Soil properties after 36 years of N fertilization under continuous corn and corn-soybean management

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Abstract. Modern agricultural systems rely on inorganic nitrogen (N) fertilization to enhance crop yields, but its overuse may negatively affect soil properties. Our objective was to investigate the effect of long-term N fertilization on key soil properties under continuous corn [*Zea mays* L.] (CCC) and both the corn (Cs) and soybean [*Glycine max* L. Merr.] (Sc) phases of a corn-soybean rotation. Research plots were established in 1981 with treatments arranged as a split-plot design in a randomized complete block design with three replications. The main plot was crop rotation (CCC, Cs, and Sc), and the subplots were N fertilizer rates of 0 kg N ha^{-1} (N0, controls), and 202 kg N ha^{-1}, and 269 kg N ha^{-1} (N202, and N269, respectively). After 36 years and within the CCC, the yearly addition of N269 compared to unfertilized controls significantly increased total exchange capacity (EC, 65% higher under N269), mainly due to the increase in exchangeable acidity (H^+), and acidified the top 15cm of the soil (pH 4.8 vs. pH 6.5). Soil organic matter (SOM) and total carbon stocks (TCs) were not affected by treatments, yet water aggregate stability (WAS) decreased by 6.7 % within the soybean phase of the CS rotation compared to CCC. Soil bulk density (BD) decreased with increased fertilization by 5% from N0 to N269. Although ammonium (NH_4^+) did not differ by treatments, nitrate (NO_3^-) increased eight-fold with N269 compared to N0, implying increased nitrification. Soils of unfertilized controls under CCC have over twice the available phosphorus level (P) and 40% more potassium (K) than the soils of fertilized plots (N202 and N269). On average, corn yields increased 60% with N fertilization compared to N0. Likewise, under N0, rotated corn yielded 45% more than CCC; the addition of N (N202 and N269) decreased the crop rotation benefit to 17%. Our results indicated that due to the increased level of corn residues returned to the soil in fertilized systems, long-term N fertilization improved WAS and BD, yet not SOM, at the cost of significant soil acidification and greater risk of N leaching and increased nitrous oxide emissions.

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1 Introduction

As the most limiting crop nutrient worldwide, nitrogen (N) fertilization use exceeds 100 Tg N yr^{-1}, leading to an estimated loss of 67 Tg N yr^{-1}, or roughly two-thirds of all synthetic N applied (Datnoff et al., 2007; FAO, 2019; Liu et al., 2010). Although inefficiencies are prevalent, modern N fertilization feeds 48% of the world's population and is responsible for 30-50% of all crop yield increases (Erisman et al., 2008). In the US Midwest region (Iowa, Illinois, Nebraska, Minnesota, Indiana, Kansas, South Dakota, Ohio, Missouri, Wisconsin, Michigan, and North Dakota), a prime agricultural region that provides more than 86% of the US corn crop (USDA-NASS, 2020), the average annual N fertilization rate for corn production in 2018 was 167 kg ha^{-1}, which amounts to over four Tg of N applied annually (USDA-ERS, 2020). Economically speaking, the profit risk is minimal for over-applying N, and under applying N is more costly when N rates are below the economic rate of return by 15-20% (Sadeghpour et al., 2017; Sawyer et al., 2006). This intense annual N fertilization accompanies the high crop productivity within the region and has been a critical source of N loss leading to contamination and hypoxia of water bodies (Khan and Mohammad, 2014) and increase nitrous oxide (N_2O) emissions (Davidson and Kanter, 2014). Yet, the long-term effects of inorganic N fertilization on the physical and chemical properties of the deep and fertile soils of the Midwest region have not been thoroughly documented.

Long-term studies in the Midwest typically record selected soil parameters, but few include a wide range of physical and chemical factors from multiple soil depths. Several long-term studies in the region have reported decreases in surface soil pH and cation exchange capacity (CEC) with increased N fertilization levels regardless of the form of N applied. This trend is particularly evident for soils under corn monocultures (Barak et al., 1997; Liebig et al., 2002; Russell et al., 2006; Stone et al., 1991). Liebig et al. (2002) and Hickman (2002) observed that the soil pH decreased under continuous corn compared to a corn-soybean rotation because continuous corn receives more N fertilizer, acidifying the soil via nitrification. Likewise, from the same long-term study as ours, 13 years prior, Jagadamma et al. (2008) found that the combination of greater N rates and continuous corn acidified the soil, yet did not change CEC. Other studies show that CEC and pH respond differently in rotated corn systems. A crop rotation and tillage study on Illinois Mollisols by Zuber et al. (2017) found increased CEC from continuous corn compared to a corn-soybean rotation due to a higher biomass of residues returned under continuous corn. Alternatively, long-term studies by Congreves et al. (2015) and Hickman (2002) on the effects of tillage and crop rotation from fertile soils did not find significant changes in CEC between continuous corn and corn-soybean rotation. A statewide assessment of long-term Illinois soils from short corn rotations (corn-soybean and corn-corn-soybean) compared to continuous corn showed that after 12 years, no rotation effect on any soil physical or chemical properties, including pH and CEC, could be detected (Hoss et al., 2018).

Reports on the effect of N fertilization on soil organic matter (SOM) and related properties are also inconsistent. For example, in Illinois, Jagadamma et al. (2007), following 23 years of N fertilization on continuous corn and corn-soybean rotations, reported an increase in soil organic carbon (SOC) accompanied by a reduction in bulk density (BD) of the surface soil with increasing N rates. The observed changes were attributed to greater residue returned to the soil with increasing corn yields under fertilized conditions. Yet in a study in Iowa, Russell et al. (2009) showed that N application increased organic matter decay rate and shortened its turnover time, resulting in generally no change in SOC by fertilization rates. Also, from four long-term sites in Iowa, Poffenbarger et al. (2017) found that when fertilized at the agronomic optimal N rate, SOC storage is 58% greater under continuous corn compared to a corn-soybean rotation. The increases in SOC storage were due to continuous corn residue production being 22% greater than the rotated corn. However, continuous corn N rates beyond the optimal decreased SOC storage by increasing C mineralization.

Adding to the complexity of crop rotation, water aggregate stability (WAS), a proxy for soil erosion potential, and bulk density (BD) have differing outcomes when considering the N rate and cropping system. Jagadamma et al. (2008), using the same setup as mentioned above in Jagadamma et al. (2007), showed that WAS increased with higher N rates in continuous corn rotation. The authors attributed this effect to crop yield increases and the quantity and chemical properties of the residues returned to the soil. Indeed, continuous corn yield increased significantly with higher N rates in their study, and corn residues contain greater amounts of phenolic acid and lignin that are known to increase soil aggregation (Jagadamma et al., 2007; Martens, 2000; Blanco-Canqui & Lal, 2004). Zuber et al. (2015) and Nouwakpo et al. (2018) confirmed these findings by showing that continuous corn increased WAS compared to soils under corn-soybean rotation or even continuous soybean. Jagadamma et al. (2008) and Zuber et al. (2015) showed no differences in BD due to rotation practices. Similarly, previously described Hoss et al. (2018) also did not find any differences in WAS or BD from rotated or continuous corn.

