INFLUENCE OF NITROGEN TIMING ON WINTER WHEAT GRAIN YIELD AND EVALUATION OF NUTRIENT MANAGEMENT ON THE STRATIFICATION OF SOIL PARAMETERS IN NO-TILL

By

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 2021

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ACKNOWLEDGEMENTS

Foremost, I am extremely grateful to God for lighting my way here.

Firstly, I would like to thank my M.S. and Ph.D. programs advisor Dr. Brian Arnall for all his support, friendship, knowledge sharing and help during this period. I would also like to express my gratitude to my committee members Drs. Hailing Zhang, Jason Warren, Neils Maness and William Raun for their full support throughout this time.

I would also like to thank my family who provided love and support at a distance, which was essential to conclude this achievement. I would like to pay my special regards to my wife Olivia Lucarelli Souza for always being by my side in all moments and to my father Luiz Antonio de Abreu e Souza for teaching me the value and beauty of science.

I would also like to acknowledge my current and former colleagues from the Precision Nutrient Team for helping in the projects and for the enjoyable moments in this journey. Without them, this project would not be possible.

Finally but not least, I would like to express my gratitude to the Brazilian community of Stillwater for all the support and pleasant moments that we have had in these years, specially Drs. Joao Arthur Antonangelo, Rolf Prade and Pedro Machado for the many discussions and helpful insights through the M.S. and Ph.D. course.

Acknowledgements reflect the views of the author and are not endorsed by committee members or Oklahoma State University.

Name: JOAO LUIS BIGATAO SOUZA

Date of Degree: DECEMBER, 2021

Title of Study: INFLUENCE OF NITROGEN MANAGEMENT ON WINTER WHEAT GRAIN YIELD AND EVALUATION OF NUTRIENT MANAGEMENT ON THE STRATIFICATION OF SOIL PARAMETERS IN NO-TILL

Major Field: SOIL SCIENCE

Abstract:

Visual symptoms of nitrogen (N) deficiency is common in winter wheat fields. Whether it is due to unavailability of suitable application equipment or unfavorable application conditions, the crop may go a significant period without fertilization. The objective of this study was to evaluate the impact of N applications made after winter wheat is under N stress for an extended period. The study was conducted across 12 site-years in Oklahoma. Treatments included control, pre-plant application and ten in-season treatments. In-season treatments were initiated at the point N deficiency was visually identified. Treatments were applied in progressive order every seven growing days to the point of 63 growing days after visual differentiation (DAVD). A negative effect on grain yield was observed when application was made post Feeks 8. The data suggested forgoing N application until Feekes 7, regardless of deficiency, had no negative impact on yield and in some cases increased yield above the pre-plant application. This documents that winter wheat producers have a much wider N application window than traditionally believed.

No-till (NT) management directly influences chemical, biological and physical proprieties in the soil, affecting nutrient dynamics. In the second chapter, our objective was to quantify soil chemical attributes stratification in continuous wheat in three long-term dry-land sites under NT at least for nine years. Available Phosphorus (P) and potassium (K), total nitrogen (TN), organic carbon (OC), pH and exchangeable aluminum (Al_{KCl}) were quantified. We found that stratification occurred for all soil attributes tested in the study, with P, K, TN, OC and Al_{KCl} being greater near the soil surface and pH being lower. Soil pH, TN, and OC stratification were generally related to the rate of annual N fertilization. Although not at the same magnitude, non-fertilized plots were likewise stratified for the tested parameters, which indicates an isolated effect of the NT on the stratification rather than the fertilizer addition. Organic carbon and TN were highly stratified in the first 5cm and little or no impact was noticed in deeper soil layers. Attention regarding sampling depth in NT areas is essential for an accurate soil attributes monitoring and fertilizer recommendation.

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CHAPTER I

RECOVERY OF YIELD WITH FERTILIZER APPLICATION POST NITROGEN STRESS IN WINTER WHEAT (TRITICUM AESTIVUM L.)

Abstract

It is common for winter wheat producers to have fields show visual symptoms of nitrogen (N) deficiency. Whether it is due to unavailability of suitable application equipment or unfavorable application conditions, the crop may go a significant period without fertilization. The objective of this study was to evaluate the impact of N applications made after winter wheat was under N stress for an extended period. The study was conducted across 12 site-years in Oklahoma. Treatments included an untreated check, pre-plant application and ten in-season treatments. In-season treatments were initiated at the point N deficiency was visually identified. Treatments were applied in progressive order every seven growing days (GDD>0) to the point of 63 growing days after visual differentiation (DAVD). A negative effect delaying N on grain yield was observed, but only when application was made post Feekes 8. Across this data set, that timing corresponded with a range of 21 to 63 DAVD. The data suggested that forgoing N application until Feekes 7, regardless of the N visual deficiency, had no negative impact on yield and in some cases increased yield above the pre-plant application. We concluded that the growth stage at which N application took place was more critical than level of N deficiency, and that N fertilizer application should be made posteriorly to the crop dormancy. This documents that winter wheat producers have a much wider N application window than traditionally believed.

