

TOTAL NITROGEN, ORGANIC CARBON AND SOIL
PH IN A LONG TERM CONTINUOUS WINTER
WHEAT (*Triticum aestivum L.*) EXPERIMENT

By

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TOTAL NITROGEN, ORGANIC CARBON, AND SOIL
PH, IN A LONG TERM CONTINUOUS WINTER
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Abstract: Continuous use of organic and inorganic fertilizers impact soil properties such as organic matter and soil pH, in addition to nutrient levels. In order to document these changes, stored samples from the last 24 years of the Magruder Plots were analyzed for organic carbon (OC), total nitrogen (TN) and soil pH. This long-term trial has received the same six-unreplicated-treatments since 1947. These consist of a combination of cattle manure, inorganic N, P, K, and lime applied when pH dropped below 5.5. Each year, a composite soil sample was taken from the surface 0-15cm in each plot, air-dried at ambient temperature, ground to pass a 2mm sieve, and stored at room temperature, 25°C. Analysis for OC and TN were conducted using a LECO dry combustion CN analyzer, and pH was measured using an electronic pH meter with a 1:1 soil to deionized water solution. Statistical analysis was performed using SAS. The use of commercial fertilizers lowered soil pH over time and did not change TN and OC. Manure application maintained 2% OM and adequate soil pH (>6.0).

Keywords: Organic Carbon (OC), total nitrogen (TN), soil pH, manure, fertilizer

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CHAPTER I

INTRODUCTION

The constant effort to fully understand soil systems is made difficult by the complexity in which biological, mechanical, physical, and chemical reactions are occurring simultaneously. Schetzl and Thompson (2005) asserted that we will never be able to completely understand all these interactions within soils. Despite the fact that our understanding of these systems is limited, it is evident that soils operate in equilibrium, and that human misuse of land has been disrupting this equilibrium. The Intergovernmental Panel on Climate Change (IPCC) has indicated that human activity is largely responsible for modifying gas concentrations in the atmosphere, especially carbon dioxide (CO₂) (Parry et al., 2007).

In order to better understand the emission of CO₂, the carbon cycle, the flux of carbon compound in the environment, has become the main interest of scientists. Based on findings on the carbon cycle, soils store in total 2,500 gigatons of carbon, 3.3 times more than the carbon in the atmosphere (Schetzl and Thompson, 2005). Most of the respiration, the release of carbon

dioxide into the atmosphere, occurring on the Earth happens in the soil. Thus, agriculture has a direct impact on the quantity of carbon in the soil because some practices can accelerate or retard soil respiration. One practice that indicates if respiration is occurring is the availability of nutrients in the soils, especially the ratio of carbon (C) and nitrogen (N). Microorganisms require the proper balance between these two nutrients in order to build essential compounds and energy molecules (Sylvia et al., 1998). However, because the amount of nitrogen in the soils is much lower than carbon, nitrogen is the limiting factor to meet appropriate characteristics of soil to yield decomposition. Thus, this study examines the impact that fertilizer application can have on total nitrogen (TN), organic carbon (OC), and soil pH level, all of which affect soil respiration and the concentration of CO₂ in the atmosphere.

CHAPTER II

REVIEW OF LITERATURE

Carbon (C)

Among all the elements present on Earth, carbon (C) is one of the most important. Its unique characteristic of having three stable oxidation states (-IV, II, and IV) allows this element to form several compounds (Essington, 2015). From chlorophyll to cellulose, carbon is present in all organic compounds. However, there are also inorganic compounds that contain carbon. Carbon dioxide (CO₂), for instance, is a molecule abundant in the atmosphere which is essential to maintain the carbon cycle running (Brady and Weil, 2008).

Carbon cycle

The amount of C in the Earth is fixed but dynamic. In this system, C fluctuates from one pool to another. The basic process involves six pools: atmosphere, vegetation, soil, surface water bodies, carbonate rocks, and fossil fuel. Initially, plants use CO₂ to synthesize organic compounds with sunlight. These compounds are primarily glucose and they are rich in energy. When plants need energy, they oxidize glucose molecules releasing CO₂ to the atmosphere through a process called respiration. Not all organic compounds are used by plants to obtain energy. Some are stored in plants' tissues which can be assimilated by animals. All the C that is not released in the atmosphere as CO₂, returns to the soil as waste or body tissue. Carbon from plants also return to soil as plant residue. In addition, gas CO₂ reacts with soil to form bicarbonate

and carbonates from carbonic acid (H_2CO_3) or it can react with water, acidifying oceans and lakes (Brady and Weil, 2008).

Essington (2015) states that the quantity of the total carbon (TC) in the soil which refers to organic C and carbonates is four times greater than the amount of C in the atmosphere. Also, the microbial respiration can release ten times more C than combustion of fossil fuel per year. Most of the CO_2 released is caused by the respiration of soil biota and roots, and the decomposition of plant residue and organic matter (Sylvia et al., 1998).

Soil organic carbon (SOC)

The main source of organic C in the soil comes from plant residue, soil biota, and soil organic matter (SOM) (Essington, 2015)

Plant residue

Plant residue is the material left on the soil after harvest which is susceptible to decomposition. Each microbial group produces specific enzymes that degrade plant residue by targeting compounds ranging in complexity from simple proteins and carbohydrates to complex lignin (Sylvia et al., 1998). Simple compounds are the principal metabolic energy source for microorganisms because it is easily decomposed, thus, more than one group of microorganisms will compete for the same biomolecule. More complex compounds are attacked by fewer groups which will break these compounds into simple biomolecules so other groups can continue the process of decomposition. The more complex the material, the fewer microorganisms can decay (Sylvia et al., 1998).

Wheat residue

Broder and Wagner (1988) published a paper reporting the representative composition of above ground wheat where 361g are cellulose following by 288g are of soluble components, 184g are hemicellulose, 141g lignin, and 9g are N out of 1000g of dry mass. In addition, wheat crop produces 1111 g/m² annually of residue biomass (Buyanovsky and Wagner, 1986).

Soil biota

Soils are inhabited by a myriad of living organisms: insects, nematodes, protozoa, algae, fungi, etc. These organisms produce several biochemical compounds required for cellular function. They also produce organic compounds that facilitate nutrient uptake and excrete extracellular substances such as enzymes, microbial mucilage, metabolic, and byproducts (waste). Compost released from decay from plant residue and animal excrement also count as biochemical compounds. The association of biochemical and living organisms summarize soil biomass (Essington, 2015).

Microbial succession

The population of microorganisms are influenced by the available amount of fresh residue. The population of microorganisms increases when availability of fresh residue increases. However, the rapid increase in population is not followed by increase of nutrient supply which results in a sharp decline of the population (Sylvia et al., 1998). In fact, some other factors can influence the microbial population such as temperature and availability of water (Sylvia et al., 1998). This process is called microbial succession: the first stage is when simple compounds such as water-soluble are the first compounds to be metabolized and stimulates almost immediately the increase of the microbial population. There is a wide range of microorganisms, called copiotrophs or r-strategists that use these compounds as a source of energy. This name

comes from the rapid rate of their growth and reproduction. They are poorly adapted in stressful environment especially with low nutrient environment. When the easy decomposable material depletes, the r-strategists decrease because they die of starvation. After the first stage, more complex components are still remaining in the soil which are decayed by fewer microorganisms which are known as k-strategies or oligotrophs. The decomposition rate declines but stays constant (Sylvia et al., 1998).

Initially, most C from residue material is converted into CO₂, but a smaller amount is converted into microbial biomass and eventually humus (Brady and Weil, 2008).

Soil organic matter (SOM)

Soil organic matter (SOM) or humus is comprised of two main groups: nonhumic and humic substances. The nonhumic part is still chemically recognizable, that is, it consists of known compounds such as carbohydrates, proteins, amino sugar, peptides, lipids, nucleic acids, and lignin. Usually, these substances do not stay in the soil for a long time because they are a source of energy for microorganisms. And the humic part is more stable and it is not chemically recognizable. In fact, they are huge molecules which can be divided in three categories: fulvic acid, humic acid, and humin. Fulvic acid are substances that contain small molecular weight but a large amount of functional groups. These functional groups are very reactive due to their large amount of oxygen in their composition. Fulvic acid substances are soluble in acid and alkaline substances. Humic acids are substances that have a large molecular weight and moderate number of functional groups. These functional groups have a lower dissociation constant so they will only deprotonate in solutions with high pH. For this reason, humic acids are soluble in basic substances but insoluble in acid substances. The last category is humin substances. They are

insoluble in acid and alkaline substances and have a very large molecular weight. They are comprised mostly by nonpolar substances (Essington, 2015).

Inorganic carbon (IC)

Inorganic C includes carbon dioxide (CO_2) from root respiration and SOM decomposition, dissolved carbon dioxide ($\text{CO}_2 \cdot \text{H}_2\text{O}$), and carbonic acid (H_2CO_3). Carbonic acid has a low acid dissociation constant which means that it has a tendency to donate protons to the solution via protonation. When carbonic acid donates a proton, bicarbonates (HCO_3^-) are produced. Bicarbonates can continue to donate a proton forming carbonate ion (CO_3^{2-}). All these reactions are highly dependent on the pH. If the solution has a significant quantity of protons, the reactions do not occur (Essington, 2015).

Nitrogen (N)

Nitrogen is an essential element for plants, animals and microbes. Like C, N composes several biomolecules, especially because of its vast valence state in the environment (Essington, 2015). This element is the main constituent of protein, but it is also present in nucleic acids and chlorophyll (Brady and Weil, 2008). Due to its high demand in plants, it is considered a macronutrient and one of the main limiting nutrients for plant growth (Brady and Weil, 2008).

Nitrogen Cycle

Earth's atmosphere is composed by 78% of N. However, all this abundance is not available for plants or animals. The triple bond between the nitrogen atoms (N_2) makes this molecule extremely inert and not usable for most plants. Instead, N needs to pass through natural process such as N fixation and lightning to break down these stable bonds and form molecules

with available N. Large amounts of N are found in the soils, especially in the SOM which contains 5% of the whole N (Brady and Weil, 2008).

Plant roots uptake the mineral form of N. However, 95-99% of the N in the soil is in the organic form, thus it needs to be mineralized to be available for plant uptake (Brady and Weil, 2008).