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Just as soil physical properties are affected by management, so are chemical properties, with N, phosphorus (P), and potassium (K) dynamics directly or indirectly affected by N fertilizer application. Losses of N from agricultural systems are widely reported and have a large impact on the environment. Numerous factors play into that loss of N, including discrepancies between fertilizer application and crop uptake, excess fertilizer N beyond crop need, low N retention in managed soils, and soil N mineralization uncertainty (Bowles et al., 2018). Behnke et al. (2018) demonstrated that fertilization strategies associated with crop rotation alter soil N dynamics, observing that N_2O emissions and soil inorganic N levels were the greatest under continuous corn due to greater N fertilizer use needed to maintain yields. Congreves et al. (2015) found that soil nutrients, including P and K, depleted differently under various crop rotations, leading to greater soil P and K levels under continuous corn and corn-soybean rotations than under continuous soybean. Further, Jagadamma et al. (2008) found that soil K levels were greater under continuous corn compared with soil K under corn-soybean rotations. Differences in soil P and K dynamics were attributed to differences in nutrients returned as crop residues, dictated by either the innate crop biomass production and quality or the amount of fertilizer applied in a cropping system.

Crop residue amounts are closely related to yield, as increases in yield translate into greater biomass production. Thus, crop rotations and their yields dictate soil nutrient cycling as each crop phase may differ in nutrient requirements, and the amount and composition of the residues returned to the soil (Ajwa and Tabatabai, 1994; Martens, 2000). In the US Midwest, corn and soybean are the dominant crops cultivated continuously or in short rotation with each other, sometimes including other crops like wheat, alfalfa, and increasingly, cover crops (Hatfield et al., 2018). Throughout the Midwest, the use of a crop rotation typically increases yields compared to monocultures due to N availability, residue management, and improved yield stability in suboptimal weather years (Behnke et al., 2018; Al-Kaisi et al., 2015; Gentry et al., 2015; Daigh et al., 2018).

The studies introduced so far have provided valuable information on the effects of different N fertilization rates and crop rotations on the soil properties of highly productive soils. However, knowledge on this relationship remains fragmented as most studies seldom assessed N rates and crop rotation together, or they were limited to certain soil properties or soil processes at the soil surface. Moreover, results are often confounded by additional practices such as tillage systems, or manure additions, or by changes in management and cropping systems during the life of the long-term experimental plots under study. Therefore, building on the work of Jagadamma et al. (2007, 2008), we hypothesized that as the N rate increases, crop yields would improve, thus returning more biomass to the soil, which will enhance SOM, WAS, and BD. However, as the N rate increases, soil pH will decrease due to nitrification rendering more H^+ ions in the soil and from depletion of exchangeable base by increased crop uptake. We expect these effects to also decrease the estimated CEC while increasing the exchangeable acidity. Crop rotation will enhance yields of both phases in the corn-soybean rotation, as is commonly observed in the Midwest. Still, the addition of soybean residues will negatively affect soil physical properties (SOM, WAS, and BD) while improving pH. The objective of this study was to evaluate the soil chemical and physical properties to a depth of 90 cm, following 36 years of N fertilization rates on corn monocultures and their rotation with soybean crops. Our results will contribute to a better understanding of how innately fertile soils respond to the long-term use of conventional management practices typical of production-scale agriculture.

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2 Materials and Methods

2.1 Experimental site description

The experimental plots were established in 1981 at the Northwestern Illinois Agricultural Research and Demonstration Center (40°55’50” N, 90°43’38” W), approximately 8 km northwest of Monmouth, Illinois. The location has mean annual precipitation of 914 mm with a mean annual temperature of 10.6°C (Illinois State Water Survey, 2010). The experimental site was on Muscatune silt loam series (fine-silty, mixed, mesic Aquic Argiudoll), of nearly flat topography (Soil Survey Staff, 2020). This soil series is dark-colored and very deep; it has moderate permeability and low surface runoff potential. This soil developed under prairie vegetation in a layer of loess 2-3 m thick over glacial till (Soil Survey Staff, 2020).

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2.2 Treatments and field management practices

Research plots were initially designed to study the effects of N fertilization on corn yields under a monoculture (CCC) and short rotation with soybeans, with each phase present every year (Cs: Corn phase within corn-soybean rotation; Sc: Soybean phase within corn-soybean rotation). The site was set up in a split-plot arrangement of rotation (CCC, Cs, and Sc) and N fertilization rates (N0, N202, and N269; kg N ha^{-1}) in a randomized complete block design with three replicates. Main plots were 18m long by 30m wide, and subplots were 18m long by 6m wide. Conventional tillage was implemented, including primary tillage with a chisel plow 20 to 25 cm deep in the fall after harvest, and secondary tillage with a field cultivator before planting in the spring. Corn and soybeans were planted in April or May each year in 76 and 38 cm rows, respectively, at the rate of 75,000 to 85,000 seeds ha^{-1} for corn, and at 340,000 to 350,000 seeds ha^{-1} for soybean. Fertilizer and pest management decisions followed the best practices for the site based on the Illinois Agronomy Handbook (Nafziger, 2009). Urea (46%N) was applied until 1996 to corn in the spring at or before plating, which switched to urea ammonium nitrate solution (UAN28%) thereafter. No N fertilizer was applied during soybean phases. Occasionally, P and K fertilizers and line were applied to every plot when necessary based on soil test results. Historical yield data for corn from both CCC (from 1982 to 2017) and CS systems (in alternate years from 1983 to 2017), and for soybean from CS system (in alternate years from 1984 to 2017) were obtained from records at the Northwestern Illinois Agricultural Research and Demonstration Center. Every year, crop yields were harvested with a plot combine (Almaco, Nevada, IA) and adjusted with 15.5 % and 13 % moisture for corn and soybean, respectively.

2.3 Soil sampling and determinations

Soil samples were collected in the spring of 2017, 36 years from the initiation of the experiment. Within each experimental unit, three individual soil cores (4.3 cm diameter) to a depth of 90 cm were taken with a tractor-mounted soil sampler with soil sleeve inserts (Amity Tech, Fargo, ND) and taken back to the lab and divided into four sections: 0-15 cm, 15-30 cm, 30-60, and 60-90 cm. Field-moist subsamples were analyzed for available N (NO_3^- and NH_4^+ in mg kg^{-1}) using KCl extraction (1:5 ratio of soil to solution) followed by flow injection analysis with a SmartChem 200 (Westco Scientific Instruments, Inc., Danbury, CN, USA). Total inorganic N (TIN) was calculated as the sum of NO_3^- and NH_4^+. The Amity Tech soil sleeves allowed us to accurately measure soil bulk density (BD, Mg m^{-3}) by keeping the soil volume exact. About 10 g of soil per subsample was oven-dried at 105°C to measure gravimetric water content at each depth, to obtain BD using the core method (Blake and Hartge, 1986). The remaining soil in each subsample was sent to a commercial laboratory (Brookside Laboratories, Inc., New Bremen, OH), for the determination of soil organic matter (SOM, %) by loss on ignition (Council on Soil Testing Plant Analysis, 1992); soil pH (1:1 soil:water) via potentiometry; available phosphorus (P, mg kg^{-1}) with Bray I extraction; Mehlich III extractable elements (Ziadi and Tran, 2008) including sulphur (S, mg kg^{-1}), calcium (Ca, mg kg^{-1}), magnesium (Mg, mg kg^{-1}), potassium (K, mg kg^{-1}), and sodium (Na, mg kg^{-1}); the summation of cations with exchangeable acidity from protons (H^+, mg kg^{-1}) was reported as total exchange capacity (EC, $cmol_c$ kg^{-1}). The total exchange capacity without H^+ was

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used as estimation for cation exchange capacity (CEC, cmol$_c$ kg^{-1}). Loss on ignition values were adjusted following Konen et al. (2002) for Illinois soils to calculate the soil organic C (SOC). Bulk density values were used to convert SOM (in %) to a basis of weight per unit area, referred to as total carbon stocks (TCs, Mg ha^{-1}) for each depth increment.

