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Effect of Fertilizer Nitrogen (N) on Soil Organic Carbon, Total N and Soil pH in Long-Term Continuous Winter Wheat (*Triticum aestivum* L.)

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ABSTRACT

Carbon sequestration via sound agronomic practices can assist in combatting global warming. Three long-term experiments (Experiment 502, Experiment 222, and The Magruder Plots) were used to evaluate the effect of fertilizer nitrogen (N) application on soil organic carbon (SOC), total nitrogen (TN) and pH in continuous winter wheat. Soil samples (0-15 cm) were obtained after harvest in 2014, analyzed and compared to soil test results from these same experiments in 1993. Soil pH decreased with increasing N fertilization, and more so at high rates. Nitrogen application significantly increased TN in Experiment 502 from 1993 to 2014, and TN tended to be high at high N rates. Fertilizer N significantly increased SOC, especially when N rates exceeded 90 kg ha^{-1}. The highest SOC (13.1 g kg^{-1}) occurred when 134 kg N ha^{-1} was applied annually. Long-term N application at high rates increased TN and SOC in the surface soil.

Keywords Nitrogen, Soil organic carbon, total nitrogen, soil pH

INTRODUCTION

Nitrogen (N) fertilizer is one of the most extensively applied nutrient sources used to improve crop yields in the world. The contribution of N to higher crop yields and its impact on water quality are well known (Schlegel, Dhuyvetter, and Havlin 1996; Biederbeck et al. 1996). Cropland where inorganic fertilizers are used could counter balance fossil fuel emissions by acting as a carbon (C) sink. Lal (2004) indicated that soil C sequestration has the capacity to reduce annual fossil fuel emissions by 0.4-1.2 Gt (5-15%) of C emitted globally.

Although a number of studies have been conducted on N fertilization under different cropping systems, their impact on soil organic carbon (SOC) and total nitrogen (TN) are not clearly understood (Biederbeck et al. 1996; Jantalia and Halvorson 2011). Other work that focused on SOC, TN and soil pH under long-term N application demonstrated that SOC increases with an increase in N rates (Raun et al. 1998). Halvorson and Reule (1999) suggested that the increase in SOC with an increase in N rates may be due to increased quantity of crop residues added to the soil. Jantalia and Halvorson (2011) reported a significant effect of fertilizer N on TN at soil depths of 0-7.6 cm and 7.6-15.2 cm under irrigated maize in a conventional tillage (CT) system. This is possibly due to accumulation of crop residues at surface depths. From a long-term perspective, this could ensure sustainability in the agricultural production systems (Lal 2007). Yang et al. (2008) showed that CT has more subsurface SOC since more crop residues are incorporated by tillage within the 20-30 cm soil depth. Their work revealed that at 0-5 cm surface depth there is more sequestration of C in no-till (NT) compared to CT.

Numerous studies also indicate that application of N has the effect of acidifying soils (Barak et al. 1997; Tuyen, Phung, and Tinh 2006; Rezig, Mubarak, and Ehadi 2013). Acidification is greatest at high rates of N application (Barak et al. 1997). Depending on the crop, lime is widely used in crop and forage production systems to correct for the acidifying effect of N fertilizers and raise soil pH (Ernani, Bayer, and Maestri 2002).

The objective of this study was to determine the effect of long-term N application on SOC, TN and soil pH and to evaluate differences in SOC, TN and soil pH over time in continuous winter wheat.

MATERIALS AND METHODS

Site Description, Soil Sampling and Statistical Analysis

Three long-term continuous winter wheat fertility experiments; Experiment 222 (E222), Experiment 502 (E502) and the Magruder Plots were used in this study. The Magruder Plots located in Stillwater, OK were initiated in 1892 with an unfertilized check plot as the only treatment. Other treatments were later introduced and applied as reported in Table 1 and described by Girma et al. (2007). Experiment 222 located in Stillwater, OK was established in 1969 while E502 located in Lahoma, OK was established in 1970. Soils at E222 and the Magruder Plots are classified as Kirkland Silt Loam (fine, mixed, thermic Udertic) and received an annual rainfall (September to August) of 798 and 541 mm in 2013 and 2014, respectively. At these sites, annual temperatures of 15.2 and 14.6^{o}C were recorded in 2013 and 2014, respectively. Soil at E502 is classified as Grant Silt Loam (fine-silty, mixed, superactive, thermic, Udic Argiustoll). This site received an annual rainfall of 746 and 586 mm in 2013 and 2014, respectively. Annual temperature at E502 was 12.8 and 13.9^{o}C in 2013 and 2014, respectively.

A randomized complete block design with four replications was used at E222 and E502. The Magruder Plots are not replicated having been established prior to the advent of modern statistics in agricultural experimentation (Girma et al. 2007). The treatment structure for these three experiments is reported in Table 1. Conventional tillage has been used since the establishment of the Magruder Plots. Experiment 222 and E502 were both changed from CT to NT in 2011. Wheat has been planted every year in the fall for all trials using varieties common to each region.