Mineralization

The term mineralization means the conversion of organic into inorganic compounds (Brady and Weil, 2008) and besides C, oxygen, hydrogen, and all other nutrients are assimilated via the mineral forms (Taiz and Zeiger, 2010). Brady and Weil (2008) also suggested that N could also be absorbed in an organic form. The mineralization process englobes two parts: ammonification and nitrification. First, heterotrophic bacteria and fungi breakdown organic compounds. Then amine groups (R-NH₂) are cleaved into smaller amino compounds by heterotrophic bacteria, fungi, and actinomycetes which are hydrolyzed and release ammonium (NH₄) (Havlin et al., 2014). Finally, ammonium is oxidized into nitrite and then into nitrate (Havlin et al., 2014).

Ammonification

Ammonification refers to the conversion of organic N into ammonium. This process involves several enzymes which are produced by microbes and soil animals. First, extracellular enzymes are released in the soil in order to break down the organic-N polymers. In sequence, monomers resulted from the previous reaction. The main extracellular enzymes depolymerize protein, aminopolysaccharides (microbial cell wall), and nucleic acids, and hydrolyze urea (Sylvia et al., 1998).

Nitrification

Nitrification is composed by two processes. First, ammonia is converted to nitrite by exclusively autotrophic nitrifying bacteria and then nitrite is converted to nitrate by autotrophic and heterotrophic bacteria and fungi (Sylvia et al., 1998).

Ammonia oxidation acidifies the soil due to the release of 1 mole of hydrogen from 1 mole of ammonia (Sylvia et al., 1998). Regardless of the source of ammonium in the soil, it will always promote an increase of hydrogen in the soil (Brady and Weil, 2008). In addition, under aerobic conditions, a small production of nitrous oxide (N₂O) is released (1%). However, when there is a lack of oxygen, the production of this gas increases becoming the major product of this reaction (Sylvia et al., 1998).

Conditions that affect mineralization

Nitrifying bacteria are more sensitive to environmental conditions than heterotrophic bacteria responsible for ammonification. It has also been found that N content increases with water supply (Schnitzer and Khan, 1975). However, the lack of oxygen affects the microorganisms' performance, especially when the pores are 60% filled with water. Above this level, the environment lack of oxygen and autotrophic nitrifying bacteria produce nitric oxide and nitrous oxide (Sylvia et al., 1998).

They perform best when the temperature is between 20 and 30 °C (Brady and Weil, 2008). However, Schnitzer and Khan (1975) stated that optimum temperatures for mineralization and nitrification were between 25 to 35°C. In addition, low temperatures affect microbial activity, thus, the accumulation of N reduces when the temperatures are lower (Schnitzer and Khan, 1975). The nitrification process ceases above 45°C (Schnitzer and Khan, 1975). Parton et

al. (1996) reported that only an increase in 2-4 °C in the Great Plains temperature could have a large effect in the system. This temperature change would increase the availability of N, but decrease SOM in the soil.

Soil pH also affects the microbial activity. The rate of mineralization decreases with low pH but does not cease (Sylvia et al., 1998; Bohn et al. 2001). Sylvia et al., (1998) state that when soil pH was below 4.5, the mineralization rate dropped but Bohn et al. (2001) reported that mineralization was affected when pH dropped at 5.5. When pH is alkaline, nitrite accumulates and inhibits the nitrite oxidizers. Thus, nitrifying bacteria are favorable in a neutral pH. However, Sylvia et al. (1998) observed high concentration of nitrate even when the pH was below 4.5. This phenomenon may happen because the *Nitrosospira* are associated with acid soils (Sylvia et al., 1998).

Assimilation

Plants absorb N as nitrate (NO_3) or ammonium (NH_4). When the conditions are favorable, the amount of nitrate are greater than ammonium. They both are absorbed by mass flow and diffusion (Havlin et al., 2014). In addition, high levels of ammonium in the living tissue can be toxic to plants and animals (Taiz and Zeiger, 2010).

Uptake of Ammonium, a positive charged ion, is facilitated by the free energy gradient through transporters. However, the absorption of nitrate, a negative charged ion, is uptake by secondary transport which requires energy to pump ion hydrogen to outside of the cells (Taiz and Zeiger, 2010). This process acidifies the soil which changes the soil pH especially near to the rhizosphere (Havlin et al., 2014). Even a small difference near root surface by 2 units can affect nutrient availability (Havlin et al., 2014).

C:N ratio

Maximum rates of decomposition are observed when there are enough available nutrients such as N, P, S. In order to synthesize new microbial cells, microorganisms require balanced amount N to meet the amount of C in the soil. On average, microbes have to incorporate one part of N for 8 parts of C because only one-third of C is assimilated while the other two-third is lost as CO₂. Thus, they need to find a material which contains 1g N to 24g C (Brady and Weil, 2008). The amount required by bacteria is twice as high as fungi (Sylvia et al., 1998).

Under those circumstances, if a material with a C:N ratio of 25:1 is added to the soil, microorganisms have to sink for enough N to synthesize more microbial cell causing depletion of inorganic N for plants. This phenomenon is called immobilization (Brady and Weil, 2008). Rates between 25 and 35, the decomposition is proceeded faster with a very efficiency to assimilate N into the microbial biomass (Sylvia et al., 1998). When the residues are low in N concentration (C:N ratio above 40), it does not have appropriate characteristics to yield decay. Thus, the inorganic pool needs to be utilized to reach the perfect amount of C otherwise, N is immobilized (Sylvia et al., 1998).

Also, soil animals play an important role in the N mineralization. 30% of the yearly N mineralization is related to protozoa and nematodes because they prey microorganisms and N is released in the soil as a waste (Sylvia et al., 1998).

Effect of management practices on soil property

Fertilizers

Adequate supply of nutrients is necessary to achieve optimum productivity for any cropping system (Havlin et al., 2014). Plants are excellent drains, and in order to guarantee a good development of crops, fertilizers need to be applied to the soil. Fertilizers are any material that provided nutrients beyond natural reserves. There are several types of fertilizers. Some are

manufactured and others are naturally obtained from waste matter for instance. However, fertilizer application is susceptible to loss because soils are a complex system with constant physical, mechanical, chemical, and biological reactions occurring at the same time (Colbert 2004). Hence, it is important to understand the impact of fertilizers to the soil to promote efficient use and avoid environmental contamination by losses (Finck, 1982).

Synthetic fertilizer

Synthetic fertilizers are man-made, and they are usually in an available form for plants. The composition of synthetic fertilizers is much more precise than the organic sources. They can contain one single element or they can contain a mix of two or more nutrients and this is specified on the label. Most N fertilizers are derived from ammonia resulting from the Haber-Bosch process while P and K fertilizers are found in geological deposits (Brady and Weil, 2008).

Organic fertilizer

Since the beginning of agriculture, soils have been used to recycle nutrients (Havlin et al., 2014). Usually, animal manure comes from confined animal feeding systems and is highly concentrated in nutrients, especially with nitrates and phosphates (Havlin et al., 2014). Despite the rich source of nutrients, manure is susceptible to nutrient loss. The volatilization of ammonia and the leaching of nitrate are the main concerns about manure application. There is also a potential of eutrophication in rivers and lakes caused by high concentration of phosphate in runoff water (Sylvia et al., 1998).

Effects on Soil Organic Carbon (SOC)

Synthetic Fertilizer

The effects of synthetic fertilizers on soils are still debatable. Long-term trials might be a powerful method to observe the effects of synthetic fertilizer on SOC over time. Klan et al. (2007) and Mulvaney et al. (2009) observed that after 40 to 50 years, synthetic fertilizer was applied on the Morrow Plot, soil C declined even with extensive residue C incorporation. They stated that the high rate of N fertilizers decreased the C:N ratio promoting a massive decomposition of crop residue and SOM. Thus, this did not only cause the release of C dioxide to the atmosphere, but also decreased the storage capacity of nutrients and water. As a result, more fertilizer was required to increase yield due to a decline in productivity. However, Powlson et al. (2009) argued that synthetic fertilizer was not the reason to cause a decrease in C content. Instead, they claimed that previous application of manure was the cause of soil oxidation and C loss. In this study, they observed a decline in C concentration in the soil even after 100-year cessation of manure application. Other scientists, such as Havlin et al. (1990), affirmed that a decrease in SOM could be overcome by fertilizer application due to an increase of biomass returning to the soil.

Organic Fertilizer

Haynes and Naidu (1998) stated that manure application resulted in increasing organic matter content in the soil. Johnston (1986) presented an experiment conducted in Rothamsted, England, where 35 tons ha⁻¹ of farmyard manure (FYM) has been added annually since 1843. This practice resulted in an exponential increase of OC content, approaching the equilibrium after 140 years. The manure plot presented a level of OC three times higher than the unfertilized plot; however, this practice did not provide the same high levels of OC before the cultivation because the decomposition rate increases when more manure is applied to the soil (Bohn et al.

2001). Also, the decomposition rate is influenced by the type of manure. In a review written by Haynes and Naidu (1998), they stated that composted manure promoted higher amounts of OC to the soil compared to fresh manure. While fresh materials still lose C throughout decomposition, composted materials were more stable and had lower C losses.

Lime

Haynes and Naidu (1998) stated that microbial population changes depending on the soil pH. Fungi communities were favored by low pH, and as pH raises the microbial communities shift to actinomycetes and then to bacteria. Also, microbial activity increased when mineralization increased. This led to two consequences. First, since microbial activity increased when mineralization increased, labile SOM was oxidized decreasing the quantities of C in the soil. Chan and Heenan (1996) stated that a decrease in OC was noticed in the first year after lime application. Controversially, when the population of microorganisms increased, it produced extracellular gelatinous polysaccharides which acted as binding agents. In addition, fungal hyphae could contribute to soil aggregation due to the development of a network. Both the polysaccharides and the network produced by hyphae helped to increase C in the soil. Haynes and Naidu (1998) suggested that an increase in OC is expected to occur after 10 years of lime application.

Effects on Total Nitrogen (TN)

Synthetic Fertilizer

As previously mentioned, N fertilizer increased the amount of N in the soil and decreased the C:N ratio favoring the decomposition of SOM (Klan et al. 2007; Mulvaney et al. 2009). Most of the synthetic fertilizers are soluble, and they are released upon their application into the soil.

This characteristic might be detrimental due to a susceptibility of N loss which does not contribute to increase N content in the soil (Chen and Avnimelech, 1986).