2.4 Statistical analyses

Linear mixed models were fit to each soil property using PROC GLIMMIX of SAS software version 9.4 (SAS Institute, Cary, NC). Factors Rotation (Rot), N fertilizer rate (Nrate), and sampling depth (D) were considered fixed effects in the analyses of variance, while replicates (blocks) were considered random effects. Depth (D) was analyzed using a repeated-measures approach with the variance-covariance structure of heterogeneous autoregressive [type=arh(1)] for each soil variable consistently selected based on the lowest Akaike's Information Criteria (Littell et al., 2006). When appropriate, least-square means were separated using the lines option of the lsmeans statement, setting the probability of Type I error (α) at 0.05, and using a Tukey correction (adjust=tukey). All plots were created within the R environment, version 3.5.3 (R Core Team, 2019), using the package ggplot2 (Wickham, 2016).

3 Results

Table 1 shows the mean values and standard error of the treatment mean (SEM) values for the soil properties of EC, soil pH, SOM, TC stocks, WAS, and BD, whereas Table 2 shows the corresponding estimates for the nutrients, N (as TIN comprised of NO_3^- and NH_4^+), and available P, and K determined under each level of N fertilization rate (N rate), crop rotation (Rot), and soil depth (D). Table 2 also includes the observed response of corn yields to fertilization and crop rotation for the duration of the study. Both tables display the probability values (p-values) and degrees of freedom (df) associated with the different sources of variation in the analysis of variance (ANOVA) of the effects of N fertilization rate (N rate), crop rotation (Rot), soil depth (D), and their interactions on the soil properties under study and the corn crop yields.

A three-way interaction effect of N rate x Rot x D was detected for soil pH ($p<0.0351$) and for EC, though the latter was marginally significant ($p< 0.1$). The interaction indicates a differential response of both variables to fertilization for each rotation at successive depths. Only under CCC, EC increased significantly with N202 and N269 at 0-15 cm; these values were greater than CEC under both phases of rotated corn (Fig. 1). No significant difference by N rate and depth was detected under Cs and Sc. Also, within the CCC rotation, soil pH decreased significantly within the 0-15 cm depth as N rates increased, while it decreased with N269 at 15-30 cm (Fig. 2). Further analysis distinguishing EC into CEC and exchangeable acidity (H^+) clearly showed that the observed differences in EC were driven by H^+ as CEC did not show significant response to N rate

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(p>0.874) while mean H^+ increased sequentially from N0 to N269, although the statistically insignificant (p=0.179; data not shown).

As expected, all soil variables showed a statistically significant main effect associated with the depth of study ($p< 0.0001$). Soil depth was the only source of variation for SOM and TCs; SOM decreased consistently with depth, while TCs were greatest at 30-60 cm. In turn, WAS was greater at intermediate depths (15-30, 30-60 cm) than within the top and lower layers (Fig. 3a). There was a significant main effect of rotation on WAS, which was greater under CCC compared with Sc, with the Cs showing intermediate values (Fig. 3b). Soil BD values were also affected by depth and the higher BD was recorded at the 15-30 cm of depth (Fig. 3c). A statistically significant effect of N fertilization was determined for BD ($p< 0.05$) with greater BD values associated with N0, and the lowest BD registered under N269 (Fig. 3d).

There were marginally significant ($p< 0.1$) two-way interaction effects of Rot x D and N rate x D on the TIN, the inorganic N, a summation of NO_3^- and NH_4^+. As shown in Fig. 4a and 4b, TIN generally decreased with depth but did not differ by N rate nor crop rotation within each depth. Unlike TIN, soil NO_3^- showed significant responses to both N rates and crop rotation, while no responses to the treatments were detected for NH_4^+ (Table 2). Thus, soil NO_3^- had significant ($p< 0.05$) interaction effects from N rate x Rot and Rot x D, and marginally significant ($p< 0.1$) effects from N rate x D. At 0-15 cm, NO_3^- was significantly greater with N269 than N0, while it did so for both N202 and N269 compared to N0 at 15-30 cm; NO_3^- did not differ by N rate at 30-60 cm and 60-90 cm depths (Fig. 4c). Soil NO_3^- was significantly greater under CCC than Cs and Sc at 0-15 cm (Fig. 4d). It was also greater under CCC than Sc at 15-30 cm; yet at the lower depths, NO_3^- did not differ by crop rotation (Fig. 4d). Under CCC, NO_3^- increased significantly with higher N rates, but it did not respond to N rate under Cs and Sc (Fig. 4e). On the other hand, soil depth was the only source of variation for NH_4^+, which decreased with depth (Fig. 4f).

Soil available P had marginally significant ($p< 0.1$) N rate x Rot interaction effect and a statistically significant ($p< 0.05$) N rate x D interaction effect. Thus, under CCC, soil available P decreased with N application and with the inclusion of soybean, thereby being the greatest with N0 from CCC than all other N rate x Rot combinations (Fig. 5a). Likewise, soil P was greater with N0 than other N rates within the topmost depth and greater than N202 at 15-30 cm (Fig. 5b).

Extractable soil K showed a statistically significant effect of the N rate x Rot ($p< 0.05$) interaction term and a marginally significant effect of the Rot x D ($p< 0.1$) interaction. As with soil P, soil K was greater with N0 under CCC than under all other N rate x Rot combinations (Fig. 5c). Soil extractable K was greater under CCC than under Cs and Sc at depths 0-15 cm and 30-60 cm. The lowest K levels were measured within the 30-60 cm of depth (Fig. 5d).

The average corn crop yield over the 36 years differed significantly ($p< 0.0001$) between rotations, N rate, and their interaction as well (Table 2). Overall, the mean corn grain yield over the experimental period was significantly ($p< 0.0001$) greater with N fertilization compared to N0 and following soybean in the corn-soybean rotation (CS) instead of after corn in the CCC

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(Figure 6). There were no significant yield increases between the top N levels, N202 and N269, over the study period. On average, corn yields increased 60% with N fertilization, the highest increases obtained when N was added into the unfertilized systems. Averaged across N rates, CS resulted in 31% higher average corn grain yield (11.054 Mg ha^{-1}) than under CCC (8.44 Mg ha^{-1}) ($p < 0.0001$) management. Increasing the rate of N application resulted in greater corn grain yield for both CCC and CS systems (Fig. 6), with values ranging from 4.90 (N0) to 10.34 (N269) Mg ha^{-1} for CCC and 8.88 (N0) to 12.16 (N269) Mg ha^{-1} for CS ($p < 0.0001$). Compared to the unfertilized controls, the averaged corn grain yield in treatments was 45% higher in the CCC system and 37% higher in the CS system, indicating a greater response to N fertilizer in the CCC system. Likewise, under N0, rotated corn yielded 45% more than CCC yet, once N was supplied, the additional gain from soybean decreased to 17%. Because the experiment was originally set up to study corn yields, soybean yields were not collected by N rate and rotation and were only reported as an average for the year. The mean crop yield for soybean through the study period was 3.83 Mg ha^{-1} (Fig. 6).