Introduction

Wheat (*Triticum aestivum* L.) is the most planted cereal crop worldwide; of which 65% of its production is utilized for human food consumption (FAO-AMIS, 2018). Considering the increasing of the human population predicted to be 10 billion by 2050 (Hitz et al., 2017), Fischer et al. (2014) estimated that at least 50% crop yield increase will be necessary to supply the population food demand by that time, under the same arable acres currently cultivated. However, wheat yields have stagnated in 27% of the production areas according to Grassini et al. (2013). Nitrogen is the most limiting nutrient in wheat production, and overall second most limiting factor after water (Szumigalski and Van Acker, 2006). In Oklahoma, winter wheat production is predominantly rain fed (Patrignani et al., 2014), which makes the improved N management necessary to increase production efficiency. Additionally, Bell et al. (1995) noted 48% of wheat yield improvement is attributable to increased N application and 28% due to improved genetics.

The utilization of in-season optical sensors can increase NUE of winter wheat by optimizing N rates when compared to conventional methods (Raun et al., 2002). A review of 26 studies showed that this type of management strategy resulted in an average NUE of 42%, approximately 10.4% more than conventional recommendation methods (Aula et al., 2020). The excess of N applied on the soil, and the low uptake efficiency by the crop, can lead to potential environmental pollution (Omara et al., 2019b). However, for this management strategy to be better utilized, in-season yield potential is required to be predicted. For an accurate yield potential estimation, winter wheat needs to be under N stress for the N application yield improvement to be calculated (Raun et al., 2002). Therefore, there is a lack of information concerning how long N applications can be delayed and yields maintained after a confirmed in-season N stress. The N application timing threshold is crucial for this optical sensing technology to be used by producers.

Nitrogen Dynamics in Winter Wheat

Previous work has shown that both genotype and environment have a great influence on crop N uptake. According to Austin et al. (1977), in a study analyzing 47 wheat genotypes, at anthesis, plants generally contained 83% of the total N taken up at physiological maturity, and, on average 68% of total N in the plant was present in the grain. Moreover, the authors also suggested a strong correlation between dry matter accumulation and N content at Feekes 10.5.3 and grain filling. Others (Justes et al., 1994; Girma et al., 2010) also reported this correlation between biomass and N accumulation. Justes et al. (1994) presented that when there is no N deficiency, the total N accumulation was determined by the plant growth rate and the soil N-nitrate availability. In addition, the authors noticed that under no stress, wheat could accumulate more N than the plants need so as to achieve maximum growth rate. Girma et al. (2010) documented that 61% of the total N accumulated by the plant was at growth stage Feekes 5 in a study performed under field conditions in Oklahoma. De Oliveira Silva (2019) found that the N uptake by hard winter wheat in Kansas at Feekes 10.5.3 would be equivalent to 82%. The crop reached this stage when the accumulative growing degrees was approximately 1500 °C.

By Feekes 5, the plants become strongly erect. Leaves are formed and all meaningful tillers contributing to yield have ceased their growth. The growing point that generates new cells for the plant begins to develop an embryo head. At this stage the number of spikelet is determined. A deficiency of N at this stage can be detrimental since the N availability directly influences the number of seeds per head as well as seed size.

During final grain fill period (Feekes 10.5.4 to 11.3), protein accumulation is source limited differently from starch that is sink-limited. Thus, the amount of protein in the grain is not sensitive to the environment but due to the sensitivity of starch accumulation to environment, and the concentration of proteins in the grain can be. Lollato and Edwards (2015) documented that in low yielding environments the starch accumulation in the grains is reduced, resulting in a high protein concentration in the grains. This negative relationship between grain yield and protein concentration is well elucidated elsewhere in the literature (Lollato et al., 2019; Simmonds, 1995). Hence, under environmental conditions where production potential is substantially higher, a starch accumulation was observed, thus significantly reducing the protein concentration in the grain (Lollato and Edwards, 2015).

Nitrogen Application Timing

In general, most of the publications on N timing in winter wheat have found that the timing of application had an effect on grain yield and grain protein concentration. Findings suggest higher N losses during the growth period commonly causing low N availability at the end of the plant life cycle, thus reducing yield and protein content (Bushong et al., 2014; Fowler and Brydon, 1989; López-Bellido et al., 2005). The highest N requirement found in the literature is during the period of the most rapid plant growth, which normally occurs after dormancy. Spring applications of N have been found to aid in the recovery of the N stress during green-up (Fowler and Brydon, 1989; Morris et al., 2006). An alternative for producers is to split the applications into multiple timings. This strategy can assist in the in-season evaluation of the crop and weather, thus giving a better indication of the best timing for N fertilization instead of an early-season decision (Dhillon and Raun, 2020).