Organic Fertilizer

Animal manure is a great reservoir of essential nutrients such as C, N, sulfur, and P. However, the availability of these elements vary widely (Bohn et al., 2001). A study described in Havlin et al. (2014), claimed that manure contained 1-6% total N, of which 50-75% was organic N, and the remaining part (20-50%) was ammonium. Also, Sylvia et al. (1998) stated that cattle manure had a narrow C:N ratio of 18, showing that decay process of this material is relatively fast. Brady and Weil (2008) reported that 35% of the N in manure was utilized during the first year, 15% during the second year, 6% during the third year, and 2% during the fourth year. In another study, 13-67% of animal manure was mineralized in 26 weeks (Chae and Tabatabai, 1986), showing that mineralization was followed by a lag period, rapid rate of increase, and finally a slow rate of N release. Furthermore, Beauchamp et al. (1982) stated that 24-33% of manure N was lost by volatilization in the first 6 days of application because ammonium can be converted into ammonia under the optimum conditions. However, the prolonged residual of N was obtained by large amounts of manure applied for several years (Power and Papendick, 1985).

Lime

Previously, it was noted that lime application in acid soils increased microbial biomass, and as a result, increased mineralization of organic N (Haynes and Naidu, 1998).

Effects on Soil pH

Synthetic Fertilizer

Based on Havlin et al. (2014), the potential acidification of urea and ammonium nitrate fertilizers is equivalent. Both fertilizers release one mole of hydrogen when they react in the soil. Thus, they decreased soil pH at the same level. Schroder et al. (2011) concluded that the decrease in soil pH was not related to N sources, but it was to the amount of N applied to the soil over of time.

Organic Fertilizer

Any source of organic material reduces the soil pH. High levels of N promote ammonium oxidation, resulting in supplement of nitrate and hydrogen to the soil (Sylvia et al. 1998; Havlin et al., 2014). Also, depending on the soil pH, organic acids and carbonic acids (weak acids) can dissociate releasing more hydrogen to soil solution. Thus, both the mineralization and the dissociation of weak acids have the capability to acidify soils (Schaetzl and Thompson, 2005). However, as mentioned by Eghball (1999) beef cattle manure contained a significant amount of calcium carbonate which contributed to neutralize acidity, especially coming from N-based recommendations.

Lime

In order to increase soil pH, it is required to use an alkaline material with carbonates, hydroxides, or silicates which can react with hydrogen. Large amounts of this material are required to react with large volumes of soil. Most lime materials react with CO₂ and water, resulting in bicarbonates. These bicarbonates are reactive and form CO₂ and aluminum hydroxide. The precipitation of aluminum hydroxide, which is an insoluble mineral, and the loss

of CO₂, results in an increase of soil pH. Also, calcium or magnesium can be incorporated within exchangeable sites which increases base saturation (Brady and Weil, 2008).

The Magruder Plot

Raun et al. (1993) conducted a study in order to evaluate long-term trials using stability analysis. The manure plot responded poorly compared to the NPK plot when the environmental mean was low (<2.0 Mg/ha) or high (>2.0Mg/ha). Even though this technique was used to compare different genotypes yields, it can be used to compare agronomic treatments specially when the conventional analysis of variance is difficult to be performed.

In 2003, a study attempting to balance N over 109 years was attempted by Davis et al. (2003) found that only 35% of the original total soil organic N remained in the soil. The average amount of N removed per year in the grain was 38.4 kg N ha⁻¹ year⁻¹. An estimated 44.5 kg N ha⁻¹ year⁻¹ came from rainfall, manure application, and symbiotic fixation. The manure plot had a computed N use efficiency (NUE) of 32.8%.

Parham et al. (2003) evaluated the evolution of the microbial population in the Magruder Plot, and it was found that cattle manure promoted an increase of bacteria but not fungi and it also promoted an increase of both r- and k-strategist microorganism communities. In contrast, chemical fertilizers promoted an increase of k-strategist bacterial communities.

Girma et al. (2007) studied yield response to amendments, yield stability, percentage of organic matter, soil nutrient status, microbial activity, and weed production in the Magruder Plots. The check plot, which remained unfertilized and under conventional tillage for over 114 years, produced more than 1 Mg ha⁻¹ yr⁻¹.

Aula et al. (2016) examined the effects of fertilizers on SOC, TN, and soil pH in the soil. It was evaluated samples from 2013 and 2014, and data from 1993. The soil pH decreased in the

manure, P, NP, NPK plots; however, the lowest level of soil pH was observed in the NPK plot. Manure and NPK resulted in increasing in TN and NPKL, presenting the highest TN when it was compared to the check plot. All the treatments presented an increase in SOC.

Research Gaps

The Magruder Plots were started in 1892. Soil test and grain yield data has now been collected and analyzed for almost 125 years. In some regards it is difficult to compare earlier data with current results due to changes in equipment and methods. For this reason, soil samples from the same time period were analyzed again using the same procedures in order to avoid method errors.

CHAPTER III

OBJECTIVE

The objective of this study is to document changes in soil organic carbon (SOC), total nitrogen (TN), and pH in the Magruder Plots over the past 25 years.

CHAPTER IV

METHODOLOGY

Site history

This experiment was initiated by Alexander Magruder in 1892 to observe potential nutrient demand in a dryland winter wheat production system. Observing a slight decline in wheat production, Magruder split the area in two parts. One received manure and the other remained unfertilized. In 1930, Horace Harper changed the experiment structure following comprehensive soil analysis. It was included N, P, K, and lime in the treatments. In 1947, the treatment structure was altered due to university campus expansion, but this time, the changes were more extreme. Six out of ten plots had to be relocated 1.6 km west from the original site. More than 450 tons of soil from 0-15 cm were transferred to the new site layer by layer (Boman et al., 1996).

Treatment structure

Currently, this experiment is composed of six-unreplicated plots. It contains four plots receiving synthetic sources of fertilizers, one receiving beef feedlot, and one unfertilized plot (Figure 1)

In the first plot, the beef of feedlot was applied every four years. Manure application is determined based on nitrogen content. Thus, 269 kg of N ha⁻¹ from feedlot manure was applied in 1991, 1995, 1999, 2003, 2007, 2011, and 2015 in the last 25 years.

The second plot is the check plot. It has been maintained unfertilized since the start of the study.

The third plot is the P plot. This plot has received 14.6 kg of P per hectare every year as a triple superphosphate.

The fourth plot is the NP plot. This plot has been receiving 67 kg of N per hectare and 14.6 kg of P per hectare every year. The source of N was ammonium nitrate prior to 2003, and the source has been urea since then. The source of P has been triple superphosphate.

The fifth plot is the NPK plot. This plot has received 67 kg of N per hectare, 14.6 kg of P per hectare, and 28.8 kg of K per hectare every year. The sources of N and P are the same as treatment 4, and the source of K has been muriate of potash (0-0-60).

The sixth plot is the NPKL plot. This plot is similar to the NPK plot, however, lime is applied when soils samples present soil pH below 5.5. Thus between 1990 to 2015, 4.9 Mg ha⁻¹ of limestone was applied one time (2009).

Site description

The Magruder Plots are located at the Agronomy Research Station in Stillwater (latitude: 36, 7.1844, N Longitude: 97, 5.3190, W) on a Kirkland silt loam 0 to 2 percent slope (fine, mixed, thermic Udertic Paleustolls). All plots are managed under dryland and have been conventionally tilled (chisel) since 1947. Varieties of winter wheat have been planted in the fall with a row spacing of 19.1 cm and 67 kg seed ha⁻¹. The size of the plots is 6 by 30 meters.

Soil sampling

For each of the 24 years, a composite surface soil sample (0 to 15 cm) was taken from each plot, air-dried at ambient temperature, ground to pass a 2 mm sieve, and stored at room

temperature. Some of the samples were missing, as such a total of 90 samples were included in this study.

Soil Analysis

SOC and TN determination

Soil organic carbon and TN were determined using the dry combustion procedure described by Schepers et al. (1989). A 200-milligram aliquot of soil was placed in the LECO (CN628) analyzer. The sample was oxidized at 1000°C under purified oxygen. The gas released from the combustion tube then flowed to an infrared spectrometer cell which reads the difference in voltage between a blank sample, containing only helium (He), and the sample containing He and CO₂. The percentage of C was calculated based on the difference in light transmitted between the two samples. An aliquot of the oxidized gas was carried over copper, then reducing N oxides to N₂. This gas was conducted to a thermal conductivity cell where N₂ was measured.

PH determination

Soil pH was determined using Fisher Scientific Accumet XL 150 pm/mV meter using a 1:1 soil to water ratio.

Statistical analysis

Analysis of variance (ANOVA) was performed using years as replications and Duncans test was used to compare means over years. In addition, a linear regression was performed as a function of time in order to understand dependent variable trends.

CHAPTER V

FINDINGS

In order to better understand the variables TN, OC, C:N ratio, soil pH, and grain yield, measurements over years were used to monitor changes. Thus, a set of tables were generated containing the regression coefficient, mean test (Duncan test), and analysis of variance (ANOVA) for each treatment (Manure, check, P, NP, NPK, NPKL) as a function of time. Also, a set of graphs were generated for each treatment with linear regression to estimate the trend from 1990 to 2015.

An additional table containing regression coefficients and means of each variable was generated and a set of graphs were created to examine the effects of lime application in the soil. As mentioned before, lime was applied in 2009, and thus, the results would be seen after 2010.

Furthermore, some variations between 1990-2006 were observed. This might be due to continuous changes in the variety sown each year that influenced results. In addition, some samples were missing and this affected the final analysis.

Manure Plot

According to Bohn et al. (2001), animal manure is a source of N and C. Since manure is applied to the soil every four years, it is expected to have a four-year-cycle of increases and decreases in TN and OC after manure application. Total N in the soil ranged from 0.8 g/kg to 1.3

g/kg with a mean of 1.09 g/kg. The mean test showed that the manure plot had the highest TN mean among all the other treatments (Table 2). Total N followed a four-year-cycle where the highest peaks were around 1.2 g/kg one year after manure application and the lowest appeared in the fourth year of manure application (Figure 2A). The C:N ratio also followed a four-year-cycle; however, it presented the lowest peaks after one year of application while the highest peaks were four years after manure had been applied (Figure 2C). The C:N ratio mean was 8.54, and it ranged from 7.64 to 10.13. This is consistent with work by Havlin et al. (2014) because this ratio is an indication of N mineralization. Thus, after organic fertilizer (manure) was applied, N became available in the soil and it decreased the C:N ratio promoting N mineralization. During the mineralization process, N was depleted, and it increased the C:N ratio.