4 Discussion

This study presents a unique opportunity to characterize important soil properties after 36 years of consistent N fertilization in continuous corn and corn-soybean rotation management. Of the soil properties assessed, only soil pH, total exchange capacity, and the levels of NO_3^-, and available P and K in the soil responded to both high N fertilization rates and crop rotation. Annual additions of N fertilizer to continuous corn greatly acidified the topsoil, from a pH of 6.5~7 with no fertilizer to a pH of 4.9 under the highest N fertilization rate (N269), yet this effect was not evidenced within any phase of the corn-soybean rotation. As more N was added within the continuous corn rotation, soil NO_3^- levels increased eight-fold within the top 30 cm, a likely consequence from increased nitrification within these systems. A study by Behnke et al. (2018a) explored the effects of 20 years of crop rotation and tillage on GHG emissions, yields, and soil properties on similar soil and reported lower pH under continuous corn than corn-soybean rotation. Liebig et al. (2002) also showed more soil acidification under continuous corn than a corn-soybean rotation with increasing N rate up to 180 kg N ha^{-1}. Barak et al. (1997) demonstrated that soil acidification was related to an increase in the concentration of H^+ in the soil associated with the use of inorganic N fertilizer. Within our experimental site, Huang et al. (2019) indeed observed that the ammonia-oxidizing gene, *amoA*, increased with a higher N rate under continuous corn, which agreed with the observed reduction in the pH of the topsoil. Therefore, Huang et al. (2019) provided genomic evidence that cropping systems with more frequent N application at higher rates increased nitrification and decreased soil pH.

As shown in Fig. 1, the significant differences in total exchange capacity by treatments were dictated by the exchangeable acidity (H^+). Indeed, this relationship was not evidenced when analysing estimated CEC alone. Conversely, the estimated means of exchangeable acidity showed clear increase with N rate within continuous corn rotation. This result agrees well with the soil acidification under high N rates and continuous corn rotation. Similarly, Barak et al. (1997) also reported significant

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Over the 36 years of experiment, N fertilization significantly increased crop yield (Fig. 6), which should convert to greater amount of residue returning to the soil. Likewise, corn returns much more residue than soybean (Lal, 2005), therefore more crop residues are expected within continuous corn rotation than a corn-soybean rotation, especially in our site without residue removal. However, the expected greater residue return did not translate to SOM content in our study, which remained unaffected by either N addition or crop rotation. This is likely due to a positive priming effect as explained by Chen et al. (2014) who proposed a versatile soil microbial shifts between two positive priming effect theories: the microbial stoichiometry theory and the microbial N mining theory. The former is driven by the copiotrophic, r-selected microbes that rapidly decompose SOM under balanced microbial growth in a C and N rich environment, while the latter is driven by oligotrophic, K-selected microbes that mine N from SOM to utilize the fresh N-limited residues (Chen et al., 2014). Thus, within high N rate management, a soil rich in C from greater residue return and N from fertilizers would trigger accelerated SOM decomposition by microbes favoured by stoichiometry theory. This leads to faster turnover between fresh residue input and SOM, thereby maintaining the SOM content comparable to that of unfertilized treatment. Likewise, SOM within continuous corn management will match that of rotated corn as the N-limited corn residues trigger the microbes to decompose SOM to mine N, thereby offsetting the greater residue return. Therefore, a positive priming effect could help explain the lack of significant changes in SOM even after decades of treatments. Moreover, our SOM determination method, loss on ignition, only accounted for the organic C components, thus perhaps overlooking possible differences in organic N amog treatments.

Crop rotation and high N rates also had notable changes to physical soil properties. We observed greater WAS under continuous corn than the soybean phase of the corn-soybean rotation (Fig. 3b). Soybean residues have lower C:N and decompose faster than corn residues (Ajwa and Tabatabai, 1994). Also, soybean residues have a lower phenolic acid content (Martens, 2000), leading to a decrease in the stability of soil aggregates, thus explaining the decreased WAS during the soybean phase of the rotated corn. Since a soil aggregate is a complex unit of soil mineral particles, microbes, and organic matter, higher WAS implies that the soil microbial community can better withstand the stresses from physical disturbance and wetting cycles (Wilpiszeski et al., 2019). An Australian study by Trivedi et al. (2017) on the relationships between crop rotation, soil aggregates, and the microbiome reported that agricultural practices with greater residue returns enhanced the soil aggregation and richer microbial inhabitants. Their results agree with ours where WAS increased under continuous corn due to greater

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Deleted: showed that long-term urea fertilization on Mollisols decreased CEC as increased crop yield depleted more soil base cations. Unlike our methods, CEC determination by Russell et al. (2006) did not include exchangeable H^+, which should increase with more nitrification induced by N fertilizer. The summation of exchangeable cations used in the present study is an indirect CEC determination method that could have overestimated CEC by including the H^+ from nitrification (Sumner and Miller, 1996; Ross and Ketterings, 1995). Alternatively, because SOM is a known source of CEC (Parfitt et al., 1995), high N rates and continuous corn rotation could be behind the observed increase in CEC. High N rates increased crop yield, and thus, the level of residue returning to the soil in continuous corn management is much higher than the level of residues returned within a corn-soybean rotation. However, the SOM content in our study was unaffected by either N addition or crop rotation, likely due to a positive priming effect. Hall et al. (2019) define a positive priming effect as the increase in SOM decomposition upon the addition of freshly added biomass. Thus, a soil rich in C and N within the continuous corn and high N rate management would trigger a positive priming effect that accelerates SOM decomposition by microbes, offsetting new residue inputs. An Iowa study by Hall et al. (2019) compared SOC decomposition and the priming effect between continuous corn and rotated corn, observing both greater SOC decomposition and priming effect with continuous corn rotation because the microbes rapidly mineralized fresh and relic SOM to obtain N upon receiving N-poor corn residues (Chen et al., 2014).

Deleted: , which rules out SOM as the source of the CEC changes residue return than rotated corn. Further analysis of the soil microbiome may uncover whether changes in WAS enhance agriculturally beneficial microbial guilds. Moreover, stable soil aggregates become a long-term source of SOM by securing it in their structure (Lynch and Bragg, 1985; Trivedi et al., 2015; Wilpiszeski et al., 2019). Indeed, the means of both WAS and SOM showed similar positive responses to continuous corn rotation (Fig. 3b; Table A1). Unlike WAS, which increased with N fertilization rates, soil BD decreased as the N rate increased as a direct result of the increased level of residues returned to the soil, an effect that in turn, increases the porosity of the soil, favoring crop root growth (Jagadamma et al., 2008). A similar degree of decrease in BD with higher N rates has been reported by Halvorson et al. (1999), who compared the effects of N fertilization from a long-term Colorado Mollisol study and reported that BD decreased by 7.4 % from 0 kg N ha^{-1} to 90 kg N ha^{-1}.

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Deleted: Since SOM is a source of CEC, as discussed earlier, more stable soil aggregates may also contribute to greater CEC. Indeed, a multivariate analysis by Zuber et al. (2017) on the effects of tillage and crop rotation on soil properties demonstrated that both WAS and CEC increased with continuous corn rotation.