Sixteen soil cores were collected from each plot in E222 and E502 while the Magruder Plots were divided into two equal halves and 16 soil cores collected from each half at 0-15 cm soil depths. These soil samples were obtained from the experimental plots following wheat harvest in July 2013 and 2014. Samples were air-dried at ambient temperature and then ground to pass a 100 mesh sieve (<0.15 mm) (Tabatabai and Bremner 1970). Samples were analyzed for TN and SOC using a LECO (TruSpec-CN) dry combustion analyzer (Schepers, Francis, and Thompson 1989). Soil pH was determined using a glass electrode and soil water ratio of 1:1. Historical soil test results for samples collected in July 1993 from these same sites were compared to that collected in this study to evaluate changes in soil pH, TN and SOC over time (1993-2014). A detailed description of how soil samples were obtained and analyzed in 1993 was reported by Raun et al. (1998).

The data obtained were analyzed using SAS software (SAS Institute Inc. 2008). PROC GLM was performed to determine the effect of N application on soil pH, TN and SOC. Least squares means were obtained using the LSMEANS statement. Treatment means were separated using the Tukey ($\alpha = 0.05$) procedure. Linear and quadratic trends were examined using non-orthogonal single degree of freedom contrasts.

RESULTS

Soil pH

For E222, N fertilization significantly reduced soil pH in the 0-15 cm soil depth in years 1993, 2013 and 2014 ($p < 0.01$) (Table 2). The effect of annual N fertilization was not detected until at least 90 kg N ha^{-1} was applied. Soil pH was observed to be 6.1, 4.8 and 4.4 in 1993, 2013 and 2014, respectively, following application of 90 kg N ha^{-1} (Table 2). The largest reduction in soil pH occurred at a rate of 134 kg N ha^{-1} where soil pH of 5.8, 4.6 and 4.3 in 1993, 2013 and 2014, respectively, were consistently lower than soil pH observed at other N rates. There was a linear relationship between soil pH and N rates with r^2 (coefficient of determination) values of 0.51, 0.51 and 0.67 in 1993, 2013 and 2014, respectively. Contrast analysis for linear and quadratic trends showed that pH was linearly related to N rate ($p < 0.01$).

There was a significant reduction in soil pH during the period considered in this study (1993 to 2014). In the unfertilized check plots, soil pH reduced from 6.3 in 1993 to 5.0 and 4.9 in 2013 and 2014, respectively (Table 2). These results showed that high N rates produced the greatest effect of increasing soil acidity. Soil pH reduction in the unfertilized check plots is suggestive of organic matter decomposition and crop removal of basic cations. Nonetheless, this dramatic change was not expected.

In E502, soil pH was significantly affected by application of N in 1993, 2013 and 2014 ($p < 0.01$). Without N input, soil pH was observed to be 5.9, 6.1 and 6.1 in 1993, 2013 and 2014, respectively (Table 2). Application of 22 kg N ha^{-1} resulted in soil pH of 5.7, 5.8 and 5.8 in 1993, 2013 and 2014, respectively. The effect of N application on soil pH was not observed until at least 45 kg N ha^{-1} was applied each year. Application of 112 kg N ha^{-1} resulted in a reduction of soil pH to 5.1, 4.9 and 4.8 in 1993, 2013 and 2014, respectively, relative to the unfertilized check plots. Contrast analysis showed that soil pH was negatively related to N rate ($p < 0.01$). Also, linear regression showed a negative relationship between soil pH and N fertilization with r^2 of 0.40, 0.80 and 0.80 in 1993, 2013 and 2014, respectively.

During the study period (1993-2014), the largest reduction in soil pH occurred at a rate of 112 kg N ha^{-1} where soil pH remained consistently low in comparison to other N rates. The slight increase in soil pH at a rate of 0 and 22 kg N ha^{-1} may be due to upward movement of lime like materials to surface soils (Schwab, Owensby, and Kulyingyong 1990). This may also explain why there was a small reduction in soil pH with time for N rates of 45, 67, 90 and 112 kg ha^{-1}. The transfer of basic cations to the surface soil may offer resistance to changes in soil pH.

In the Magruder Plots, soil pH was observed to decrease in manure; phosphorus (P); nitrogen and phosphorus (NP); and nitrogen, phosphorus and potassium (NPK) treated plots relative to the unfertilized check plot in 1993, 2013 and 2014 (Table 4). The unfertilized check plot soil pH was

5.8, 5.5 and 5.2 in 1993, 2013 and 2014, respectively. The lowest soil pH was observed in the NPK plot where soil pH was observed to be 5.2, 4.7 and 4.4 in 1993, 2013 and 2014, respectively. Generally, there was high acidity where fertilizer N was applied without lime compared to P and NPKL (nitrogen, phosphorus, potassium and lime) treatments. The manure plot retained a relatively high soil pH of 7.5, 6.4 and 6.2 in 1993, 2013 and 2014, respectively, compared to other treatments.

These results showed that with time (1993-2014), lime addition prevented large reductions in soil pH observed in NP and NPK plots and in fact raised soil pH by 0.3 relative to the unfertilized check plot. Ability of P to form an insoluble compound with acidic cations such as Al^{3+} and Fe^{3+} may explain why soil pH has remained above 5.0 at the end of the 22 year period (1993-2014) considered in this study.