Organic C did not always follow the four-year pattern as indicated by the non-significant regression coefficient ($p < 0.05$). Organic C fluctuated between 7.39 g/kg and 10.65 g/kg with a mean of 9.31 g/kg (Figure 2B). In the manure plot, 2.03% of OM was maintained due to periodic addition of organic amendment. Haynes and Naidu (1998), Johnston (1986) and Bohn et al. (2001) stated that manure application can increase OC; however, as mentioned by Haynes and Naidu (1998), fresh manure is more susceptible to C loss than composted materials. Also, the decomposition rate increases in proportion to manure application. Therefore, even though the application of manure can increase OC in the soil, it is difficult to achieve the same high levels before cultivation. However, manure application and the NPKL plot had the highest OC mean among all four treatments (Table 3).

Soil pH over time had a non-significant slope component ($p < 0.05$) with an average pH of 6.48 in the manured plot. It was the highest soil pH among the four treatments (Table 5). This level of soil pH is consistent with Girma et al. (2007) and Zhang (1998) who also noticed a soil

pH above 6.20 in the same plot. Even though manure application can contribute to soil acidification due to N mineralization and subsequent nitrification or additions of organic acids (Sylvia et al. 1998; Havlin et al., 2014), manure may have a significant amount of calcium carbonate (Eghball 1999) which increases soil pH. At this soil pH, as was evidenced by Parham et al. (2003), communities of bacteria are favored over that of fungi. Sharpley and Smith (1995) stated that manure application increases inorganic P fractions, especially, bicarbonate P which is the major contributor to Ca-bound in the soil, favoring the formation of hydroxyapatite.

Grain yield did not have a significant positive or negative trend ($p < 0.05$); however, the average mean was 2.12 Mg/ha (Table 6). Manure application was calculated based on N but it is not calibrated to remedy other limiting elements such as P or K. Furthermore, the actual content of manure varies widely, and this may negatively affect the grain yield when it is compared to NP, NPK, and NPKL plots that had statistically higher grain yield than the manure plot (Table 6).

Check Plot

According to Schaetzl and Thompson (2015) cultivation accelerates soil oxidation promoting C loss as CO₂ due to physical disturbance of the aggregates and oxygen supply to microorganisms. Hence, the depletion of OC decreases the capability of the soil to store nutrients as mentioned by Sylvia et al. (1998) where 20-80% of the CEC is dependent on OM. Nevertheless, after long periods of cultivation, SOM tends to stabilize where inputs in SOM balances out with losses as decomposition proceeds. However, OC has not achieved the stability, and has been decreasing with time ($p < 0.05$) (Figure 3B). Organic C started at 6.8 g/kg in 1990 and was 6.30 g/kg in 2015. The average content of OC was 6.75 g/kg which was the lowest level of OC among all the treatments.

Total N fluctuated over the years, ranging from 0.77 g/kg to 1.08 g/kg with a significant decrease over time ($p < 0.05$) (Figure 3A). This result is not consistent with Rice et al. (1986) who stated that after five years of cultivation in a conventional tillage system, TN stabilized at 1.5 mg/g. The TN mean in the check plot was statistically similar to that of the P plot and both had the lowest level of TN among all the treatments (Table 2).

The ratio between C and N was variable, similar to TN, but inversely proportional after 2009. This suggests that when TN decreases, the C:N ratio increases.

Figure 3D shows that soil pH decreasing with time (0.017 pH unit per year) ($p < 0.05$). Initially, soil pH started at 6.10 in 1990 and decreased to 5.59 by 2015. This result is consistent with the literature where SOM oxidation contributes to increased soil acidity by forming CO_2 which reacts with water and produces a weak acid. This acid dissociates and releases H^+ to the soil solution due to the acid coefficient constant equaling 6.35 (Brady and Weil, 2008). On average, soil pH was 5.8 that was high compared to other treatments and the only one below that observed in the manure plot.

Grain yield averaged 1.01 Mg/ha. This result is consistent with Girma et al. (2007) who noted that grain yield in the check plot was 1 Mg/ha without any fertilizer application for more than 114 years of continuous wheat production under conventional tillage. The P plot also had a statistically similar grain yield mean, at 1.20 Mg/ha. Alternatively, those plots receiving inorganic N (NP, NPK, NPKL) had grain yields above 2.2 Mg/ha.

P Plot

Schaetzl and Thompson (2015) explained that calcium is an effective agent to stabilize organic matter. Thus the application of triple superphosphate, which contains calcium, can promote resistance of OM to microbial decomposition and prevent C loss. However, OC has

been decreasing by 0.04 g/kg per year ($p < 0.05$), resulting in the minimum value of 6.24 g/kg and maximum value of 9.39 g/kg (Figure 4B) with the mean of 7.1 g/kg as shown in Table 2. This result might suggest that SOM has not achieved stability because the P plot was derived from a larger check plot which received manure application from 1892 to 1929 (Davis et al., 2003). Total N had a non-significant regression coefficient ($p < 0.05$) and ranged from 0.79 g/kg to 1.11 g/kg with a mean of 0.92 g/kg (Table 2). This mean is statistically similar to the check plot. Because a decrease in OC would be associated with mineralized N, a decrease in the C:N ratio was expected. Nonetheless, the ratio between C and N showed a non-significant regression coefficient ($p < 0.05$), ranging from 6.24 to 9.39 and a mean of 7.78 (Figure 4C) (Table 4).

The regression model suggested that soil pH decreased 0.01 per year over 25 years ($p < 0.05$) (Figure 4D). However, the raw data showed that in 1990, soil pH was 6.18 and dropped to 5.42 in 2015. Aula et al. (2016) suggested that P application had little or no effect on soil pH. Therefore, other factors contributed to the decrease in soil pH such as SOM oxidation, mineralization of N, and nutrient uptake (Brady and Weil, 2008). On average, soil pH in the P plot was 5.58.

The average grain yield in the P plot was similar to the check plot at 1.2 Mg/ha and also had one of the lowest grain yields in comparison to the other treatments. Even though P was the first most limiting nutrient in 1930-1957, crop response changed once N was depleted from SOM and as was mentioned by Girma et al. (2007).

NP Plot

According to Klan et al. (2007) and Mulvaney et al. (2009), application of N decreases the C:N ratio, favoring the decomposition of SOM. Total N over time had a non-significant regression coefficient ($p < 0.05$) but fluctuated between 0.91 g/kg and 1.22g/kg (Figure 5A).

Nitrogen fertilizer is extremely susceptible to loss by leaching, volatilization, denitrification, and plant uptake (Brady and Weil, 2008). Thus, total N might not reflect the inorganic additions from N fertilizer but rather the organic portion of SOM. It maintained a TN average of 1.04 g/kg which was statistically similar to NPK and NPKL plots but lower than the manure plot (Table 2).

Organic C had a non-significant regression coefficient ($p < 0.05$), ranging between 7.44 g/kg to 9.03 g/kg (Figure 4B) and a mean of 8.30 g/kg (Table 3). This mean was statistically similar to the NPK plot, but higher than the check plot and the P plot, yet lower than the manure plot. The NP plot was also split from the original check plot that had received manure between 1892 to 1929. However, in contrast to the P plot, OC has become stable. Nitrogen stimulates plant growth and development of the root system (Brady and Weil, 2008). Roots produce organic substances that promote an increase in the microbial population which excretes polysaccharides that act like a binding agent, stabilizing SOM (Haynes and Naidu, 1997).

The C:N ratio over time had a non-significant regression coefficient ($p < 0.05$) ranging from 7.36 to 9.03 (Figure 5C) with a mean of 8.54 (Table 4).

Nitrogen fertilizer can increase soil acidity due to nitrification of ammonium (Brady and Weil, 2008). In Table 5, the slope coefficient shows that soil pH decreased by 0.01 per year ($p < 0.05$). In 1990, soil pH was 5.39 and 4.94 by 2015. The average soil pH in the NP plot was statistically similar to the NPK plot. Also, the change of fertilizer was not noticeable in the soil pH (Figure 5D) as observed by Schoroder et al. (2011) who concluded that different sources of N did not present a significant acidity difference in the soil. At low pH values, plants tend to accumulate ammonium because nitrifying bacteria are sensitive to low pH (< 5.5) as Bohn et al. (2001) suggested, but Boer and Kowalchuk (2001) concluded that autotrophic bacteria are the main nitrifying agents in acidic soils which can supply nitrate to the soil. At a low pH (< 4.7),

Al^{+3} can be a predominant ion in the soil solution and that can be toxic to plants. It hydrolyzes water and releases H^+ which decreases soil pH (Brohn et al., 2001). As a result, the availability of some nutrients decreases. In addition, Sylvia et al. (1998) stated that mineralization decreases when soil pH is below 4.5. However, these conditions did not affect grain yield which fluctuated between 0.17 Mg/ha to 4.09 Mg/ha (Figure 5E) and achieved a mean of 2.2 Mg/ha. This mean was statistically similar to NPK and the manure plot. Nitrogen fertilization stimulates microbial activity by supplying N to the microbial community and then slowly releases N to the crop. This phenomenon is known as the priming effect (Weaterman and Kurtz, 1973), and it happens when a low to moderate rate of N fertilizer (<67kg/ha) is applied in the soil (Raun et al., 1998).

NPK Plot

A similar trend was found in the NPK plot, where TN, OC, and C:N ratio had non-significant regression coefficients ($p < 0.05$). Total N ranged from 0.87 g/kg to 1.12 g/kg (Figure 6A) with a mean of 1.03 g/kg (Table 2). This mean was statistically similar to the NP and NPKL plots but lower than the manure plot. Organic C ranged between 7.5 g/kg to 10.2 g/kg (Figure 6B) and had a mean of 8.26 g/kg. Again, it was similar to the NP plot but lower than the manure plot (Table 3). The C:N ratio ranged between 7.3 and 9.7 with a mean of 8.26 (Figure 6C).

Furthermore, soil pH decreased by 0.02 units per year ($p < 0.05$). In 1990, soil pH started at 5.5 and decreased to 4.86 by 2015 (Figure 6D). Average soil pH in the NPK and NP plot were the lowest compared to the other treatments, at 5.0 and 5.13 respectively. This could be due to nitrification of N.

The average grain yield was 2.4 Mg/ha and it was the highest grain yield among all treatments, making it statistically similar to the NPKL plot.