One of the greatest limitations to the studies discussed previously is the lack of a wide breadth of N application timings in a single study. The majority of the work focuses on multiple rates applied at only a few timings. An additional limitation is the lack of documenting if and when the crop displays signs of N stress. The objective of this study was to evaluate how long N application can be delayed during the winter wheat crop cycle without affecting grain yield and protein concentration. For this, subsequent N applications were performed after the point at which N deficiencies were identified.

Material and methods

The study was conducted over the 2016-2017, 2017-2018, 2018-2019, and 2019-2020 growing seasons. Five locations were utilized for the study: Lake Carl Blackwell research farm (LCB) near Perry, OK; Cimarron Valley research station near Perkins, OK; Cross Country research farm near Stillwater, OK; the Raymond Sidwell research station near Lahoma, OK; and the Ballagh Family research farm near Newkirk, OK. Soil classification, soil characteristics, and previous crop for each location is described in Table 1.

Prior to the initiation of the trials, 0-15 cm composite soil samples, composed of 15 soil cores, were collected from the entire trial area, as outlined in Zhang and Arnall (2013), and submitted for analysis to Soil, Water and Forage Analytical Laboratory (SWFAL) located in Stillwater, OK. The pH analysis was performed using 1:1 soil to deionized water ratio. For NO_3-N, 1 M KCl extraction was used and analyzed on a flow injection analyzer using cadmium reduction chemistry. For phosphorus (P) and potassium (K), Mehlich 3 extractions were analyzed using inductively coupled plasma (ICP). Soil analysis results are described in Table 2.

At Perkins2017, Perkins2018, and Stillwater2017, 84 kg ha^{-1} of diammonium phosphate (DAP) was applied in-furrow at planting, due to soil acidity at Perkins and the low soil test P at Stillwater, which would limit the yield potential (Zhang and Raun, 2006).

At all locations, treatments were arranged in a randomized complete block design (RCBD) with three replications; sites Newkirk2019, LCB2019, Newkirk2020 and LCB2020 were replicated four times. Plot size was 3.1 m by 6.1 m. All the areas utilized in this study were under no-till management. Due to the field at LCB being in a systematic crop rotation, the project establish two studies at that site during growing seasons. Best management practices recommended by Oklahoma State University were followed for all pest management.

Each trial evaluated eleven different N fertilization timings (one applied pre-plant and ten in-season timings) along with an unfertilized check; creating a total of 12 treatments (Table 3). All timings received a rate of 100 kg N ha^{-1} which was applied as ammonium nitrate (AN, NH_4NO_3) The rate of 100 kg N ha^{-1} was chosen as a yield limiting rate to represent any impact on N fertilization efficiency (Fowler and Brydon, 1989). The hypothesis to the approach was that if N was unlimited, the study would be unable to quantify occasional difference in efficiencies gained or lost due to application timing. Ammonium nitrate was applied over the commonly used urea N source (46-0-0) to reduce volatilization N loss (Ernst and Massey, 1960; Tian et al., 1998). The AN was applied to the plots via surface broadcast.

The initiation of in-season application was based on the visual N deficiency between the pre-plant treatment and the non-fertilized check. Locations were visited weekly after sowing. At the point at which a visual difference of crop biomass or leaf greenness was identified, it was considered visual responsive. At this point a GreenSeeker® hand held sensor was used to collect normalized difference vegetation index (NDVI) values from the pre-plant fertilized treatment and non-fertilized check plot (Table 4). In-season treatments were applied at 0, 7, 14, 21, 28, 35, 42, 49, 56, and 63 growing degree days > 0 (GDD>0) after visual deficiency (DAVD). Daily temperature values were retrieved from the Mesonet Wheat Growth Day Counter for the station closest to the research area (www.mesonet.org). GDD>0 is calculated as:

$$1\ GDD > 0 = \frac{Day\ Max\ Temperature + Day\ Min\ Temperature}{2} - 4.4\ °C \qquad (1)$$

Only if

$$\frac{Day\ Max\ Temperature + Day\ Min\ Temperature}{2} - 4.4\ °C > 0°C \qquad (2)$$

or

$$\frac{Day\ Max\ Temperature + Day\ Min\ Temperature}{2} - 4.4\ °C < 30\ °C \qquad (3)$$

At grain maturity, the center 1.5 m of the plots were harvested with a Massey Ferguson 8-XP plot combine (Kincaid Equipment Manufacturing; Haven, KS). Data for grain yield and percent moisture content were recorded by the onboard Harvest Master Yield monitoring computer (Juniper Systems; Logan, UT) and grain samples were collected from each plot at harvest. To standardize all grain yields, the moisture content was adjusted to 12.5 %. Grain protein was determined post-harvest using near infrared spectroscopy Diode Array NIR Analysis Systems model DA 7200, Perten (Kungens Kurva, Sweden).