Total Nitrogen (TN)

For samples obtained from E222, TN was not significantly affected by N application in 1993 and 2014 ($p > 0.1$). However, there was a tendency for TN to increase with increasing N rate (Table 5 and Figure 1). In 1993, TN remained relatively stable at 0.8 g kg^{-1} when N was applied at a rate ranging from 0 to 134 kg ha^{-1}. Total N observed in 2014 increased from 1.4 to 1.5 g kg^{-1} at a rate of 0 and 134 kg N ha^{-1}, respectively. These results indicated a small increase in TN with addition of N over unfertilized check plots. In 2013, there was a significant effect of N fertilization on TN in the surface soil (0-15 cm) ($p < 0.1$) (Table 5). Results showed a linear increase in TN with an increase in N rates ($p < 0.05$) (Table 5). Total N was largest at N rates of 90 and 134 kg ha^{-1} where TN was observed to be 1.5 and 1.6 g kg^{-1}, respectively. Single degree of freedom contrasts showed a significantly higher amount of TN at 90 and 134 kg N ha^{-1} over the unfertilized check plots with TN of 1.3 g kg^{-1} ($p < 0.05$).

There was a significant increase in TN between 1993 and 2014 for all the different N rates including the unfertilized check. During this 22 year period (1993-2014) the largest increase in TN was observed at an application rate of 90 kg N ha^{-1} where TN increased by 0.1 g kg^{-1} relative to the unfertilized check plots. These results suggest accumulation of a slightly higher amount of TN in the surface soil with an increase in N rate.

In E502, TN in the surface soil was significantly affected by N fertilization in 2013 ($p < 0.1$) and 2014 ($p < 0.01$). Without application of N, TN was 1.1 and 1.0 g kg^{-1} in 2013 and 2014, respectively (Table 6 and Figure 1). Fertilization at an annual rate of 90 kg N ha^{-1} resulted in a significant increase in TN compared to lower N rates. Total N exhibited a linear trend with an increase in N rates ($p < 0.05$). Furthermore, contrast analysis showed that application of 90 and 112 kg N ha^{-1} resulted in higher TN (1.2 g kg^{-1}) compared to 45 kg N ha^{-1} with TN of 1.0 g kg^{-1} ($p < 0.1$) in 2014. In 1993, there was no significant effect of N application on TN in the surface soil. There was a tendency for TN to increase with an increase in rates of N application. Overall, TN exhibited a linear relationship with N rates ($p < 0.1$).

For the period (1993 to 2014) considered in this study, TN increased significantly at all N rates and in the unfertilized check plots. Total N tended to be largest at rates above 67 kg N ha^{-1} (Figure 1). Application of N at a rate of 112 kg N ha^{-1} resulted in TN of 0.7, 1.3 and 1.2 g kg^{-1} in 1993, 2013 and 2014, respectively, and this was consistently greater than TN observed at other rates. These results showed a significantly higher amount of TN at 112 kg N ha^{-1}. Overall, TN at rates less than or equal to 90 kg N ha^{-1} did not differ significantly within each of the 3 years and the significant increase in TN with time may be due to other environmental factors.

In the Magruder Plots, results showed that TN increased for all treatments at 0-15 cm in 1993, 2013 and 2014, relative to the unfertilized check plot (Table 3 and Figure 3). In the unfertilized check plot, TN was observed to be 0.6, 1.2 and 0.9 g kg^{-1} in 1993, 2013 and 2014, respectively. Application of NPK resulted in an increase in TN to 0.9, 1.8 and 1.2 g kg^{-1} in 1993, 2013 and 2014, respectively. Manure addition increased TN by 0.1, 0.3 and 0.4 g kg^{-1} in 1993, 2013 and 2014, respectively, relative to the unfertilized check plot. Addition of NPKL retained a higher soil TN compared to the unfertilized check. Total N observed in the unfertilized check plot did not change significantly following application of P alone.

Changes in TN during the period (1993-2014) considered in this study are reported in Table 3. Results suggested an increase in TN with time for the different treatments. In the unfertilized check plot, TN increased from 0.6 g kg^{-1} in 1993 to 1.2 and 0.9 g kg^{-1} in 2013 and 2014, respectively. Long-term application of N in both organic and inorganic forms consistently maintained a slightly higher amount of TN in comparison to application of P alone, an indication of the importance of N in maintaining soil fertility.

Soil Organic Carbon (SOC)

In 1993, 2013 and 2014, there was a significant effect of N application on SOC of surface soils in E222 ($p < 0.05$). Soil organic C reported in the unfertilized check plots was 7.9, 10.7 and 10.7 g kg^{-1} in 1993, 2013 and 2014, respectively (Table 5 and Figure 2). This increased to 8.8, 13.1

and 12.4 g kg^{-1} in 1993, 2013 and 2014, respectively, when N was applied at a rate of 134 kg ha^{-1}. Soil organic C started to increase significantly following application of at least 90 kg N ha^{-1}. Soil organic C followed a linear trend with added N in 1993 and 2013 ($p < 0.01$). In 2014, SOC followed both linear and quadratic trends, which indicated a slight decline in SOC at 134 kg N ha^{-1}. Additionally, contrast analysis revealed a significantly higher SOC at 134 kg N ha^{-1} compared to 45 kg N ha^{-1} ($p < 0.1$).