NPKL Plot

Lime was applied when soil pH was below 5.5; however, re-analyzing the soil samples revealed that in some years the soil samples had pH values below 5.5. This discrepancy among the pH readings might be related to equipment operation as Summer (1994) mentioned. Despite this issue, soil pH increased from 4.48 to 5.64 after 5 years of lime application. Combining all years, the NPKL plot did not illustrate a significant trend over time for any of the variables. However, the C:N ratio did increase when the analysis focused on years 2010 to 2015. This result suggested that mineralization was occurring. However, it did not affect TN or OC. They fluctuated between 8.14 g/kg and 10.49 g/kg for OC and 0.91 g/kg to 1.18 g/kg for TN (Figure 8A and Figure 8B). Buerkert et al. (2012) stated that in the first year after application, lime favors mineralization and the release of N and C. Furthermore, the long-term effect of lime application stimulates root growth by lowering aluminum in soil solution and that increases OC (Haynes and Naidu 1997).

The NPKL plot had the highest grain yield among all treatments (Table 6) at 2.5 Mg/ha as a result of lime application which increased the nutrient availability to the crop and decreased aluminum activity (Brady and Weil, 2008).

CHAPTER VI

CONCLUSION

Manure application every four years at a rate of 269 kg N/ha resulted in the highest mean for all dependent variables except grain yield which was below NPK and NPKL plots. Manure application at the rate of 269 kg N/ha could maintain 2% OM and adequate soil pH.

Soil test parameters in the check plot continue to decrease, evidenced in the TN and OC data reported. It also had the lowest mean among all the treatments for all variables evaluated. The P plot showed that even though P was the most limiting factor in 1930-1957, this deficiency has been corrected and now the most limiting nutrient is N.

All plots receiving inorganic N showed a decrease in soil pH over time and it did not change TN and SOC.

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TABLES

Table 1. Treatment structure with source of fertilizer and rate, the Magruder Plots, OK 1991-2015.

Year	Trt	N source	Rate	P source	Rate	K source	Rate
			kg ha ⁻¹		kg ha ⁻¹		kg ha ⁻¹
1991-2003	1	Manure‡	269	-	0	-	0
	2	-	0	-	0	-	0
	3	-	0	TSP	34	-	0
	4	NH ₄ NO ₃	67	TSP	34	-	34
	5	NH ₄ NO ₄	67	TSP	34	Potash	34
	6†	NH ₄ NO ₅	67	TSP	34	Potash	34
2004-2015	1	Manure‡	269	-	0	-	0
	2	-	0	-	0	-	0
	3	-	0	TSP	34	-	0
	4	Urea	67	TSP	34	-	34
	5	Urea	67	TSP	34	Potash	34
	6†	Urea	67	TSP	34	Potash	34

‡ Manure was applied in 1991, 1995, 1999, 2003, 2007, 2011, and 2015.

† Lime was applied in 2009

Table 2. Linear regression analysis between treatments for Total Nitrogen (TN), testing the intercept=0 and slope=1 (PR>t,0.05) and ANOVA evaluating treatments using years as replications, Duncans mean separation procedure (PR>F,0.05) 1990-2015, Stillwater, OK.

Treatment	n	Test variable	Estimate	PR> t	Mean†	Standard deviation
Manure Plot	14	Intercept=0	-0.4949	0.9531	1.0964a	0.1240
		Slope=1	0.0008	0.8501		
Check Plot	15	Intercept=0	12.1107	0.0263	0.904c	0.0943
		Slope=1	-0.0056	0.0374*		
P Plot	15	Intercept=0	2.7598	0.5916	0.9273c	0.0827
		Slope=1	-0.0009	0.7208		
NP Plot	15	Intercept=0	-5.2590	0.3991	1.04b	0.1039
		Slope=1	0.0031	0.3154		
NPK Plot	15	Intercept=0	0.3886	0.9493	1.0386b	0.0972
		Slope=1	0.0003	0.9152		
NPKL Plot	14	Intercept=0	-2.4270	0.7480	1.0542ab	0.1200
		Slope=1	0.0017	0.6455		
Source	DF	Type III SS	Mean Square	F Value	PR> F	
Treatment	5	0.4594	0.0918	21.4500	<.0001	
Year	14	0.6016	0.0429	10.0300	<.0001	

† Means followed by the same letter are not significantly different.

Table 3. Table 3. Linear regression analysis between treatments for Organic Carbon (OC), testing the intercept=0 and slope=1 (PR>t,0.05) and ANOVA evaluating treatments using years as replications, Duncans mean separation procedure (PR>F,0.05) 1990-2015, Stillwater, OK.

Treatment	n	Test variable	Estimate	PR> t	Mean†	Standard deviation
Manure Plot	14	Intercept=0	-2.7442	0.9631	9.3128a	0.8765
		Slope=1	0.0060	0.8392		
Check Plot	15	Intercept=0	118.1658	0.0029	6.7506d	0.7320
		Slope=1	-0.0556	0.0044*		
P Plot	15	Intercept=0	106.1994	0.0196	7.1806c	0.7924
		Slope=1	-0.0494	0.0276*		
NP Plot	15	Intercept=0	-8.9663	0.8189	8.3013b	0.6320
		Slope=1	0.0086	0.6602		
NPK Plot	15	Intercept=0	67.2050	0.0575	8.5433b	0.5892
		Slope=1	-0.0293	0.0920		
NPKL Plot	14	Intercept=0	46.6313	0.3931	9.3123a	0.8661
		Slope=1	-0.0187	0.4899		
Source	DF	Type III SS	Mean Square	F Value	PR> F	
Treatment	5	80.9850	16.1970	47.9100	<.0001	
Year	14	23.4838	1.6774	4.9600	<.0001	

† Means followed by the same letter are not significantly different.

Table 4. Linear regression analysis between treatments for the C:N ratio, testing the intercept=0 and slope=1 (PR>t,0.05) and ANOVA evaluating treatments using years as replications, Duncans mean separation procedure (PR>F,0.05) 1990-2015, Stillwater, OK.

Treatment	n	Test variable	Estimate	PR> t	Mean	Standard deviation
Manure Plot	14	Intercept=0	14.8129	0.7578	8.5364ab	0.7045
		Slope=1	-0.0031	0.8959		
Check Plot	15	Intercept=0	35.3739	0.3613	7.4960e	0.6253
		Slope=1	-0.0139	0.4691		
P Plot	15	Intercept=0	99.1241	0.0851	7.782de	0.9640
		Slope=1	-0.0456	0.1096		
NP Plot	15	Intercept=0	43.2727	0.1651	8.0193cd	0.5077
		Slope=1	-0.0176	0.2522		
NPK Plot	15	Intercept=0	70.6830	0.0940	8.2673bc	0.7004
		Slope=1	-0.0311	0.1346		
NPKL Plot	14	Intercept=0	71.7109	0.1749	8.7043a	0.8534
		Slope=1	-0.0314	0.2292		
Source	DF	Type III SS	Mean Square	F Value	PR> F	
Treatment	5	14.5255	2.9051	13.1600	<.0001	
Year	14	29.9025	2.1358	9.6800	<.0001	

† Means followed by the same letter are not significantly different.

Table 5. Linear regression analysis between treatments for soil pH, testing the intercept=0 and slope=1 ($PR > t, 0.05$) and ANOVA evaluating treatments using years as replications, Duncan's mean separation procedure ($PR > F, 0.05$) 1990-2015, Stillwater, OK.

Treatment	n	Test variable	Estimate	PR> t	Mean [†]	Standard deviation
Manure Plot	14	Intercept=0	42.6790	0.1072	6.4892a	0.4003
		Slope=1	-0.0181	0.1656		
Check Plot	15	Intercept=0	39.8887	0.0245	5.822b	0.2995
		Slope=1	-0.0170	0.0489*		
P Plot	15	Intercept=0	40.2083	0.0025	5.5846c	0.236
		Slope=1	-0.0173	0.0067*		
NP Plot	15	Intercept=0	39.8705	0.0035	5.1326d	0.2416
		Slope=1	-0.0173	0.0084*		
NPK Plot	15	Intercept=0	64.6254	0.0002	5.058d	0.3399
		Slope=1	-0.0297	0.0004*		
NPKL Plot	14	Intercept=0	53.2535	0.0843	5.4864c	0.5073
		Slope=1	-0.0238	0.1172		
Source	DF	Type III SS	Mean Square	F Value	PR> F	
Treatment	5	20.3067	4.0613	58.1600	<.0001	
Year	14	5.1531	0.3680	5.2700	<.0001	

[†] Means followed by the same letter are not significantly different.

Table 6. Linear regression analysis between treatments for grain yield, testing the intercept=0 and slope=1 (PR>t,0.05) and ANOVA evaluating treatments using years as replications, Duncans mean separation procedure (PR>F,0.05) 1990-2015, Stillwater, OK.

Treatment	n	Test variable	Estimate	PR> t	Mean	Standard deviation
Manure Plot	14	Intercept=0	-14.7955	0.7709	2.126c	0.7296
		Slope=1	0.0085	0.7389		
Check Plot	15	Intercept=0	6.1479	0.7933	1.0766d	0.3770
		Slope=1	-0.0025	0.8288		
P Plot	15	Intercept=0	-12.0359	0.6223	1.2046d	0.3948
		Slope=1	0.0066	0.5882		
NP Plot	15	Intercept=0	-76.3918	0.1738	2.2606bc	0.9385
		Slope=1	0.0393	0.1622		
NPK Plot	15	Intercept=0	-24.0798	0.6219	2.4586ab	0.7892
		Slope=1	0.0132	0.5872		
NPKL Plot	14	Intercept=0	-47.0810	0.2052	2.5813a	0.6119
		Slope=1	0.0249	0.1817		
Source	DF	Type III SS	Mean Square	F Value	PR> F	
Treatment	5	31.5455	6.3091	54.7200	<.0001	
Year	14	34.7608	2.4829	21.5300	<.0001	

† Means followed by the same letter are not significantly different.

Table 7. Linear regression analysis for NPKL plot testing the intercept=0 and slope=1 (PR>t,0.05) and NPKL plot mean for TN, OC, C:N ratio, soil pH, and grain yield 2010-2015, OK.