Our results also demonstrated that soil nutrients N, P, and K were sensitive to high N rates and crop rotation. The total surface inorganic N marginally increased with increasing N rates (Fig. 4a) and under continuous corn management (Fig. 4b). This trend in TIN was driven by increases in NO_3^-, as the level of NH_4^+ was not affected by management, displaying a typical decrease with increasing soil depth (Fig. 4f). Soil NH_4^+ did not respond to ammonium inputs from UAN, our fertilizer source, because it readily nitrified in the soil (Coskun et al., 2017; Lin et al., 2001; Di and Cameron, 2002), which is consistent with our results of increasing soil acidification and NO_3^- at higher N rates from the continuous corn rotation (Fig. 4e). A multivariate study by Behnke et al. (2020b) on the effects of crop rotation and tillage on soil properties and microbial N cycling genes also supports our findings by observing ammonia-oxidizing bacteria *amoA* correlating negatively with soil concentrations of NH_4^+ and positively with soil NO_3^- levels. As for a crop rotation effect, Behnke et al. (2020b) also found that bacterial *amoA* gene counts increased with continuous corn (annually receiving about 246 kg N ha^{-1}) compared to unfertilized continuous soybean after 25 years of treatments in place. In this study, however, the N fertilization effect is completely confounded with the rotation, as the treatments include the comparison of continuous corn versus continuous soybean management (unfertilized) and their corn-soybean rotation (receiving N fertilizer during the corn phase). Increased levels of NO_3^- in the soil under continuous corn have been related to increased N_2O emissions (Behnke et al., 2018a) by potentially stimulating the microbial nitrification and denitrification steps of the microbial N cycle. Behnke et al. (2020b) found that both *amoA* and *nirK* gene counts, used as indicators of changes in the nitrification and denitrification steps of the microbial N cycle (Hirsch and Mauchline, 2015), increased with continuous corn compared to continuous soybean. In conjunction with the studies of Behnke et al. (2018a) and Huang et al. (2019), our results strongly suggest that excessive and continuous N application will leave the soil more vulnerable to nutrient loss via nitrate leaching and nitrous oxide emissions. Moreover, our results indicate that rotated corn increases corn yields and reduces nitrate levels, which agrees with Behnke et al. (2018a). Further studies should verify these claims by concurrently assessing the changes in the activity of nitrifier and denitrifier microbial guilds in the soil, levels of NO_3^- and N_2O emissions, along with potential nitrification and denitrification rates for each N fertilization level during the growing season.

Congreves et al. (2015) found that soil nutrients, including P and K, were depleted differently under various crop rotations, leading to greater soil P and K levels under continuous corn and corn-soybean rotations than under continuous soybean. Differences in soil P and K dynamics were attributed to differences in nutrients returned as crop residues, dictated by either the innate crop biomass production and quality, or the amount of N fertilizer applied in a cropping system. Our results showed the available levels of soil P and K were significantly greater with no fertilizer and under continuous corn management (Figs. 5a and 5c). As the corn yield increased with increasing N rates, the crop uptake of P and K increased accordingly (Behnke et al., 2018a; Zuber et al., 2015). The highest P and K levels were also found within the topsoil where residues accumulate, and the N fertilizer is applied (Figs. 5b and 5d). While these nutrients decreased in the intermediate depths that are primarily explored by the crop root system, they slightly increased in the deeper layers that are closer to the parent mateirals. These nutrients were slightly greater within the deeper layers, closer to the soil parent material, and out of reach from roots of typical row crops (Anderson, 1988; Simonsson et al., 2007). Given the importance of P levels in these intensively managed systems to trigger environmental consequences due to unintentional P losses via runoff from agricultural fields, further studies should explore how N fertilization and rotation practices influence soil P dynamics and stocks throughout the soil profile, informing P budgets for comprehensive agroecosystem P management.

In 2004, Jagadamma et al. (2008) characterized the top 30cm of the soil at our experimental site, and while the results of this precursor study mostly agreed with ours, differences between studies could be explained by differences in methodology and depth of study. Like our findings, Jagadamma et al. (2008) showed that higher N rates acidified the soil and decreased BD and K^+ level under continuous corn rotation; they also found greater soil N with higher N rates, yet they did not separate the contributions of NO_3^- and NH_4^+, in their report of total N. Jagadamma et al. (2008) found that WAS, increased with higher N rates under continuous corn rotation but not corn-soybean rotation, which is comparable to our results (Fig. 3b). In contrast, the authors did not find significant changes in CEC (Jagadamma et al., 2008), attributed to the use of a different CEC determination method that included exchangeable acidity (H^+ and Al_3^+), and to the study of the top 30cm of the soil as a whole. Furthermore, Jagadamma et al. (2008) reported that SOC increased with higher N rates and continuous corn rotation, while our results did not find statistically significant changes in SOM (Table 1). This is likely the result of our consideration of the effects of our replicates (blocks) as random effects in the ANOVA, as we are concerned with inferences beyond these particular replicates (Federer and King, 2007). Jagadamma et al. (2008) considered the replicate effects as fixed effects instead, as their ANOVA was used as a preliminary variable selection technique for a subsequent multivariate approach aiming to predict crop yields based on soil properties. The authors identified soil C stocks, WAS, soil C:N ratio, K^+, and water content as the subset of soil properties that could best predict corn yields in the continuous corn systems (R^2=067, p<0.001). However, no soil property was related to crop yields within the corn-soybean system. In this regard, our averaged grain yields for the total duration of the experiment did not vary significantly from those until 2004 reported by Jagadamma et al. (2008). The averaged yield of both, continuous and rotated corn, increased with N inputs, and plateaued after 140 kg N ha^{-1}; the averaged rotated corn yield was greater than the averaged continuous corn yield over all N rates (Jagadamma et al., 2018). Compared to these 2004 results, the results of our study showed that unfertilized corn yield increased no more than 2% over these 13 years, while the fertilized corn yields increased by 6% under continuous corn, and 7.7% under rotation with soybeans, respectively. Overall, the comparison between this study and Jagadamma et al. (2008) did not find any unexplained inconsistency, suggesting that this agricultural system remained stable in the 13 years since the first report.

5 Conclusions

Our evaluation following 36 years of N fertilization and crop rotation on soil physical and chemical properties highlight the unique features of Mollisols. Long-term management practices that reduce soil pH are known to pose a significant threat to the environment through increased greenhouse gas emissions and N losses through accelerated nitrification. Furthermore, the reduction in pH requires routine management in the form of liming or other soil remediation methods. We found nearly a two-unit reduction in soil pH and an eight-fold increase in soil nitrates observed from the highest fertilizer rate within the continuous corn system compared to those of unfertilized control, implying substantial nitrification, confirming our hypothesis. By adding soybean to our system, we observed a decrease in the soil aggregate stability to water during the soybean phase, due to lower quantity and different chemical properties of soybean residues. Increased yields with fertilization within the continuous corn system improved the bulk density of the soil, likely associated to the volume of residues returned, thus confirming our hypothesis. On the other hand, an contradicting our original hypothesis, soil organic matter (as percentage or as stock) was unaffected by our treatments, a response that is potentially associated to a priming effect of the microbial guilds. The Mollisols investigated in this study are resilient, yet management practices that trigger acidification and N pollution should be further scrutinized. Our results suggest that the typical chemical and physical properties studied here might not provide enough characterization of the status of the system, and a deeper understanding of the microbial nutrient cycling within these highly productive agroecosystems, is vital. Future work emphasizing coupled measurements of greenhouse gas emission with soil metagenomic and functional analysis will validate the implications of our findings. Moreover, future efforts should further characterize the chemical and physical properties of soil organic matter, and draw a more complete picture of the C an N budget in the agroecosystem. Likewise, exploring management practices that encourage temporal and spatial diversification to alleviate nutrient losses, such as cover cropping or split N application, would help answer questions on the sustainability of current practices.

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Code and Data availability: Data will be deposited in Mendeley upon manuscript acceptance.