Soil organic C has significantly increased with time (1993-2014) for the different N rates including unfertilized check plots. From 1993 to 2014, SOC increased in the unfertilized check plots from 7.9 g kg^{-1} in 1993 to 10.7 g kg^{-1} in 2014, respectively. The increase in SOC was highest at a rate of 90 kg N ha^{-1} where SOC increased by 1.4 g kg^{-1}. Results showed that application of 90 and 134 kg N ha^{-1} consistently maintained large amounts of SOC in the surface soil over the 22 year period, further demonstrating the importance of N in the accumulation of SOC in the surface soil (0-15 cm).

For E502, SOC increased with increasing N rates and the effect was greatest at rates above 90 kg N ha^{-1} (Table 6 and Figure 2). In 2014, SOC reached 10.2 g kg^{-1} when 112 kg N ha^{-1} was applied compared to 8.9 g kg^{-1} in unfertilized check plots. At the same rate (112 kg N ha^{-1}), SOC of 9.4 and 10.5 g kg^{-1} were the highest reported in 1993 and 2013, respectively. Soil organic C displayed a linear trend with an increase in N rates ($p < 0.01$). Soil organic C observed in 1993, 2013 and 2014 at rates less than or equaled to 90 kg N ha^{-1} did not differ significantly from SOC reported in unfertilized check plots except at 67 kg N ha^{-1} in 2014.

There was a significant difference in SOC observed over the years (1993-2014). In the unfertilized check plots, SOC increased from 8.4 g kg^{-1} in 1993 to 9.3 and 8.9 g kg^{-1} in 2013 and 2014, respectively. The largest accumulation of SOC during the 22 year period occurred at a rate of 112 kg N ha^{-1} where SOC was observed to be 9.4, 10.5 and 10.2 g kg^{-1} in 1993, 2013 and 2014, respectively. This further illustrates the importance of N particularly at rates that exceed 90 kg ha^{-1} in maintaining high amounts of SOC in the surface soil (0-15 cm).

For samples collected from the Magruder Plots, there was an increase in SOC in the surface soil (0-15 cm) for all the treatments relative to the unfertilized check plot in 1993, 2013 and 2014 (Table 3 and Figure 4). Manure application resulted in a significant increase in SOC by 4.1 and 3.8 g kg^{-1} in 2013 and 2014, respectively, relative to the unfertilized check plot. In 1993, SOC followed a similar trend where it increased by 2.0 g kg^{-1} relative to the unfertilized check plot. Soil organic C was observed to be high where N was applied. The least amount of SOC with N applied in combination with other nutrient sources was found to be 9.1 g kg^{-1} in 1993 and this was higher than the largest amount of SOC (8.3 g kg^{-1}) with P as the only added crop nutrient, further demonstrating the importance of N in soil fertility.

Over time (1993 to 2014), SOC in the unfertilized check plot decreased from 7.8 g kg^{-1} in 1993 to 7.7 and 7.1 g kg^{-1} in 2013 and 2014, respectively (Table 3 and Figure 4). Soil organic C decreased from 11.6 g kg^{-1} in 1993 to 10.2 g kg^{-1} in 2014 when NPK was annually applied. There was a slight change in SOC in the P plot from 8.2 g kg^{-1} in 1993 to 8.3 g kg^{-1} in 2014. From 1993 to 2014 application of manure maintained a relatively large amount of SOC compared to other treatments.

DISCUSSION

Soil pH

Nitrogen fertilization significantly decreased soil pH across all locations reported in this study. An increase in N rate was accompanied by a decrease in soil pH. The lowest soil pH tended to occur at high rates of N applied. Over time (1993- 2014), soil pH decreased significantly for the different N rates in E222. A similar trend was reported in E502, but only at the rate of 67 kg N ha^{-1}. A combination of crop removal of basic cations and decomposition of organic matter may be responsible for the reduction of soil pH in the unfertilized check plots in E222. A small reduction in soil pH occurred at a rate of 134 kg N ha^{-1} since the initial soil pH (5.8) in 1993 was already lower than pH under low and moderate rates of N fertilization. The initial soil pH prior to the start of this experiment in 1969 might have been high and addition of 134 kg N ha^{-1} led to a rapid decline in soil pH. However, only a small reduction in soil pH was detected between 1993 and 2014. Soil pH experienced an insignificant change between 1993 and 2014 for most N rates in E502. Schwab, Owensby, and Kulyingyong (1990) noted that cycling of bases from deeper soil layers to surface soil and or leaching of H^+ can raise soil pH. The decrease in soil pH with increased N rates and with time could be attributed to the nitrification process in which hydrogen ions are produced and released to the soil solution. This is consistent with past work showing a decrease in soil pH with increased N fertilization (Tuyen, Phung, and Tinh 2006; Barak et al. 1997; Schroder et al. 2011; Rezig, Mubarak, and Ehadi 2013).

In the Magruder Plots, soil pH decreased across all treatments with time except in P and NPKL plots where soil pH increased relative to the untreated check. Despite soil pH decreasing in the manure plot compared to the unfertilized check plot, soil pH (6.2 in 2014) remained higher than in any other plots. Eghball (1999) attributed the high soil pH in manure treated plots to large amounts of basic cations found in manure, which tend to raise or slowdown the rate of decline of soil pH. Eghball, Ginting, and Gilley (2004) showed in their study that animal manure has a capacity to raise soil pH in crop fields where it has been applied. Application of NPK with lime prevented a large decrease in soil pH observed in the NPK plot, but it was still lower than pH observed in the manure plot. The slight rise in soil pH in the P plot relative to the unfertilized check plot suggested that addition of P has little effect on soil pH and in fact may increase soil pH due to P fertilizers being calcium-phosphates and their ability to form insoluble compounds with acidic cations such as Fe^{3+} and Al^{+3}.