Treatment	n	Test variable	Estimate	PR> t	Mean	Standard deviation
TN	6	Intercept=0	26.4465	0.6185	1.0603	0.0942
		Slope=1	-0.0126	0.6322		
OC	6	Intercept=0	-311.1756	0.5453	8.8407	0.9256
		Slope=1	0.1590	0.5345		
CN	6	Intercept=0	-500.9014	0.2111	8.3417	0.4822
		Slope=1	0.2530	0.2092		
Soil pH	6	Intercept=0	-280.3533	0.0263	5.4217	0.3058
		Slope=1	0.1420	0.0248		
Grain yield	6	Intercept=0	-545.0871	0.2111	2.8897	0.8507
		Slope=1	0.2723	0.2092		

FIGURES

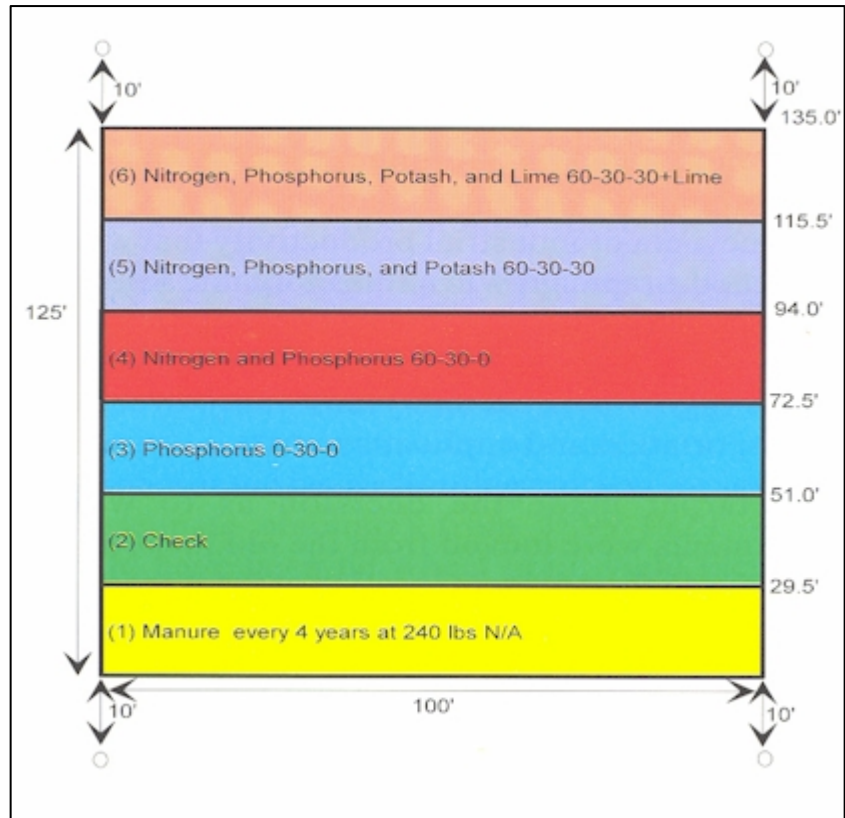


Figure 1. Treatment structure in the Magruder Plots, 1947-present.

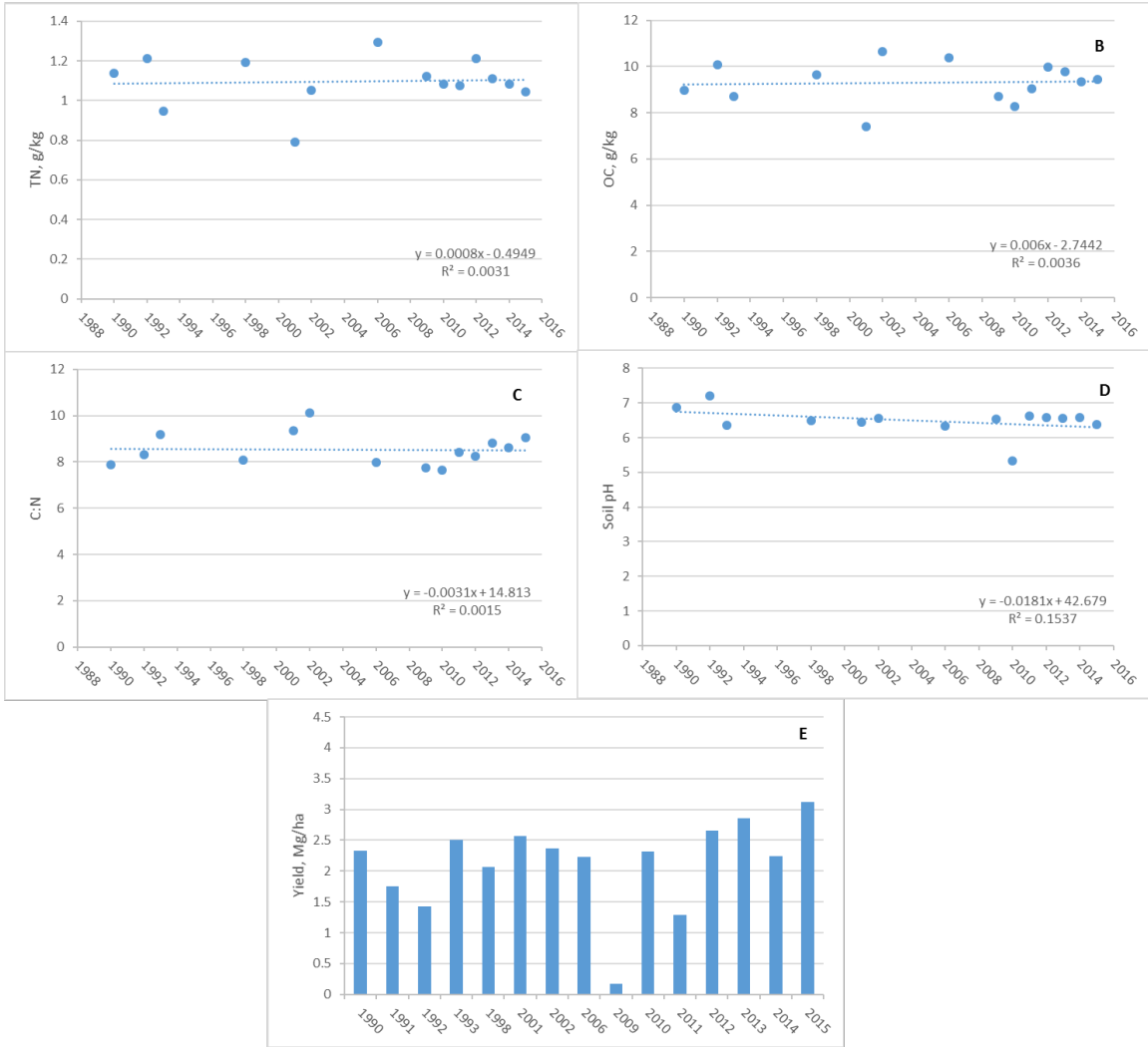


Figure 2. Linear regression analysis for total nitrogen (A), organic carbon (B), C:N ratio (C), soil pH (D), and grain yield (E) for the manure plot, as a function of time, 1990-2015, OK. 269 kg N/ha applied once, every four years.

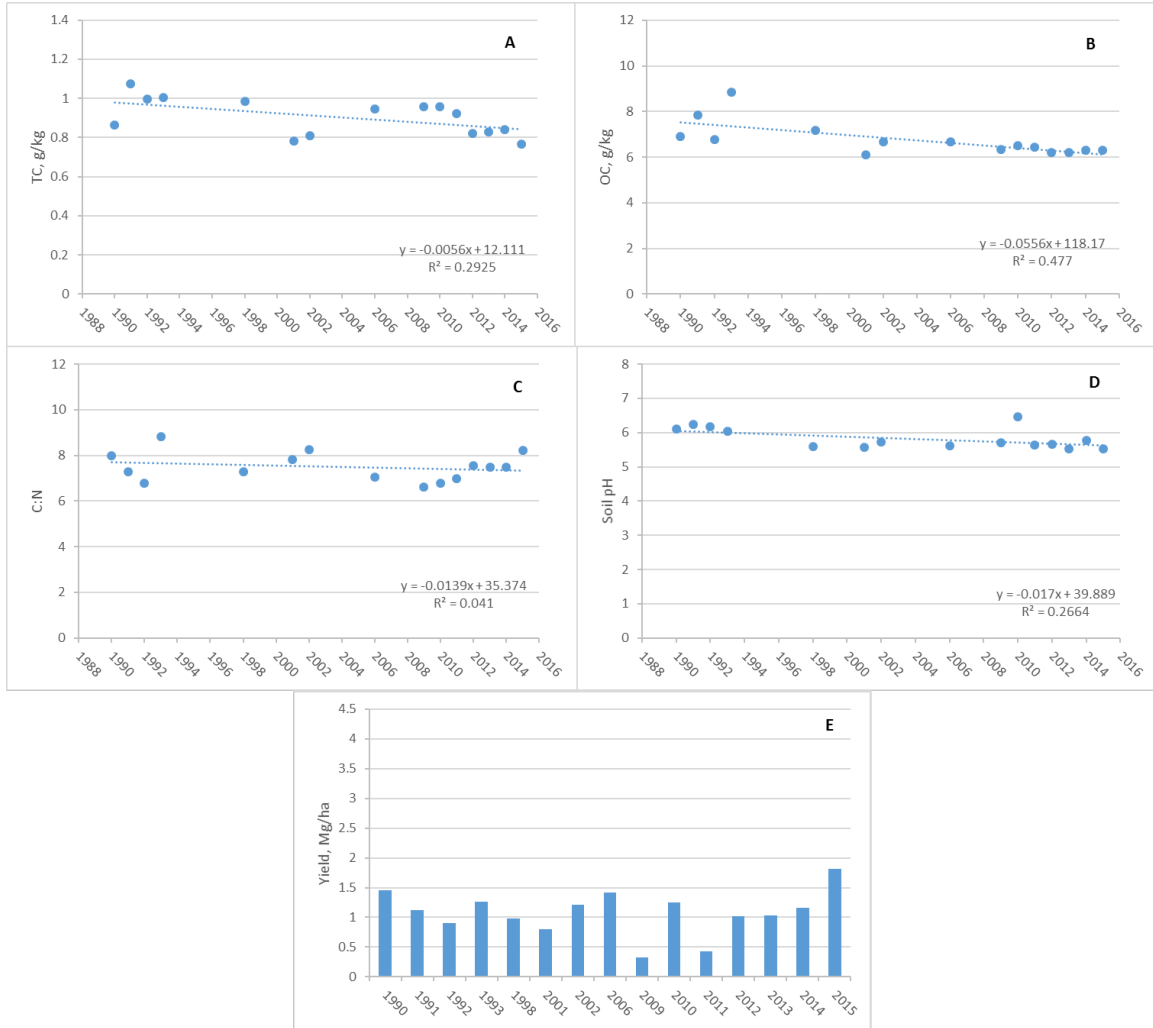


Figure 3. Linear regression analysis for total nitrogen (A), organic carbon (B), C:N ratio (C), soil pH (D), and grain yield (E) for the check plot, as a function of time, 1990-2015, OK.

