enzyme activities in the upper layer were high and primarily affected by management practices. Soils in the middle layer had rapidly increasing clay and gravimetric moisture contents, K, and CEC, while SOC, total N, and extractable P concentration gradually decreased in this layer. Microbial biomass, enzyme activities, and fungal to bacterial ratio decreased with depth. Soils in the claypan layer were P- and N-limited for microbes. Soil hydrolase activities increased in the upper part of the claypan layer and then decreased with depth, especially in HM systems. Carbon accumulation was not observed. The inherent soil properties, such as the particle size, determined the amount of potential hydrolase activity changes in the claypan layer. Our results indicate that agricultural practices primarily control soil biological activity in the topsoil, while inherent soil properties dictate the potential enzyme activities in the clay layer, with production system (HM versus crop) impacting enzyme activities. Additional research is required to delineate causative factors impacting enzyme activity in the clay layer.

Incorporating more grasses in the crop rotation or as cover crops may allow nutrients to be extracted from deeper within the soil profile, enhancing the utilization of the entire soil profile and providing additional nutrient resources to cash crops. By simultaneously examining soil physical, chemical, and biological property in different land use

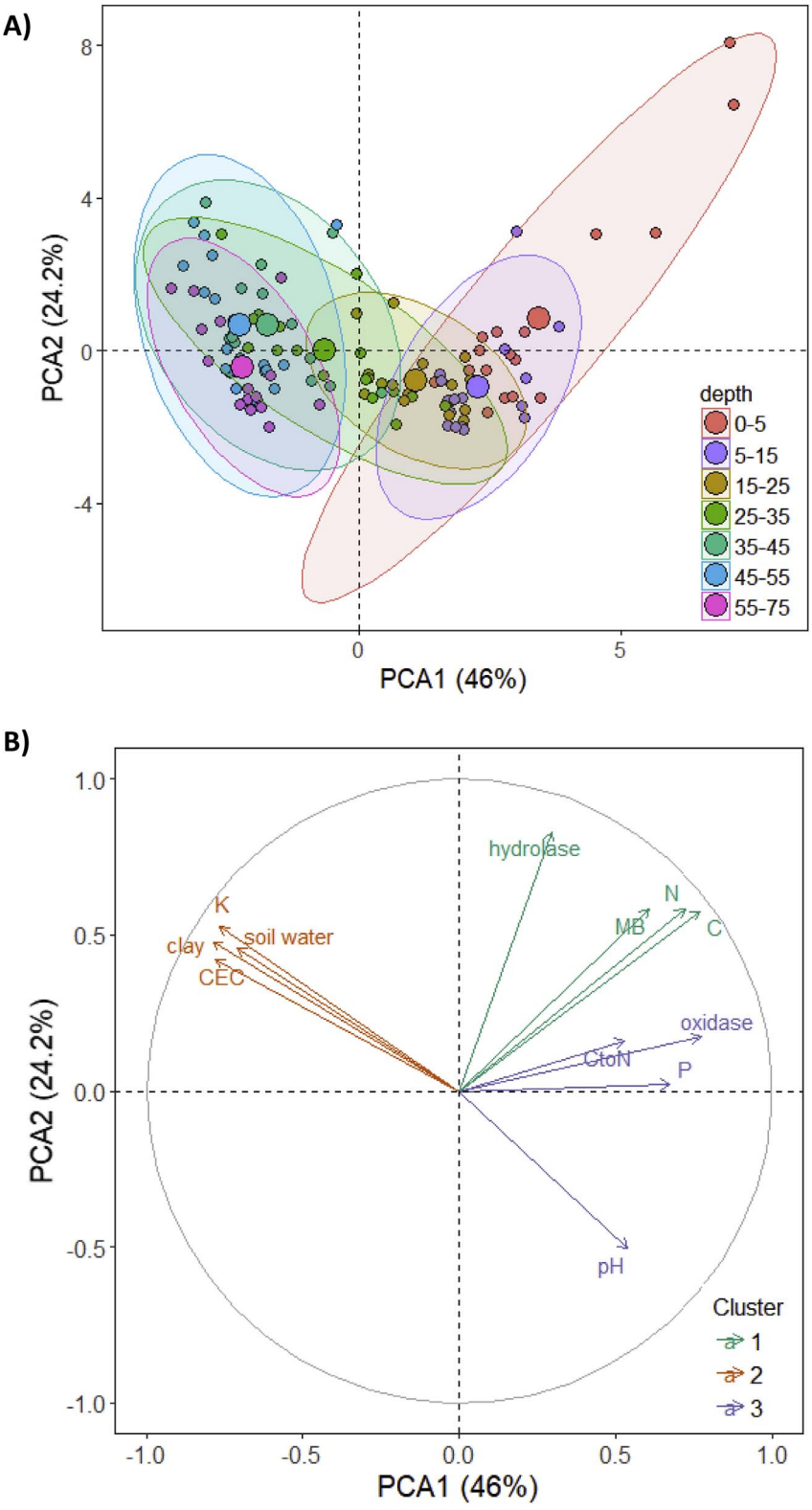

Fig. 6. Impact of depth on measured soil properties, with each experimental unit summarized by a single point using principal component analysis (PCA). A) Change in soil parameters with depth. The centroid was marked as a big solid point, indicating the group mean point of the site scores on each axis. B) Soil properties cluster using K-means algorithm. MB: PLFA microbial biomass.

practices with depth, we were able to delineate the impact of human activity and inherent soil properties on soil microbial properties.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.03.012.

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