Total Nitrogen (TN)

Total N was significantly affected by the different rates of N fertilization (0-15 cm) in E502 in 2013 and 2014. There was a general pattern for TN to increase as the rate of N application was increased. The increase in TN was observed to be greatest when at least 90 kg N ha^{-1} was applied. There was also a significant increase in TN with time from 1993 to 2014 for the different rates of N fertilization. Rates above 67 kg N ha^{-1} maintained a relatively high amount of TN during the 22 year period (1993-2014) compared to lower N rates. In E222 there was a significant increase in TN with time but different rates of N application only had a significant effect on TN in 2013. The increase in TN as the rate of N fertilization was increased could be attributed to increased quantity of crop residues returned to the soil. The increase in TN as the rate of N application was increased is consistent with results from several other studies (Raun et al. 1998; Jantalia and Halvorson 2011; Halvorson and Jantalia 2011). They reported a significant increase in TN with an increase in N rates and with more pronounced effect at high N rates. Jantalia and Halvorson (2011) reported a decrease in C:N ratio as N fertilization rate was increased. This is suggestive of crop residues with high N content that when returned to the soil, TN would be expected to increase. Halvorson and Jantalia (2011) observed that increased above ground biomass and N input was associated with sequestration of C and N to the surface soil. Ortas, Akpinar, and Lal (2013) also reported a significantly higher amount of TN in plots treated with mineral N (2.3 g kg^{-1}) compared to unfertilized check plots with TN of 1.8 g kg^{-1}. Although it was not within the scope of this study to investigate the effect of conversion from CT to NT, it appears that change in tillage method might have played a role in the changing trend in TN and SOC storage within the soil profile in E222 and E502. In a long-term tillage system study, Dalal et al. (2011) reported a significantly higher effect of NT on TN of the surface soil (0-10 cm) compared to CT. Malhi and Kutcher (2007) also observed a significant increase in TN in the surface soil (0-15 cm) under zero tillage in comparison to CT after five years.

Total N in the Magruder Plots increased in all treatments over the time period evaluated (1993-2014) relative to the unfertilized check plot and appeared to be slightly higher in NP, NPK, NPKL and manure plots than in P plot. Ortas, Akpinar, and Lal (2013) observed no significant difference between TN in mineral fertilizer plots and manure plots. They found TN to be slightly higher in mineral fertilizer plots (2.3 Mg ha^{-1}) compared with manure plot (2.1 Mg ha^{-1}) in the surface soil (0-15 cm). Glendining et al. (1996) found a small increase in TN at low N rates after

135 years of continuous winter wheat production. However, they found TN to have increased by 21% in plots that received 144 kg N ha^{-1} during the same period due to large amounts of organic N returned to the soil. This demonstrated the importance of N in the accumulation of TN in the soil surface particularly at rates above 90 kg N ha^{-1}.

Soil Organic Carbon (SOC)

Soil organic C did not increase significantly until at least 67 kg N ha^{-1} was applied (Tables 5 and 6). Soil organic C followed a linear pattern where an increase in rates of N fertilization was accompanied by increased SOC. Rates that exceeded 67 kg N ha^{-1} resulted in increased sequestration of atmospheric CO_2. There was also a significant increase in SOC with time from 1993 to 2014. The largest increase in SOC between 1993 and 2014 occurred at a rate of 90 kg N ha^{-1} where it increased by 1.37 g kg^{-1} relative to the unfertilized check plots in E222. In E502, SOC was consistently higher at an application rate of 112 kg N ha^{-1} over the 22 year period (1993-2013) considered in this study.

The increase in SOC with increased N rates may be attributed to increased C sequestered in plant biomass, and later returned to the soil as crop residue (Aulakh et al. 2001; Dolan et al. 2006). Raun et al. (1998) and Halvorson and Reule (1999) reported similar results. Their work found that SOC was significantly influenced by application of N fertilizer. They found an increase in SOC with additional N applied. Tillage in E222 and E502 was changed from CT to NT in 2011 and this plays an important role in SOC storage in the soil profile in addition to influencing N. This may explain why SOC increased with time in unfertilized check plots. In the Magruder Plots, a decrease in SOC was observed in the unfertilized check plot, further illustrating the importance of NT for SOC to accumulate in the soil. In a comprehensive review of N fertilization and tillage effect on SOC, Alvarez (2005) found that CT resulted in accumulation of SOC in the soil. His work showed that N application was only effective to increase SOC when residues were retained on the soil surface.

In the Magruder Plots, SOC increased with time (1993-2014), especially the manure and NP plots. Soil organic C was observed to decrease with time in the unfertilized check and NPK plots. Manure maintained the highest amount of SOC between 1993 and 2014 compared to other treatments. This showed that manure played an important role in improving SOC, which is an important soil property. Zhang et al. (2009) reported a significant increase in SOC following application of manure. Their work indicated that application of inorganic fertilizers without manure did not affect SOC. Dalal (1989) reported that application of N without residue retention or implementation of no-till has no significant effect on C sequestration in the soil.