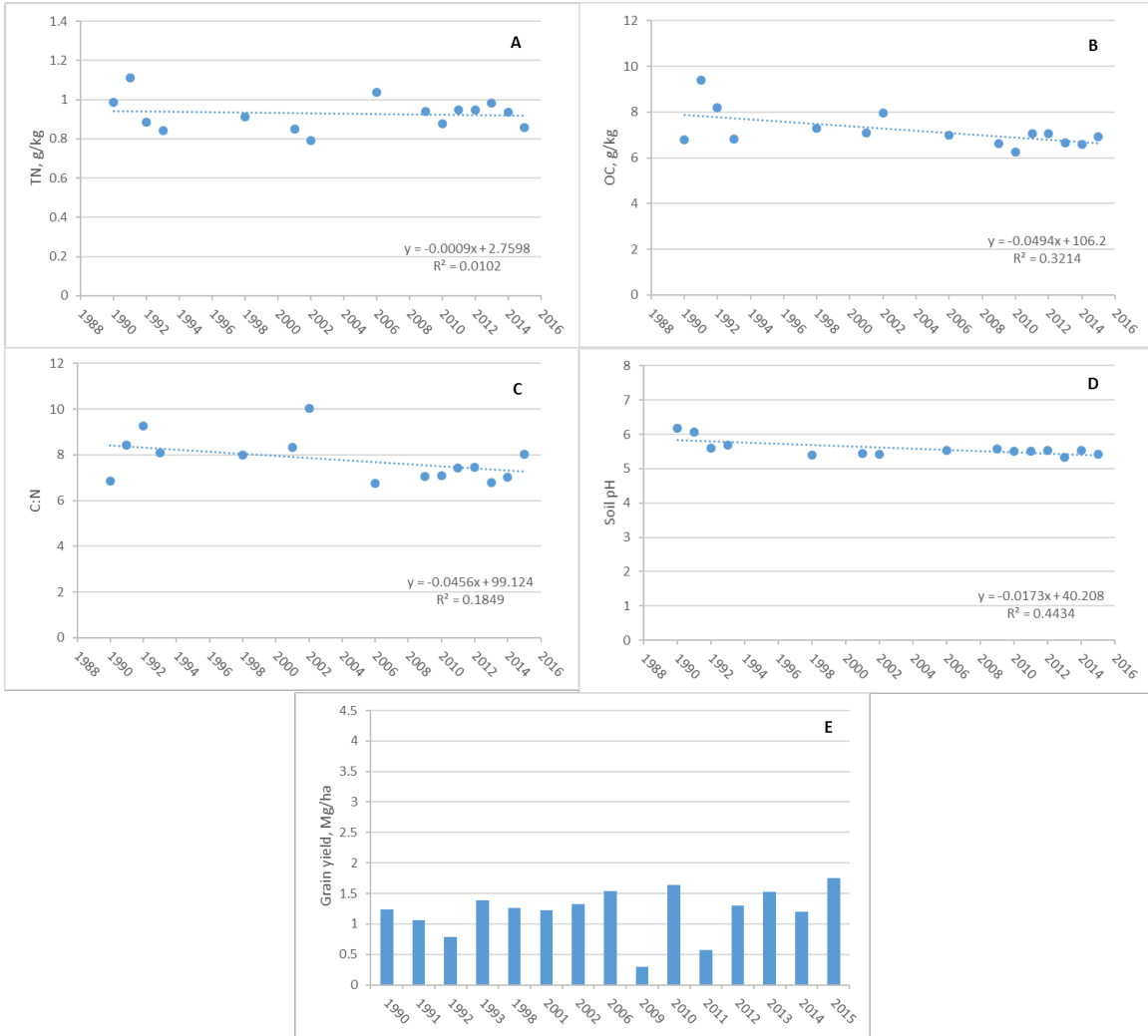


Figure 4. Linear regression analysis for total nitrogen (A), organic carbon (B), C:N ratio (C), soil pH (D), and grain yield (E) for the P plot, as a function of time, 1990-2015, OK. 14.6 kg/ha triple superphosphate was applied as a source of P.

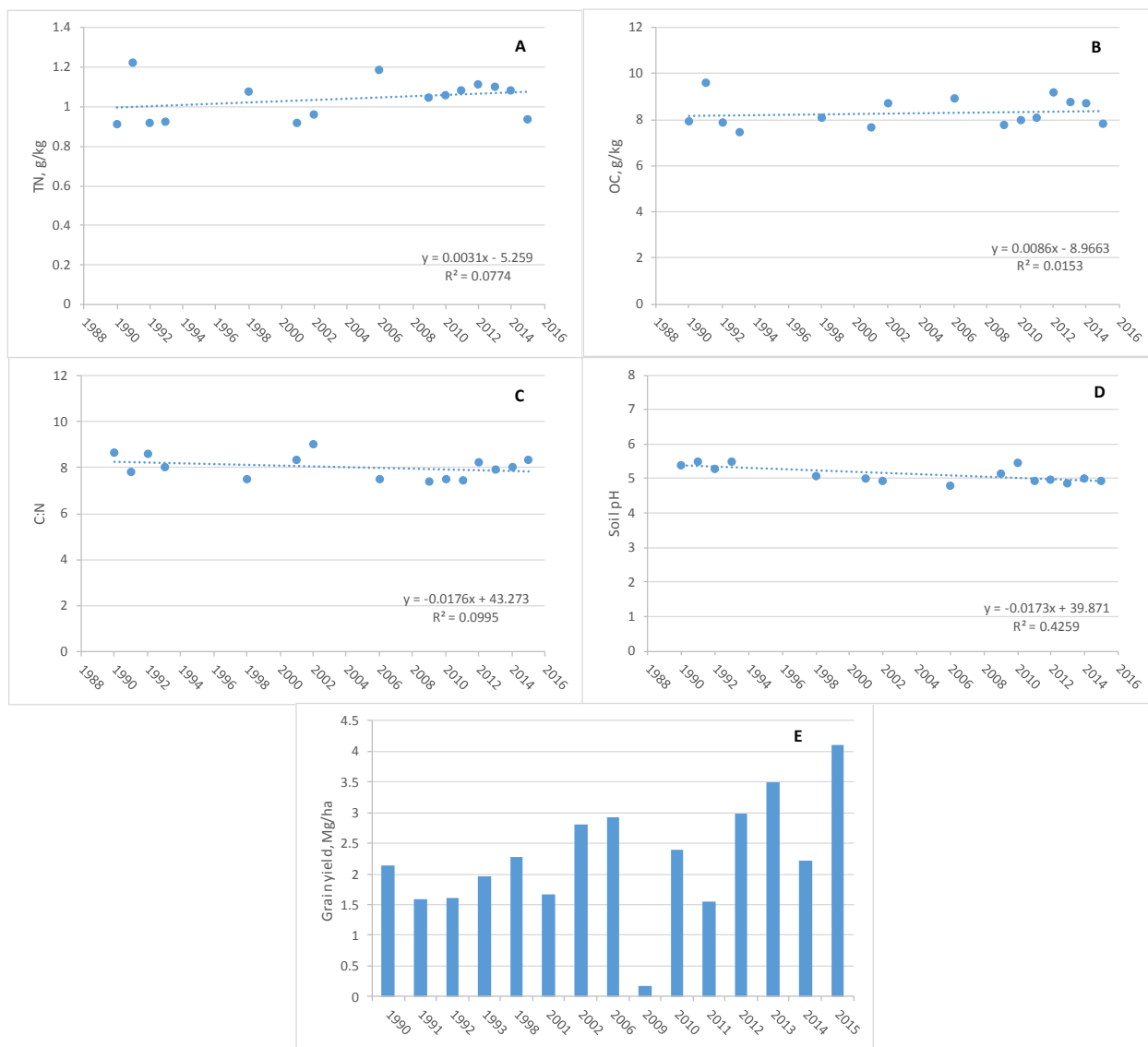


Figure 5. Linear regression analysis for total nitrogen (A), organic carbon (B), C:N ratio (C), soil pH (D), and grain yield (E) the NP plot, as a function of time, 1990-2015, OK. 67kg/ha of ammonium nitrate (NH_4NO_3) was applied from 1990-2004 shifting to 67kg/ha of urea from 2004-present in addition to 14.6 kg/ha of triple superphosphate.