Data was analyzed using JMP 15 PRO® (SAS institute) for year-specific crop production factors, such as grain yield and protein. Data was differentiated using ANOVA methods and Dunnett's to separate the means at $p = 0.05$. Controls utilized on the test were the check (trt1) (data not shown), pre-plant (trt2) and 0DAVD (trt3). For the pre-plant and 0DAVD comparisons the check treatment was removed from the data.

Results and discussion

Timing of Response to Nitrogen

As the design of this study was to evaluate N fertilizer application after visual N deficiencies were observed, the N treatments were applied at a range of dates across all site-years. Table 5 provides the dates for all treatment applications. Even though the planting date and application of the pre-plant were very similar across all sites, the date of 0DAVD ranged from November 11 to March 28. Even in the same season within the same field, LCB, previous crop impacted 0DAVD by 40 and 45 days in first and second crops years, respectively. The difference in 0DAVD date across site-years, along with the impact of location on the accumulation on GDD’s>0, created a range of dates for 63DAVD from February 12 to May 14, spanning a window of growth stages from Feekes 4 to Feekes 10.5. This range of application dates presented an opportunity to evaluate N application over a wide range of physiological growth stages, and yet, creates a challenge in that there is an inconsistency in growth stages evaluated across site-years.

Grain Yield and Protein Response to Nitrogen

To provide a general overview of all site-years, Figures 1 through 3 graphically represent the grain yield and grain protein concentration for each treatment. In order to determine if a N response occurred, a multiple comparison utilizing Dunnett's test (Pre-plant N application as a control) was performed by location (Table 6). A significant main effect of N was found when comparing the pre-plant N applied treatment to the non-fertilized check in 9 of the 12 site-years considering grain yield. Perkins2018, LCB2018b, and LCB2019 were the locations at which three was no difference between the pre-plant N and non-fertilized check. However, the grain yield of 0DAVD was statistically greater than the check at LCB2018b, and LCB2019, data not shown. The completion of this study resulted in eleven N responsive locations

In-season applications at LCB 2018b and LCB2020 started earlier in the growing season than the visual differentiation between the check plots and that pre-plant. The actual differentiation for LCB2018b and LCB2020 were noticed at application of treatment 5 (14DAVD) and treatment 4 (7DAVD), respectively. Nitrogen was applied prior to visual deficiency as the crop was already in an advanced stage and the range of application of the other treatments would override the agronomic interest.

Impact of nitrogen application timing on grain yield

In order to analyze the impact on in-season application timing on yield and grain protein concentration, a multiple comparison utilizing Dunnett's test was performed using pre-plant treatment as control for grain yield and protein (Table 6). The multiple comparison analysis was also performed utilizing the 0DAVD as another reference for grain yield and protein (Table 7). The first allows the evaluation of in-season N application, while the latter addresses the purpose of the study, which was to evaluate the ability of winter wheat to recover from N stress.

As shown in Table 6, only 8 of the 117 (6.8%) in-season application comparisons with pre-plant application were significantly less considering yield. All eight of these comparisons resulted when N application was delayed until March 30th (approximately Feekes 8) or later. When compared to the 0DAVD (Table 7) ten of the 117 (8.5%) comparisons showed a significant decrease in yield. Of these ten significant comparisons, nine were in-season applications and one was pre-plant application. Six of these in-season applications were found in the Newkirk2019 and LCB2019 trials. All of these applications were made on April 15th (approximately Feekes 8) or later. While not always significant, there was a numeric decrease in yield when compared to 0DAVD noted in the majority of the N applications made during the month of April or later (Figures 1, 2 and 3).

In the 2016/17 cropping year, one (3%) of the 38 in-season–application-comparisons for grain yield made to the pre-plant N application showed a significant decrease in yield, while 16 (42%) resulted in a significant yield increase. Due to the early visual differentiation at Perkins2017 and Stillwater2017, the range of in-season applications was completed by mid/late February. Therefore, because of the early N application start, we were not able to verify the point that the N stress could no longer be recovered from. However, results displayed that delaying application was possible up to 63 days after the development of N deficiency without grain yield or protein concentration loss. Conversely, there was no timing effect during the 2017/18 and 2018/19 cropping season in which grain yield was significantly greater than the pre-plant or 0DAVD (Table 6 and 7). One of the 39 in-season applications made to the pre-plant resulted in a significant increase on yield for the 2018-19 and 2019-20 crop seasons and four yielded significantly less. These four applications were made in Newkirk2020 after April 6th, which corresponded to the growth stage of Feekes 9.

Across the eleven N responsive site-years, grain yields achieved from applications made post visual differentiation was equal to, or greater than, that of the pre-plant application if the N was applied before Feekes 8. This result was such regardless of yield level or rainfall distribution, which ranged from 897 to 1390 mm across all site/years (Table 1).