Author Contributions: Conceptualization, M.B.V.; methodology, N.K., and G.D.B.; formal analysis, M.B.V., and N.K.; resources, M.B.V..; data curation, M.B.V.; writing—original draft preparation, N.K., and M.B.V.; visualization, N.K.; writing—review and editing, G.D.B.; and M.B.V.; supervision, project administration, and funding acquisition, M.B.V. All authors have read and agreed to the published version of the manuscript.

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Tables

Table 1. The treatment mean values (Mean) and standard error of the means (SEM) of the soil properties: total exchange capacity (EC, cmolc kg^{-1}), soil pH, soil organic matter (SOM, %), total carbon stock (TCs, Mg ha^{-1}), water aggregate stability (WAS, %), and bulk density (BD, Mg m^{-3}) determined by the main effects of N fertilization rate (N rate, 0, 202, and 269 in kg N ha^{-1}), crop rotation [Rot, continuous CCC; corn phase of corn-soybean rotation (Cs), and soybean phase of corn-soybean rotation (Sc)], and soil depth (D, cm). Probability values (p-values) and degrees of freedom (df) associated with the different sources of variation in the analysis of variance are shown below.

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	EC		pH		SOM		TCs		WAS		BD	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
N rate												
0	20.00	0.56	6.73	0.08	2.78	0.10	53.76	1.77	71.42	1.38	1.41	0.02
202	20.56		6.59		2.81		53.39		73.26		1.38	
269	21.58		6.49		2.86		52.56		72.49		1.34	
Rot												
CCC	22.20	0.85	6.31	0.12	2.98	0.10	54.55	1.77	74.99	1.38	1.34	0.03
Cs	19.32		6.79		2.82		53.73		72.22		1.38	
Sc	20.62		6.71		2.66		51.43		69.95		1.40	
D												
0-15	23.04	0.67	6.40	0.09	3.87	0.08	52.50	1.55	64.80	1.21	1.37	0.02
15-30	21.00	0.60	6.55	0.08	3.50	0.08	50.52	1.52	76.96	1.30	1.45	0.02
30-60	19.00	0.53	6.69	0.07	2.53	0.07	67.64	1.86	79.39	1.32	1.30	0.02
60-90	19.82	0.55	6.78	0.07	1.37	0.04	42.27	1.38	68.40	1.48	1.37	0.02

Source of Variation	df	CEC	pH	SOM	TCs	WAS	BD
N rate	2	0.0019	0.0057	0.8108	0.8115	0.5158	0.0240
Rot	2	0.1560	0.0616	0.1250	0.2639	0.0167	0.3104
N rate x Rot	4	0.0020	0.0124	0.9789	0.9473	0.2068	0.9287
D	3	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
N rate x D	6	0.0126	0.0033	0.3125	0.4628	0.3743	0.5247
Rot x D	6	0.0004	<.0001	0.2840	0.7150	0.8282	0.3100
N rate x Rot x D	12	0.0950	0.0351	0.3482	0.7476	0.5991	0.9474

Table 2. The treatment mean values (Mean) and standard error of the means (SEM) of the soil nitrate (NO_3^-, mg kg^{-1}), ammonium (NH_4^+, mg kg^{-1}), total inorganic nitrogen (TIN, mg kg^{-1}), available phosphorus (P, mg kg^{-1}), and potassium (K, mg kg^{-1}) determined by the main effects of N fertilization rate (N rate, 0, 202, and 269 in kg N ha^{-1}), crop rotation [Rot, continuous CCC; corn phase of corn-soybean rotation (Cs), and soybean phase of corn-soybean rotation (Sc)], and soil depth (D, cm). Average corn phase yield (Mg ha^{-1}, 1981-2017) determined for each N rate and rotation are included in the last column. Probability values (p-values) and degrees of freedom (df) associated with the different sources of variation in the analysis of variance are shown below.

	NO_3^-		NH_4^+		TIN		P		K		Yield	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
N rate												
0	2.24	0.84	8.42	3.05	10.70	3.06	17.47	1.56	113.39	4.57	6.89	0.41
202	5.12		8.39		13.52		11.42		99.22		11.10	
269	6.21		6.74		12.88		11.86		102.11		11.25	
Rot												
CCC	7.65	0.84	6.15	4.74	13.72	4.16	14.19	1.56	119.86	4.62	8.44	0.40
Cs	3.15		5.54		8.73		14.14		99.11		11.05	
Sc	2.78		11.86		14.65		12.42		95.75			
D												
0-15	8.62	1.35	14.38	2.86	23.06	2.99	26.44	1.96	188.70	7.92		
15-30	5.19	0.56	8.58	2.80	13.77	2.65	10.70	1.28	93.81	2.39		
30-60	2.61	0.30	4.85	2.87	7.46	2.68	6.04	0.44	59.93	2.41		
60-90	1.69	0.21	3.59	2.92	5.17	2.72	11.15	0.57	77.19	3.05		

Source of Variation	df	NO_3^-	NH_4^+	TIN	P	K	Yield
N rate	2	0.0075	0.6428	0.5520	0.0207	0.0908	<.0001
Rot	2	0.0005	0.6122	0.5638	0.6632	0.0047	<.0001
N rate x Rot	4	0.0055	0.4296	0.2296	0.0973	0.0171	<.0001
D	3	<.0001	<.0001	<.0001	<.0001	<.0001	
N rate x D	6	0.0643	0.2630	0.0547	0.0029	0.3401	
Rot x D	6	0.0097	0.8515	0.0972	0.6705	0.0870	
N rate x Rot x D	12	0.1996	0.7622	0.6334	0.1265	0.2185	

Figure legends

Figure 1. Mean values of total exchange capacity (EC, $cmol_c\ kg^{-1}$) with the proportions of each major exchangeable cations and acidity (H^+) comprising EC are shown by N fertilization rate (Y-axis), crop rotation (panel columns), and depth (panel rows). Bars show the mean values and each of their two error bars represent the standard errors of the treatment means each for the sum of exchangeable cations, and exchangeable acidity. The N fertilization rates were 0, 202, and 269 (kg N ha^{-1}). Crop rotations are continuous corn (CCC), and the corn-soybean rotation (CS) as corn Cs, and soybean (Sc) phase, respectively. For a given depth and within each rotation, mean EC values for each N rate followed by the same lowercase letter are not statistically different ($\alpha = 0.05$). For a given depth and across rotations, mean EC values for each N rate followed by the same uppercase letter are not statistically different ($\alpha = 0.05$). Lack of statistically significant differences among treatment means within a given depth are indicated by "NS".