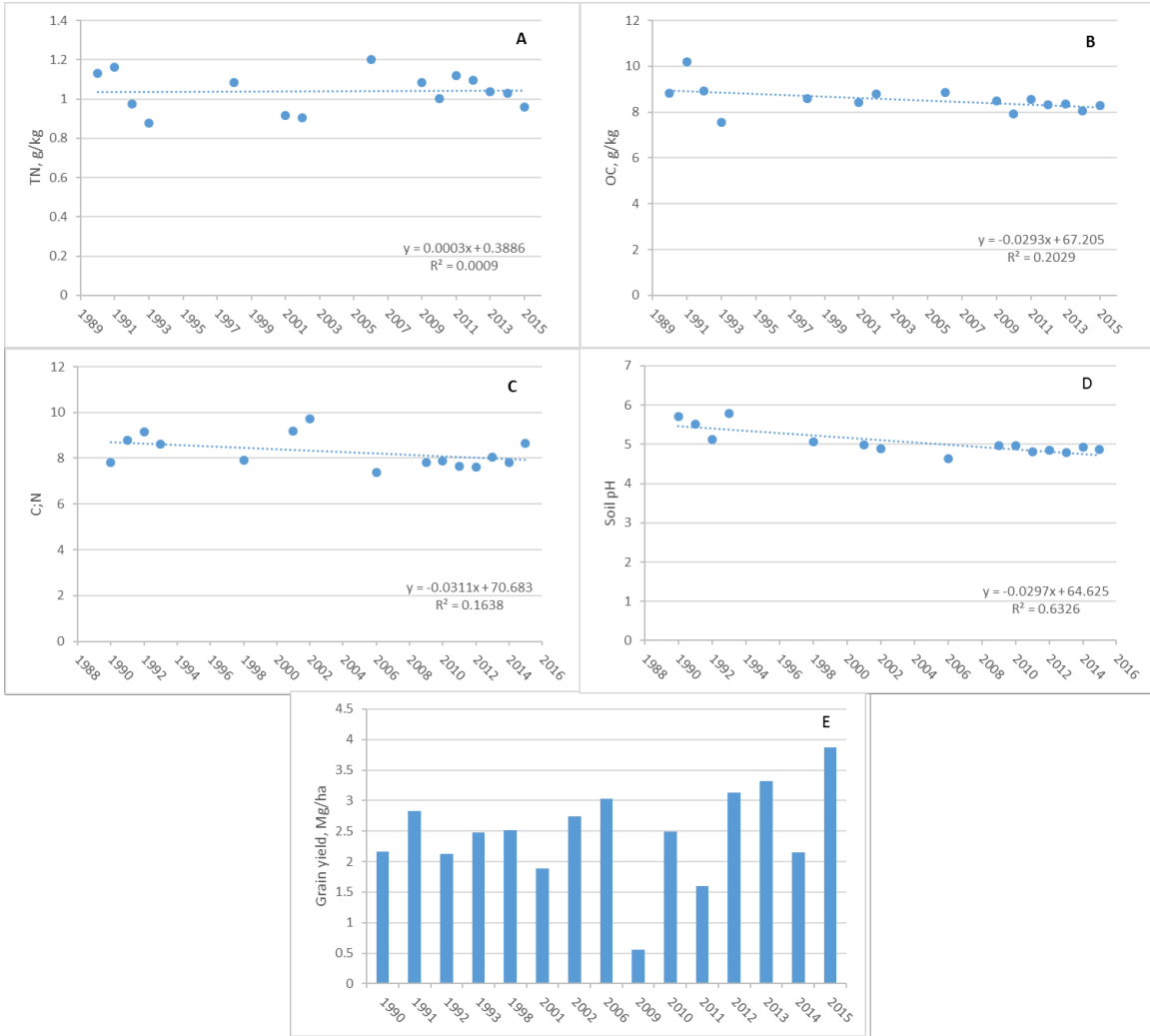


Figure 6. Linear regression analysis for total nitrogen (A), organic carbon (B), C:N ratio (C), soil pH (D), and grain yield (E) the NPK plot, as a function of time, 1990-2015, OK. 67kg/ha of ammonium nitrate (NH_4NO_3) was applied from 1990-2004 shifting to 67kg/ha of urea from 2004-present in addition to 14.6 kg/ha of triple superphosphate and 28.8 kg/ha of KCl.

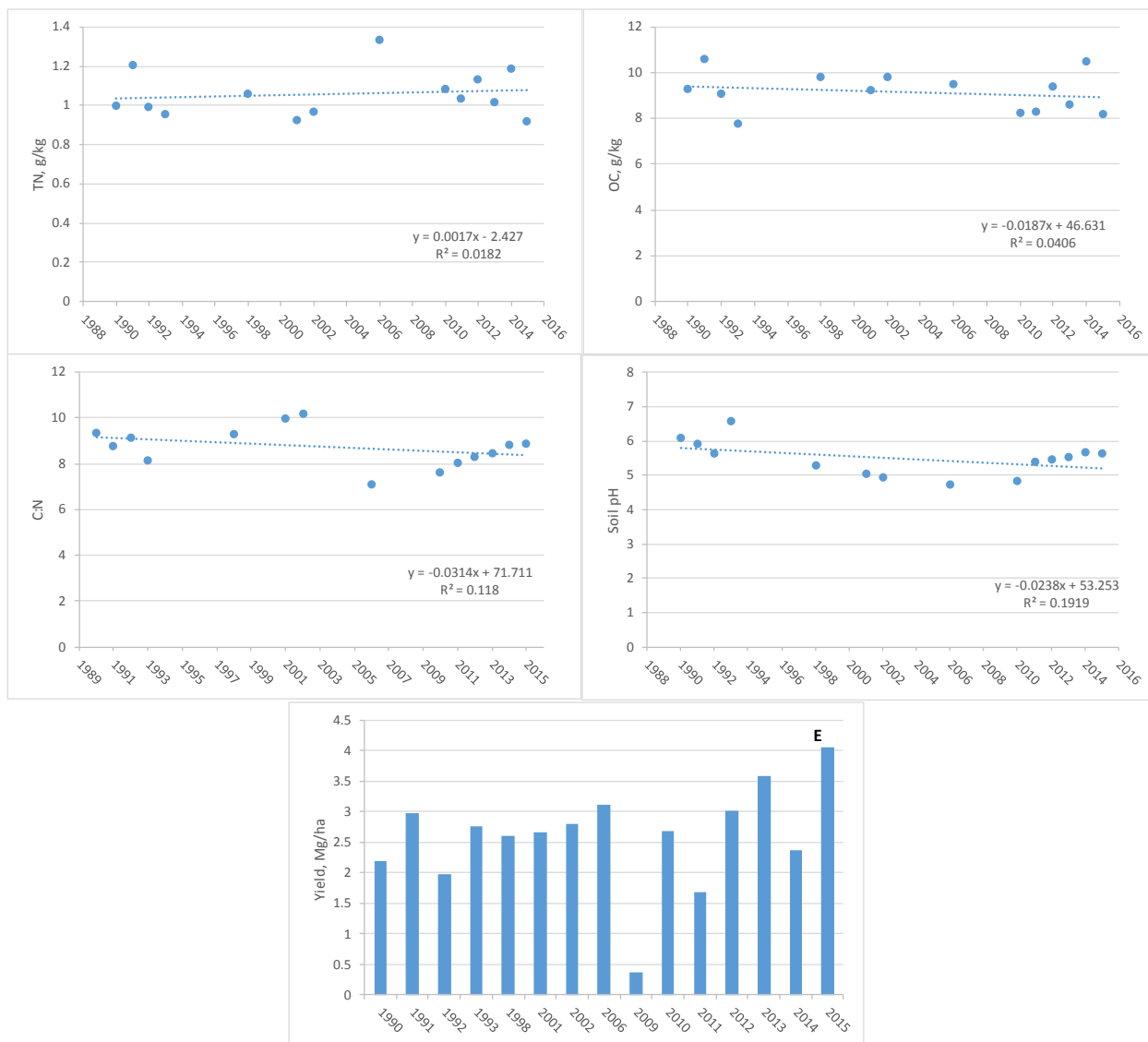


Figure 7. Linear regression analysis for total nitrogen (A), organic carbon (B), C:N ratio (C), soil pH (D), and grain yield (E) the NPKL plot, as a function of time, 1990-2015, OK. 67kg/ha of ammonium nitrate (NH_4NO_3) was applied from 1990-2004 shifting to 67kg/ha of urea from 2004-present in addition to 14.6 kg/ha of triple superphosphate and 28.8kg/ha of KCl. Limestone was applied in 2009

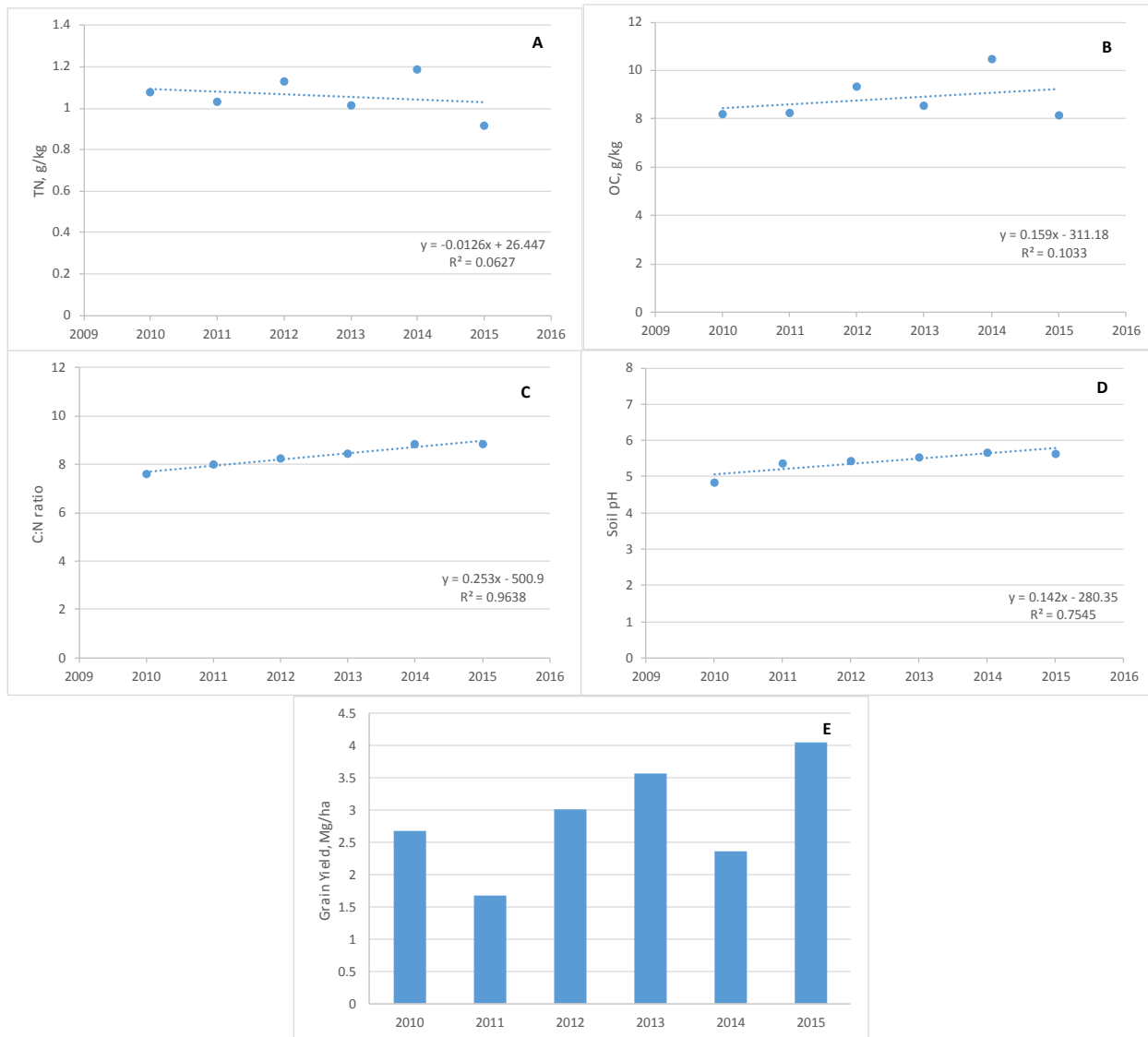


Figure 8. Linear regression analysis for total nitrogen (A), organic carbon (B), C:N ratio (C), soil pH (D), and grain yield (E) the NPK plot, as a function of time, 2010-2015, OK. 67kg/ha of urea was applied from 2004-present in addition to 14.6 kg/ha of triple superphosphate and 28.8 kg/ha of KCl. Limestone was applied in 2009.

VITA

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Master of Science

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