Impact of nitrogen application timing on grain protein concentration

Grain protein concentration decreased only once when compared to the pre-plant across all locations (Table 6). This one timing, LCB2018b at 64DAVD, was made on May 2nd. During this time the crop was in the early stages of grain-fill. Of the 117 comparisons for protein concentration performed against the pre-plant treatment across all locations, in-season application of N significantly increased protein 48 times (41%). It should be noted that Perkins2018, the one location that did not have a significant yield response to N application, had nine positive grain protein responses to N fertilization. Perkins2017, was the only site without a significant positive protein concentration response to an application. Perkins2017 was the location with the earliest date for the 63DAVD, which may have influenced this result. As seen in Figures 1-3, protein concentration increased with time. However, the increase in protein concentration was most dramatic when the timing of N application progressed past Feekes 8. Others found similar results regarding the increase of grain protein when the timing of the N fertilization was delayed (Flower et al., 1989; Woolfolk et al., 2002).

Just as with yield, the number of significant positive responses to N application was reduced when in-season treatments were compared to 0DAVD (Table 7). This indicates that delaying N application until first visual deficiency will likely produce higher protein values than when all N is applied pre-plant.

N Stress Recovery

While the visual differences between the pre-plant fertilized plot and the non-fertilized control was highly variable, the data documents that the N applications made at or shortly after the point at which N deficiency was visually evident, resulted in yields equivalent or greater than the pre-plant application in every environment tested in all four cropping seasons.

Nonetheless, the pre-plant application was statistically equivalent in yield when compared to the application made at the point of visual deficiency (trt 3) with the exception of (LCB2017a). In this site year, pre-plant yielded 0.53 Mg ha^{-1} less than the 0DAVD, 3.84 and 4.37 Mg ha^{-1} respectively. Recovery of yield equivalent to the pre-plant occurred seven times at 63DAVD, twice at 56DAVD, and one location at 42DAVD and 35DAVD each while the 0DAVD consistently out yielded the pre-plant. However, the ability of the crop to recover to equivalent levels of the 0DAVD after prolonged deficiency was similar to that of the pre-plant comparison. Eight locations documented full recovery until 63DAVD and one location at 56DAVD, 35DAVD, and 28DAVD.

While applying N after visual deficiency resulted in grain yields that were equal to or better than pre-plant until the point of Feekes 8, grain protein concentrations increased as application dates moved beyond Feekes 8, regardless of the date that deficiency developed. These results are not surprising, as protein accumulation begins approximately 10 days after Feekes 10.5.3 (Parker, 1985). In addition, final grain protein concentration can be greatly influenced by grain yield and berry size. Lollato and Edwards (2015) documented that in low yielding environments, the starch accumulation in the grains was reduced, resulting in a higher protein concentration in the grain. However, in environments where productive potential was substantially higher, a starch accumulation occured, thus significantly reducing the grain protein content. The extreme delaying on N resulted in a reduced final grain yield and a high N availability during Feekes 10.5.3.

Conclusions

The application of N and its timing had a significant effect on the yield and protein concentration of winter wheat. Conversely to the conventional thought, data from this study suggest that the agronomic optimum timing was not related with timing of N deficiency. Although a negative effect on grain yield was observed, it was only in the scenarios where N was applied after late March, which for this work corresponded to Feekes 8 growth stage. This study documented no negative impact on grain yield or protein concentration when N application was made after a deficiency had occurred. Noteworthy was that yield and protein values of the in-season N applicatoin were for all site years equal to or greater than that of the preplant treatments.

A significant finding of this work was that reaching maximum achievable yield levels was not related to the point in time at which the crop goes under N stress. Rather, optimum grain yield recovery was mostly associated with the N applications made when the crop was most vigorously growing, during Feekes 6 to 8 growth stages. These results were consistent across a diverse range of soils, cultivars, yield levels, and precipiation amount and distribution. Similar results were found in the literature where no major application timing effect was evidencied when application were performed up to Feekes 6 (Harris et al., 2016; Wallace et al., 2020).

Evaluation of N timing on protein concentration displayed a trend for increasing protein values with an increasing delay of application up to May, approximately Feekes 10. With the expectation of receiving a price premium for protein increase in the grain, and targeting the highest possible efficiency to reduce costs, it is possible for producers to make just one in-season aplication and manage for both higher yields and optimum protein content.

These results are significant for the winter wheat growers of the southern Great Plains as this research documented not only the ability, but also the necessity to move away from pre-plant and fall N applications for winter wheat grain production. The window for N application is likely much greater than most wheat producers would have ever considered. This work showed that not only could N be delayed and yield not sacrificed, but when delayed, yield will be maintained and protein concentration increased.