Deleted: cation

Deleted: C

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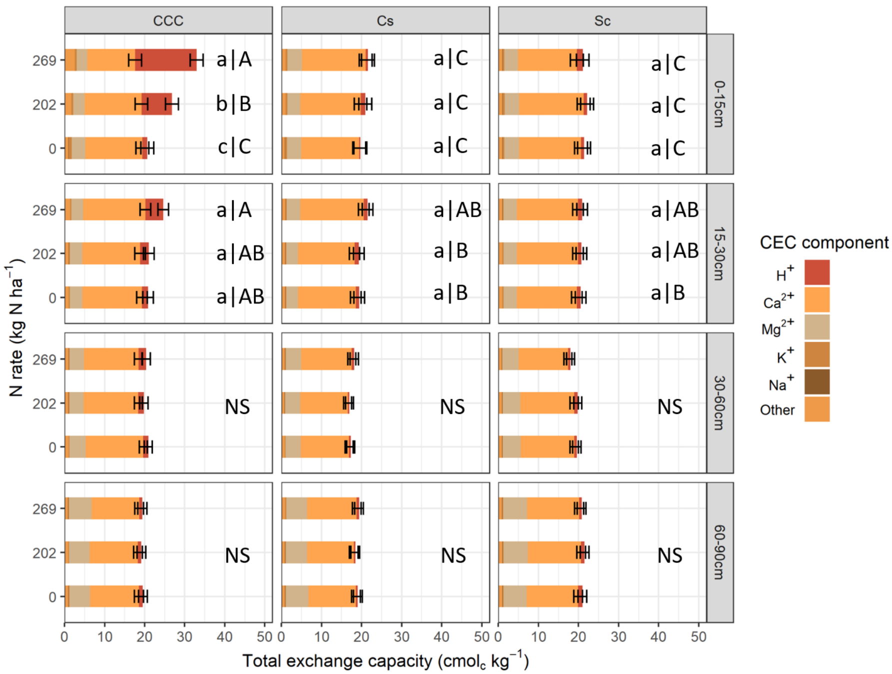
Figure 2. Mean soil pH under each N fertilization rate (Y-axis), crop rotation (panel columns), and depth (panel rows). Bars show the mean values and the error bars represent the standard error of the treatment means. The N fertilization rates were 0, 202, and 269 (kg N ha^{-1}). Crop rotations are continuous corn (CCC), and the corn-soybean rotation (CS) as corn Cs, and soybean (Sc) phase, respectively. For a given depth and within each rotation, mean pH values for each N rate followed by the same lowercase letter are not statistically different ($\alpha = 0.05$). For a given depth and across rotations, mean pH values for each N rate followed by the same uppercase letter are not statistically different ($\alpha = 0.05$). Lack of statistically significant differences among treatment means within a given depth are indicated by "NS".

Figure 3. Mean water aggregate stability of the soil aggregates (WAS, %) determined for each depth (a) and crop rotation (b). Mean soil bulk density (BD, Mg m^{-3}) determined for each depth (c) and N fertilization rate (d). In both plots, bars show the mean values and the error bars represent the standard error of the treatment means. The N fertilization rates were 0, 202, and 269 (kg N ha^{-1}). Crop rotations are continuous corn (CCC), and the corn-soybean rotation (CS) as corn Cs, and soybean (Sc) phase, respectively. Within each panel, means followed by the same lowercase letters are not statistically different ($\alpha = 0.05$).

Figure 4. Averaged total inorganic N (TIN, mg kg^{-1}) determined at different depths of soils under different N fertilization rates (a) and different crop rotations (b). Mean values of soil NO_3^- (mg kg^{-1}) are also shown in c) by N fertilization rate at each successive depth (c), and by rotation at each depth (d). Panel e) shows the interaction effect of N fertilization rate and crop rotation on NO_3^- levels. Panel f) depicts the mean NH_4^+ values measured at each depth. In all cases, bars show the mean values and error bars represent the standard error of the treatment means. The N fertilization rates were 0, 202, and 269 (kg N ha^{-1}) Crop rotations are continuous corn (CCC), and the corn-soybean rotation (CS) as corn Cs, and soybean (Sc) phase, respectively. Within each panel, means followed by the same lowercase letters are not statistically different ($\alpha = 0.05$). Lack of statistically significant differences among treatment means within a given depth are indicated by "NS".

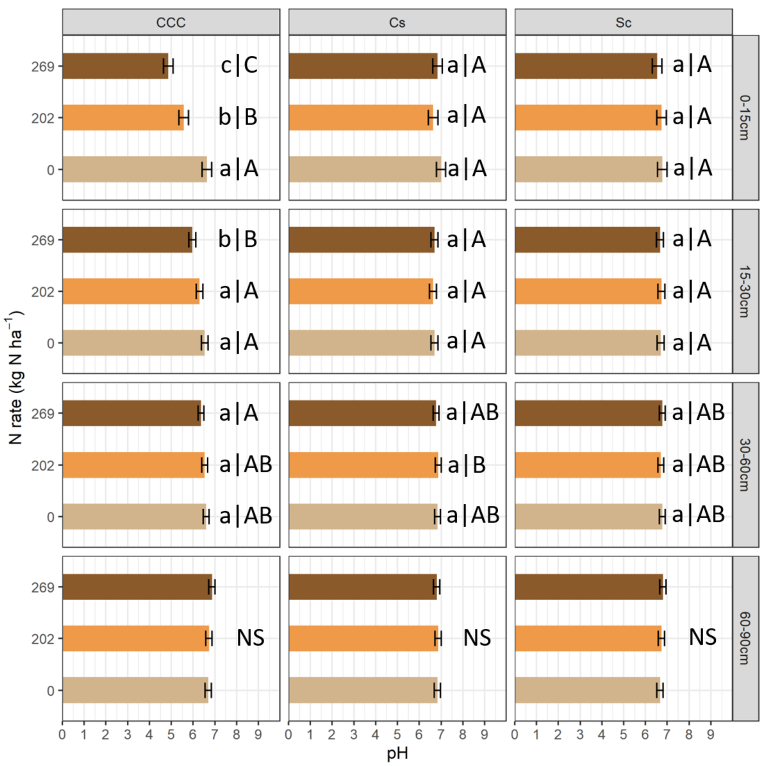
Figure 5. The mean soil P (mg kg^{-1}) by N fertilization rate and crop rotation (a) and by depth and N fertilization rate (b), and soil K (mg kg^{-1}) by N fertilization rate and crop rotation (c) and by depth and crop rotation (d). In all cases, bars show the mean values and error bars represent the standard error of the treatment means. The N fertilization rates were 0, 202, and 269 (kg N ha^{-1}). Crop rotations are continuous corn (CCC), and the corn-soybean rotation (CS) as corn Cs, and soybean (Sc) phase, respectively. Within each panel, means followed by the same lowercase letters are not statistically different (α = 0.05). Lack of statistically significant differences among treatment means within the same depth are indicated by “NS”.

Figure 6. Average corn yield (Mg ha^{-1}) during the 36 years of the study for each level of N fertilization and for each cropping system. Average soybean yield (Mg ha^{-1}) is included as reference (dashed line). Error bars represent the standard error of the treatment means. The N fertilization rates were 0, 202, and 269 (kg N ha^{-1}). Crop rotations are continuous corn (CCC), and the corn-soybean rotation (CS) as corn Cs, and soybean (Sc) phase, respectively.



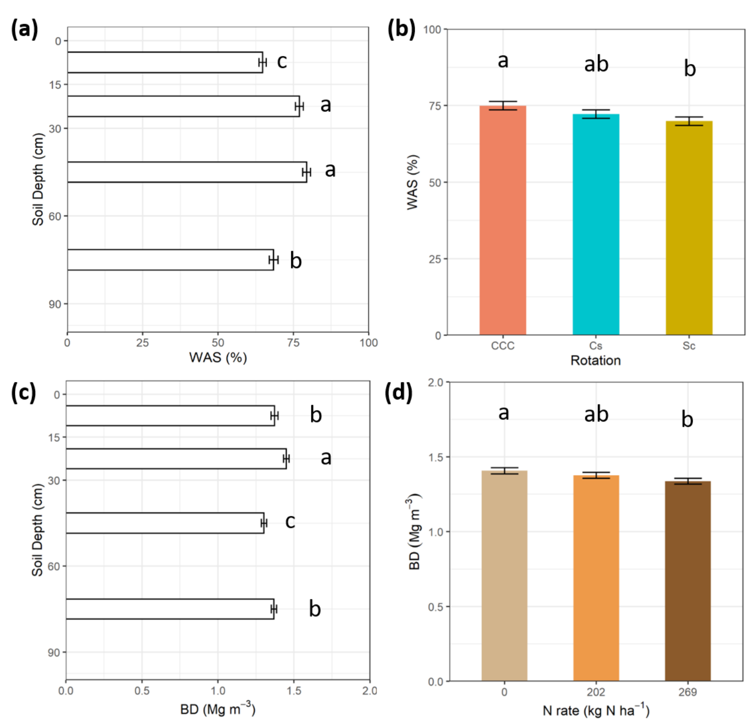
CCC
Cs
Sc
0-15cm
15-30cm
30-60cm
60-90cm
269
202
0
a|A
b|B
c|C
a|C
a|AB
a|B
NS
N rate (kg N ha^{-1})
Total exchange capacity (cmol$_c$ kg^{-1})
0
10
20
30
40
50
CEC component
H$^+$
Ca^{2+}
Mg^{2+}
K$^+$
Na$^+$
Other