CONCLUSIONS

Long-term application of fertilizer N has led to acidification of surface soils (0-15 cm) particularly at high rates where soil pH decreased significantly to as low as 4.3 compared to the initial values ranging from 5.1 to 7.5. This increased acidity necessitates the use of lime to raise soil pH and retain grain yields at acceptable levels. Manure application reduces the need for lime since soil pH variations were within the range for production of most crops.

It was evident from this study that significant accumulation of SOC and TN in the surface soil occurred when at least 90 kg N ha^{-1} was annually applied. This may improve the physical, chemical and biological properties of the surface soil. Manure if available could also provide a valuable option for increasing SOC in the surface soil profile with resultant improvement in soil health.

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Table 1 Treatment structure for Experiment 222, Stillwater, OK., Experiment 502, Lahoma, OK. and the Magruder Plots, Stillwater, OK.

Experiment	Treatment	Fertilizer Rate (kg ha^{-1})		
		N	P	K
222	1	0	29	30
	2	45	29	30
	3	90	29	30
	4	134^	29	30
502	2	0	20	56
	3	22	20	56
	4	45	20	56
	5	67	20	56
	6	90	20	56
	7	112	20	56
Magruder Plots	1$^{>}$	269	0	0
	2	0	0	0
	3	0	14.6	0
	4	67.3	14.6	0

	5	67.3	14.6	27.9
	6^{+}	67.3	14.6	27.9

>N source was cattle manure, applied once every four years

Fertilizer N Source was 46-0-0; Inorganic P: 0-46-0; Inorganic K: 0-0-62

^ N split applied as 67 kg N ha^{-1} in fall and 67 kg N ha^{-1} in spring

Complete treatment structure and experimental design for Experiments 222, 502 and the Magruder Plots can be found at http://nue.okstate.edu/Long_Term_Experiments.htm

Table 2 Analysis of variance and associated contrasts for soil pH (0-15 cm) as affected by N fertilization, Experiment 222, Stillwater, OK, and Experiment 502, Lahoma, OK.

	SOV	df	Soil pH		
			1993	2013	2014
				Mean Squares	
E222	N rate	3	0.15636**	0.1382**	0.3115***
E502	N rate	5	0.28691***	0.7520***	1.0315***
				Treatment means	
E222					
0			6.26† ± 0.03[a]	5.04 ± 2.26[a]	4.94 ± 0.21[a]
45			6.13 ± 0.08[ab]	4.85 ± 2.18[ab]	4.61 ± 0.15[ab]
90			6.14 ± 0.15[ab]	4.75 ± 2.13[ab]	4.38 ± 0.12[b]
134			5.80 ± 0.24[b]	4.60 ± 2.06[b]	4.33 ± 0.18[b]
SED			0.11	0.11	0.13
Contrast linear			***	***	***
Contrast quadratic			ns	ns	ns
E502					
0			5.94 ± 0.22[a]	6.06 ± 0.19[a]	6.09 ± 0.22[a]
22			5.74 ± 0.21[a]	5.82 ± 0.17[ab]	5.83 ± 0.30[ab]
45			5.73 ± 0.43[a]	5.60 ± 0.26[bc]	5.63 ± 0.24[bc]

67				5.65 ± 0.11[ab]	5.38 ± 0.16[cd]	5.31 ± 0.16[cd]
90				5.49 ± 0.39[ab]	5.09 ± 0.33[de]	5.00 ± 0.19[de]
112				5.14 ± 0.41[b]	4.92 ± 0.22[e]	4.76 ± 0.13[e]
SED				0.17	0.10	0.12
Contrast linear				***	***	***
Contrast quadratic				ns	ns	ns

***, ** Significant at 0.01 and 0.05 probability levels, respectively; ns-significant; Mean of four replicates ± standard deviation; † Means followed by the same letter in the same column are not significantly different ($p < 0.05$); SED-Standard error of the difference between two equally replicated means; SOV-Source of variation; E222 and E502 refer to Experiment 222 and Experiment 502, respectively

Table 3 Total N and SOC of surface soil (0-15 cm) as affected by long-term fertilization, Magruder Plots, Stillwater, OK., 1993, 2013 and 2014

Treatment	TN (g kg^{-1})				SOC (g kg^{-1})	
	1993×	2013	2014	1993	2013	2014
Manure	0.74	1.49† $\pm$ 0.04^{bc}	1.31 $\pm$ 0.10^{a}	9.83	11.75 $\pm$ 0.92^{a}	10.85 $\pm$ 0.78^{a}
Check	0.60	1.20 $\pm$ 0.04^{d}	0.90 $\pm$ 0.05^{b}	7.83	7.70 $\pm$ 0.45^{c}	7.10 $\pm$ 0.54^{c}
P	0.62	1.37 $\pm$ 0.02^{cd}	1.03 $\pm$ 0.03^{ab}	8.17	7.95 $\pm$ 0.54^{bc}	8.25 $\pm$ 0.72^{bc}
NP	0.70	1.66 $\pm$ 0.07^{ab}	1.14 $\pm$ 0.14^{ab}	9.09	9.89 $\pm$ 0.07^{ab}	9.46 $\pm$ 0.30^{abc}
NPK	0.85	1.83 $\pm$ 0.02^{a}	1.20 $\pm$ 0.02^{ab}	11.60	10.20 $\pm$ 0.14^{a}	10.19 $\pm$ 0.86^{ab}
NPKL	0.77	1.72 $\pm$ 0.10^{a}	1.17 $\pm$ 0.10^{ab}	9.94	10.80 $\pm$ 0.14^{a}	9.91 $\pm$ 0.28^{ab}
SED		0.06	0.09		0.54	0.01