This study provides significant evidence that N management strategies that rely upon optical sensing technologies the area around an N-rich reference strip to become deficient, can be utilized to limit risk and minimize yield loss. The work also supports the strategy of yield prediction based upon NDVI measurements. Dhillon et al. (2020) reported that the optimum GDD > 0 needed to predict grain yield using NDVI from long-term fertility trials was between 97 and 112, or the Feekes 5 to Feekes 6 growth stages. The ability to delay N fertilization until after Feekes 6 allows for improved prediction of grain yield, determination of response to N, and more accurate recommendations of N.

The final conclusion of this project is that the timing of N application should not be based on the prescence of N deficiency. Rather the timing should be based on the weather and enviroment during application. While this project used NH_4NO_3 as its N source to limit the impact of N loss via volatilization, the primary source for in-season N in the region are dry urea and urea ammonium nitrate solution. Both sources have well documented loss due to volatilization. Providing evidence that the optimum application window is quite wide allows producers more flexibility to avoid those environments which will likley lead to N loss.

Further studies should take in consideration the timings of the applications in other environments and conditions as this study was limited to rainfed conditions of limited region in Oklahoma. Expanding the study into other environments, range of planting dates and cultivars would provide a more robust understanding of the influence of N application timing on grain yield and protein, and how producers should manage it.

TABLES

Table 1 Soil series classification, description, previous crop, planting date, seed rate and rainfall (September to August) for all experimental sites utilized in the study

Trial/Year	Soil Series and Description	Previous Crop	Planting Date	Cultivar	Seed Rate (kg ha^{-1})	Total Rainfall (mm)
Perkins2017	Teller; (fine-loamy, mixed, active, thermic Udic Agriustoll)	Wheat	10/13/2016	Bentley	84	997
Stillwater2017	Kirkland; (Fine, mixed, superactive, thermic Udertic Paleustolls)	Wheat	10/13/2016	Bentley	84	1003
LCB2017a	Port; (Fine-silty, mixed, superactive, thermic Cumulic Haplustolls)	Canola	10/12/2017	Double Stop	84	896
LCB2017b	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents)	Wheat	10/12/2017	Double Stop	84	896
Perkins2018	Konawa; (fine-loamy, mixed, active, thermic Ultic Haplustalf). Teller; (fine-loamy, mixed, active, thermic Udic Agriustoll)	Wheat	10/12/2017	Bentley	84	817
Lahoma2018	Grant; (Fine-silty, mixed, superactive, thermic Udic Argiustolls)	Wheat	10/12/2017	Bentley	84	1222
LCB2018a	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents)	Wheat	10/11/2017	Double Stop	84	937
LCB2018b	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents	Fallow	10/11/2017	Double Stop	84	937
Newkirk2019	Agra-Foraker (Fine, mixed, superactive, thermic Udertic Paleustolls)	Alfalfa	10/24/2018	Bentley	84	1390
LCB2019	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents)	Wheat	11/7/2018	Double Stop	84	1368
Newkirk2020	Agra-Foraker (Fine, mixed, superactive, thermic Udertic Paleustolls)	Wheat	10/18/2019	SY Monument	84	892
LCB2020	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents)	Wheat	10/15/2019	Double Stop	84	861

Table 2 Soil test results and nutrient concentrations, 0-15 cm for all experimental sites (Lake Carl Blackwell, Perkins, Lahoma, Stillwater) utilized in the study

Location/Year	pH	NO3--N	P	K
		……………….	mg kg^{-1}	……………….
Perkins2017	5.6	15.5	18	96.5
Stillwater2017	6.1	15.5	5	54
LCB2017a	5.9	15.5	12	62
LCB2017b	5.7	10.5	14	49.5
Perkins2018	5.6	13	12.5	71.5
Lahoma2018	5.6	10.5	25	139
LCB2018a	5.8	2.5	10.5	45.5
LCB2018b	6.2	8.5	16.5	75.5
Newkirk2019	7.1	8	24	107
LCB2019	5.3	14.5	110	373
Newkirk2020	7.7	12	56	482
LCB2020	6.3	38	98	494

Table 3 Treatment structure implemented at all locations. Fertilizer timing of in-season treatments was based on growing degree days after visual deficiency (DAVD).

Treatment	Fertilizer Timing	Nitrogen Rate (kg ha^{-1})
1	Pre-plant	100
2	Check	0
3	0 DAVD	100
4	7 DAVD	100
5	14 DAVD	100
6	21 DAVD	100
7	28 DAVD	100
8	35 DAVD	100
9	42 DAVD	100
10	49 DAVD	100
11	56 DAVD	100
12	63 DAVD	100

Table 4 Documented normalized difference vegetation index (NDVI) from the pre plant (treatment 1) and non-fertilized check (treatment 2) at visual differentiation in all site/years. Fertilizer applications and collection on NDVI initiated when the pre plant treatment within at least one block was visually determined to be greener or had more biomass than the non-fertilized check.