Figure 1



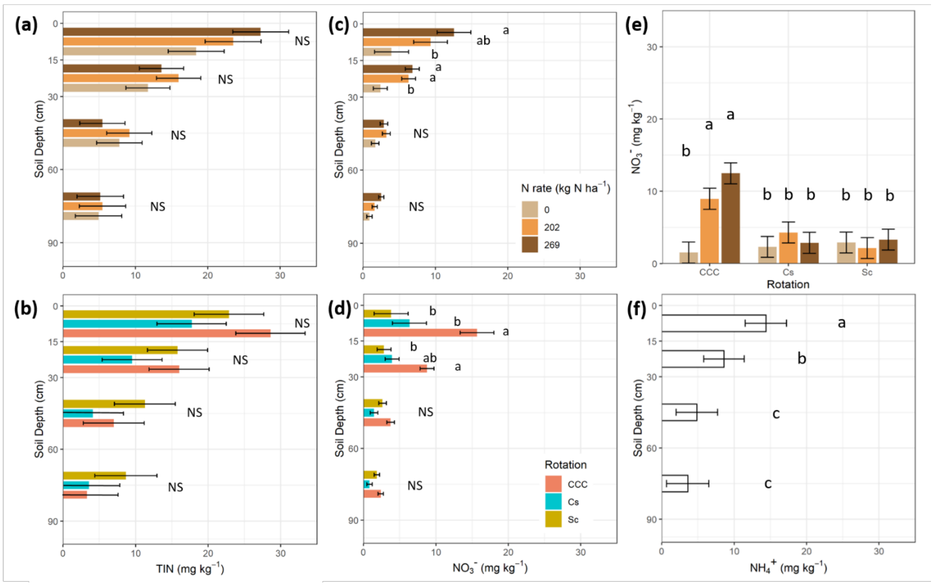
CCC
Cs
Sc
0-15cm
15-30cm
30-60cm
60-90cm
N rate (kg N ha^{-1})
269
202
0
c|C
b|B
a|A
a|A
a|A
a|A
a|A
a|A
a|A
b|B
a|A
a|A
a|A
a|A
a|A
a|A
a|A
a|A
a|A
a|AB
a|AB
a|AB
a|AB
a|B
a|AB
a|AB
a|AB
NS
NS
NS
0 1 2 3 4 5 6 7 8 9
pH

Figure 2



(a)
Soil Depth (cm)
WAS (%)
c
a
a
b
(b)
WAS (%)
Rotation
CCC
Cs
Sc
a
ab
b
(c)
Soil Depth (cm)
BD (Mg m^{-3})
b
a
c
b
(d)
BD (Mg m^{-3})
N rate (kg N ha^{-1})
0
202
269
a
ab
b

Figure 3



(a)
(b)
(c)
(d)
(e)
(f)
Soil Depth (cm)
TIN (mg kg^{-1})
NO$_3^-$ (mg kg^{-1})
NH$_4^+$ (mg kg^{-1})
N rate (kg N ha^{-1})
0
202
269
Rotation
CCC
Cs
Sc
NS

Figure 4

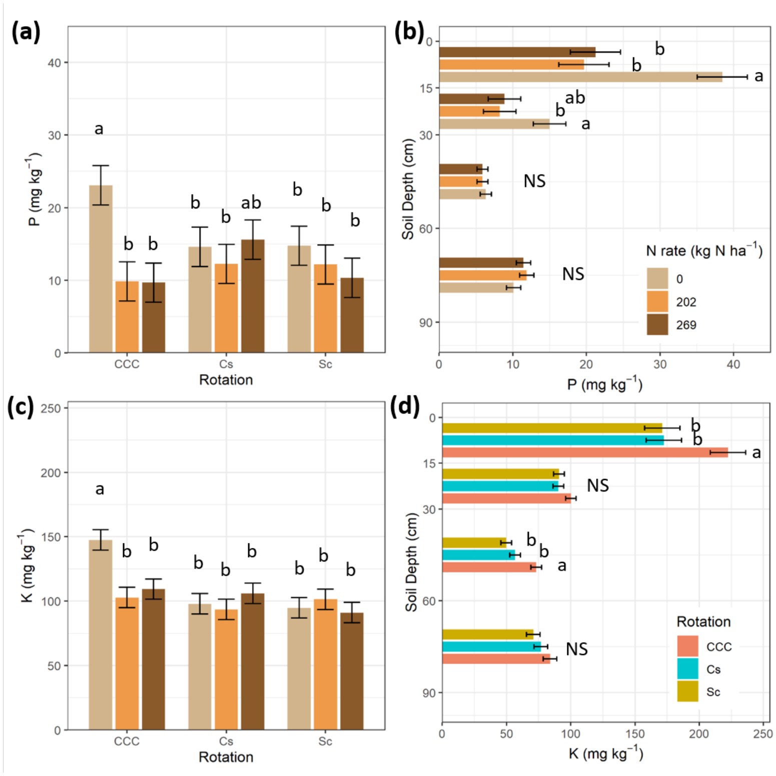

(a)
P (mg kg^{-1})
Rotation
CCC
Cs
Sc
(b)
Soil Depth (cm)
P (mg kg^{-1})
N rate (kg N ha^{-1})
0
202
269
NS
(c)
K (mg kg^{-1})
Rotation
(d)
Soil Depth (cm)
K (mg kg^{-1})
Rotation
CCC
Cs
Sc
NS

Figure 5

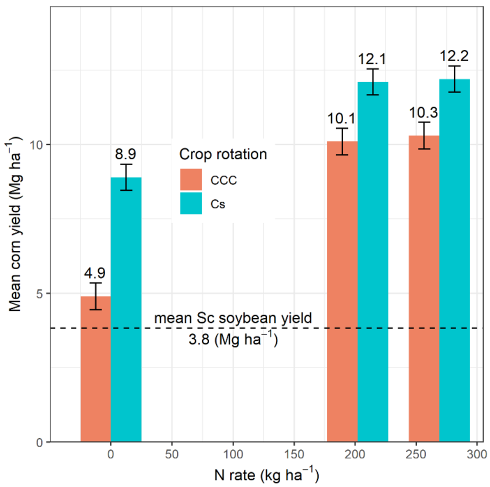

Mean corn yield (Mg ha^{-1})
N rate (kg ha^{-1})
Crop rotation
CCC
Cs
mean Sc soybean yield
3.8 (Mg ha^{-1})
4.9
8.9
10.1
12.1
10.3
12.2
0
5
10
0
50
100
150
200
250
300

Figure 6