Mean of two replicates ± standard deviation; SED- standard error of the difference between two equally replicated means † Means followed by the same letter within the same column indicates no significant difference (P<0.05); × In 1993 analysis was based on one data point since the plots were not divided into two equal halves

Table 4 Surface soil pH (0-15 cm) as affected by long-term fertilization, Magruder Plots, Stillwater, OK., 1993, 2013 and 2014

Treatment	Soil pH		
	1993×	2013	2014

Manure	7.47	6.41 ± 0.28[a]	6.24 ± 0.15[a]
Check	5.77	5.47 ± 0.12[b]	5.16 ± 0.09[b]
P	5.65	5.31± 0.11[bc]	5.07 ± 0.04[b]
NP	5.25	4.83 ± 0.11[cd]	4.53 ± 0.10[c]
NPK	5.22	4.70 ± 0.05[d]	4.42± 0.08[c]
NPKL	5.48	5.45 ± 0.00[b]	5.17 ± 0.03[b]
SED		0.14	0.09

Mean of two replicates ± standard deviation; SED- standard error of the difference between two equally replicated means; † Means followed by the same letter within the same column indicates no significant difference (P<0.05); × In 1993 analysis was based on one data point since the plots were not divided into two equal halves

Table 5 Analysis of variance and associated contrasts for TN and SOC (g kg^{-1}) 0-15 cm, Experiment 222, Stillwater, OK. in 1993, 2013 and 2014

Source of Variation	df	TN				SOC		
		1993	2013	2014	Mean	1993	2013	2014
Replication	3	0.0234	0.0843**	0.04697*		3.5358***	0.3828	1.8664**
N rate	3	0.0028	0.0488*	0.02036		0.5728**	4.2484**	2.8214***
Error	9	0.0022×	0.0128	0.01395		0.1476	0.6947	0.2747
N Rate					Means			
0		0.79 ±	1.31 ±	1.37 ±		7.90 ±	10.69 ±	10.74 ±
45		0.81 ±	1.48 ±	1.37 ±		8.27 ±	11.90 ±	11.70 ±
90		0.78 ±	1.53 ±	1.47 ±		8.37 ±	12.55 ±	12.58 ±
134		0.84 ±	1.56 ±	1.51 ±		8.82 ±	13.08 ±	12.42 ±
SED		0.03	0.08	0.08		0.27	0.59	0.37
Contrast								
N rate-Linear		ns	**	*		***	***	***
N rate-		ns	ns	ns		ns	ns	*
45 vs 90		ns	ns	ns		ns	ns	**

45 vs 134		ns	ns	ns				*	*	*

***, **, * Significant at 0.01, 0.05 and 0.1 probability level respectively; ns not significant; Mean of four replicates ± standard deviation; †Means followed by the same letter in the same column are not statistically different ($p < 0.05$); SED Standard error of the difference between two equally replicated means; × Error degree of freedom was 14 due to missing data

Table 6 Analysis of variance and associated contrasts for TN and SOC (g kg^{-1}) 0-15 cm, Experiment 502, Lahoma, OK., 1993, 2013 and 2014

Source of Variation	df	TN				SOC		
		1993	2013	2014		1993	2013	2014
					Mean			
Replication	3	0.0106^{ns}	0.3054***	0.0341***		0.1856^{ns}	0.8710*	1.3264**
N rate	5	0.0165^{ns}	0.0328*	0.0237***		0.4807**	1.0647**	1.0323**
Error	15	0.0085×	0.0119	0.0052		0.1276	0.2824	0.3284
N Rate					Means			
0		0.61 ±	1.06 ±	0.99 ±		8.40 ±	9.25 ±	8.87 ±
22		0.52 ±	1.14 ±	1.00 ±		8.60 ±	9.37 ±	9.65 ±
45		0.71 ±	1.24 ±	1.05 ±		8.78 ±	9.75 ±	9.64 ±
67		0.65 ±	1.14 ±	1.01 ±		8.91 ±	10.36 ±	10.19 ±
90		0.66 ±	1.19 ±	1.16 ±		9.04 ±	9.80 ±	10.01 ±
112		0.70 ±	1.32 ±	1.16		9.37 ±	10.53 ±	10.23 ±
SED		0.07	0.08	0.05		0.25	0.38	0.41
Contrast								
N rate-Linear		*	**	***		***	***	***
N rate-		ns	ns	ns		ns	ns	ns

45 vs 67		ns	ns	ns		ns	ns	ns
45 vs 90		ns	ns	*		ns	ns	ns
45 vs 112		ns	ns	*		**	*	ns