Location	Mean Pre	Mean Check	Mean Difference	Prob>F
Perkins2017	0.56	0.44	0.12	0.05
Stillwater2017	0.61	0.48	0.13	0.09
LCB2017a	0.61	0.55	0.07	0.01
LCB2017b	0.39	0.33	0.05	0.16
Perkins2018	0.64	0.54	0.10	0.09
Lahoma2018*	0.47	0.46	0.01	0.79
LCB2018a*	0.35	0.31	0.05	0.14
LCB2018b*	0.32	0.31	0.01	0.59
Newkirk2019	0.30	0.29	0.02	0.05
LCB2019	0.35	0.24	0.11	0.09
Newkirk2020	0.45	0.39	0.06	0.09
LCB2020	0.49	0.42	0.06	0.12

Prob>F demonstrates one-way ANOVA of NDVI by treatment at each site/year. For the trials of LCB2018b and LCB2020 the actual visual differentiation was noticed at treatment 5 and treatment 4, respectively.

Table 5 Dates for all pre-plant and in-season nitrogen (N) applications dates for the experimental sites (Lake Carl Blackwell, Perkins, Lahoma, Stillwater and Newkirk) utilized in the study evaluating the impact N fertilizer timing on winter wheat, conducted in north central Oklahoma over the 2016-2017 and 2017-2018 growing seasons.

Treatments	2	3	4	5	6	7	8	9	10	11	12
	Pre-plant	0DAVD	7DAVD	14DAVD	21DAVD	28DAVD	35 DAVD	42DAVD	49DAVD	56DAVD	63DAVD
Perkins2017	10/13/2016	11/10/2016	11/17/2016	11/24/2016	na	12/21/2016	12/29/2016	1/12/2017	1/23/2017	2/2/2017	2/12/2017
Stillwater2017	10/13/2016	11/10/2016	11/17/2016	11/24/2016	na	12/21/2016	1/2/2017	1/19/2017	1/30/2017	2/12/2017	2/20/2017
LCB2017a	10/12/2016	12/21/2016	1/9/2017	1/19/2017	1/30/2017	2/12/2017	2/20/2017	3/1/2017	3/9/2017	3/19/2017	3/27/2017
LCB2017b	10/12/2016	1/30/2017	2/12/2017	2/20/2017	3/1/2017	3/9/2017	3/19/2017	3/27/2017	4/4/2017	4/11/2017	4/19/2017
Perkins2018	10/12/2017	12/22/2017	1/23/2018	2/5/2018	2/19/2018	3/3/2018	3/11/2018	3/18/2018	3/26/2018	4/3/2018	4/12/2018
Lahoma2018	10/12/2017	12/21/2017	1/22/2018	2/1/2018	2/26/2018	3/6/2018	3/15/2018	3/23/2018	3/30/2018	4/6/2018	4/17/2018
LCB2018a	10/11/2017	12/22/2017	1/23/2018	2/5/2018	2/26/2018	3/6/2018	3/15/2018	3/22/2018	3/30/2018	4/9/2018	4/18/2018
LCB2018b	10/11/2017	2/5/2018	2/26/2018	3/6/2018	3/15/2018	3/22/2018	3/30/2018	4/9/2018	4/18/2018	4/26/2018	5/2/2018
Newkirk2019	10/24/2018	3/7/2019	3/15/2019	3/22/2019	3/28/2019	4/5/2019	4/12/2019	4/19/2019	4/29/2019	5/7/2019	5/14/2019
LCB2019	11/7/2018	3/8/2019	3/15/2019	3/22/2019	3/28/2019	4/7/2019	4/15/2019	4/22/2019	4/29/2019	5/7/2019	na
Newkirk2020	10/18/2019	2/4/2020	2/25/2020	3/3/2020	3/11/2020	3/23/2020	3/29/2020	4/6/2020	4/14/2020	4/21/2020	4/28/2020
LCB2020	10/15/2019	2/3/2020	2/21/2020	3/5/2020	3/12/2020	3/23/2020	3/29/2020	4/9/2020	4/16/2020	4/21/2020	4/28/2020

na = not applicable; Different colors in the table display different months.

Table 6 Winter wheat grain yield (kg ha^{-1}) and protein (%) as affected by the timing of application of 100 kg N ha^{-1} at all trials locations in Oklahoma in 2016-2017, 2017-2018, 2018-2019 and 2019-2020. Multiple comparison utilizing Dunnett's test (Pre-plant application treatment as a control) is demonstrated by the asterisks evaluating the effect of nitrogen application on winter wheat grain yield and protein. Boxes shaded pink represent grain yield or protein concentration which were statistically less than the pre-plant application, while the green shaded boxes represents significant increase in grain yield or protein.