***, **, * Significant at 0.01, 0.05 and 0.1 probability level respectively; ns not significant; Mean of four replicates ± standard deviation; †Means followed by the same letter in the same column are not statistically different ($p < 0.05$); SED Standard error of the difference between two equally replicated means; × Error degree of freedom was 14 due to missing data

Figure 1. Effect of N fertilization on TN of surface soil (0-15 cm) in Experiment 222 (E222), Stillwater, OK, Experiment 502 (E502) and Lahoma, OK in 1993, 2013 and 2014

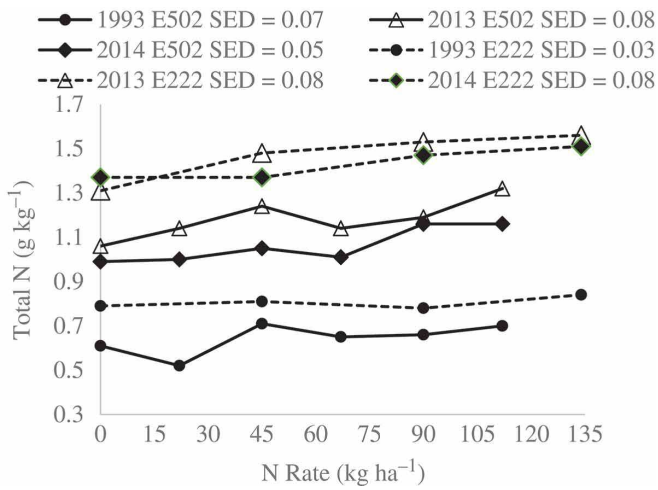

Figure 2. Effect of N fertilization on soil organic carbon (SOC) of surface soil (0-15 cm) in Experiment 222 (E222), Stillwater, OK, Experiment 502 (E502), Lahoma, OK in 1993, 2013 and 2014

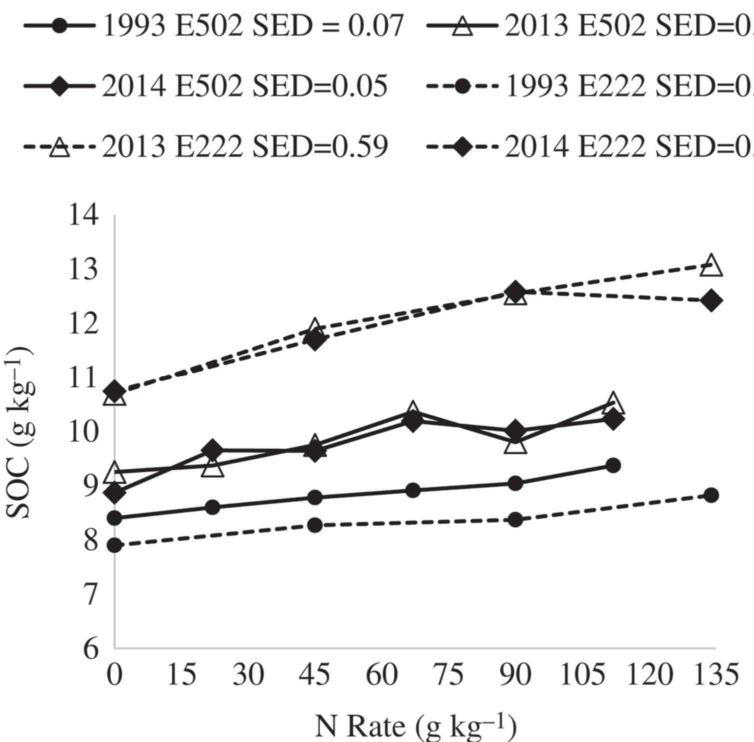

Figure 3. Effect of different treatments on TN at the Magruder Plots in 1993, 2013 and 2014 at 0-15 cm surface soil depths

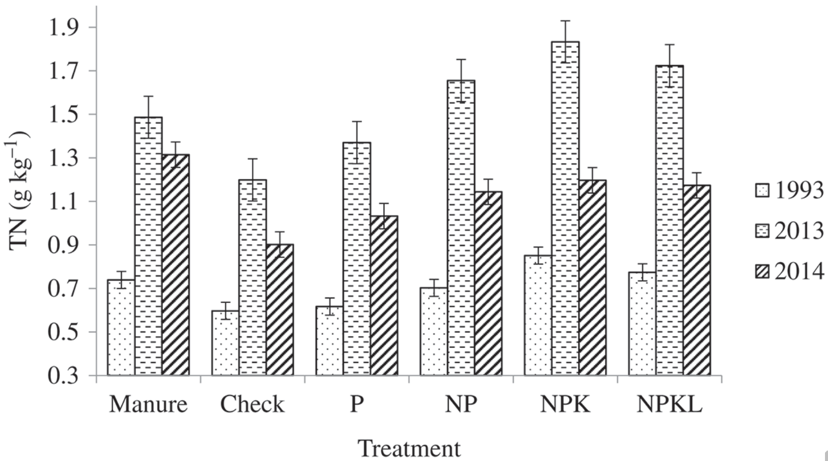

Figure 4. Effect of different treatments on soil organic carbon (SOC) at the Magruder Plots in 1993, 2013 and 2014 at 0-15 cm surface soil depths