		Treatments										
		1	3	4	5	6	7	8	9	10	11	12
		Check	0 DAVD	7 DAVD	14 DAVD	21 DAVD	28 DAVD	35 DAVD	42 DAVD	49 DAVD	56 DAVD	63 DAVD
Perkins2017	Yield	***	ns	ns	ns	na	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	na	ns	ns	ns	ns	ns	ns
Stillwater2017	Yield	*	ns	ns	ns	na	*	ns	ns	*	ns	**
	Protein	ns	ns	ns	ns	na	ns	ns	**	***	*	***
LCB2017a	Yield	***	**	ns	*	ns	*	***	***	***	***	***
	Protein	ns	ns	ns	ns	ns	ns	ns	ns	ns	***	***
LCB2017b	Yield	***	ns	ns	*	***	***	***	***	ns	ns	**
	Protein	ns	ns	ns	ns	*	ns	ns	***	***	***	***
Perkins2018	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	**	**	*	*	ns	***	*	***	***	***	***
Lahoma2018	Yield	***	ns	ns	ns	ns	ns	ns	ns	*	ns	*
	Protein	**	ns	ns	ns	ns	ns	ns	**	**	*	**
LCB2018a	Yield	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	**
LCB2018b	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	***	ns	ns	ns	ns	ns	**	**	***	***	**
Newkirk2019	Yield	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	*	ns	***	**	**	***	***	***	***	***	***
LCB2019	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	na
	Protein	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	na
Newkirk2020	Yield	***	ns	ns	ns	ns	ns	ns	*	*	***	***
	Protein	**	ns	ns	ns	ns	ns	*	***	**	***	***
LCB2020	Yield	***	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	ns	***	***	***	***	***

*Significance levels are indicated by the asterisks as: * is significant at the 0.05 probability level; ** is significant at the 0.01 probability level; *** is significant at 0.001of probability level; ns: not significant at 0.05 probability level;* na: not available; *For the trial of LCB2020 the actual visual differentiation was noticed at treatment 4.*

Table 7 Winter wheat grain yield (kg ha^{-1}) and protein (%) as affected by the timing of application of 100 kg N ha^{-1} at all trials locations in Oklahoma in 2016-2017, 2017-2018, 2018-2019 and 2019-2020 crop seasons. Multiple comparison utilizing Dunnett's test (treatment 3 application as a control) is demonstrated by the asterisks evaluating the effect of nitrogen application on winter wheat grain yield and protein concentration. Boxes shaded pink represent grain yield or protein concentration which were statistically less than the pre-plant application, while the green shaded boxes represents significant increase in grain yield or protein.

		2 Pre-plant	4 7 DAVD	5 14 DAVD	6 21 DAVD	7 28 DAVD	8 35 DAVD	9 42 DAVD	10 49 DAVD	11 56 DAVD	12 63 DAVD
Perkins2017	Yield	ns	ns	ns	na	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	na	ns	ns	ns	ns	ns	ns
Stillwater2017	Yield	ns	ns	ns	na	ns	ns	ns	ns	ns	*
	Protein	ns	ns	ns	na	ns	ns	**	ns	ns	***
LCB2017a	Yield	**	ns	ns	ns	ns	ns	**	**	*	ns
	Protein	ns	ns	ns	ns	ns	ns	ns	*	***	***
LCB2017b	Yield	ns	ns	ns	**	**	**	**	ns	ns	***
	Protein	ns	ns	ns	ns	ns	ns	*	*	***	***
Perkins2018	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	**	ns	ns	ns	ns	ns	ns	ns	ns	*
Lahoma2018	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
LCB2018a	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	ns	ns	ns	**	*
LCB2018b	Yield	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein	ns	ns	ns	ns	ns	**	**	***	***	**
Newkirk2019	Yield	ns	ns	ns	ns	ns	ns	*	***	***	***
	Protein	ns	**	ns	ns	ns	***	***	***	***	***
LCB2019	Yield	ns	ns	ns	ns	ns	**	ns	**	ns	na
	Protein	ns	**	ns	ns	ns	***	***	***	***	na
Newkirk2020	Yield (kg ha-1)	ns	ns	ns	ns	ns	ns	ns	ns	**	***
	Protein (%)	ns	ns	ns	ns	ns	ns	**	*	***	***
LCB2020	Yield (kg ha-1)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Protein (%)	ns	ns	ns	ns	ns	***	***	***	***	***

*Significance levels are indicated by the asterisks as: * is significant at the 0.05 probability level; ** is significant at the 0.01 probability level; *** is significant at 0.001of probability level; ns: not significant at 0.05 probability level;* na: not available; *For the trial of LCB2020 the actual visual differentiation was noticed at treatment 4*

FIGURES

Figure 1 Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Perkins, Stillwater, Lake Carl Blackwell (a) and Lake Carl Blackwell (b) at Oklahoma in 2016-2017

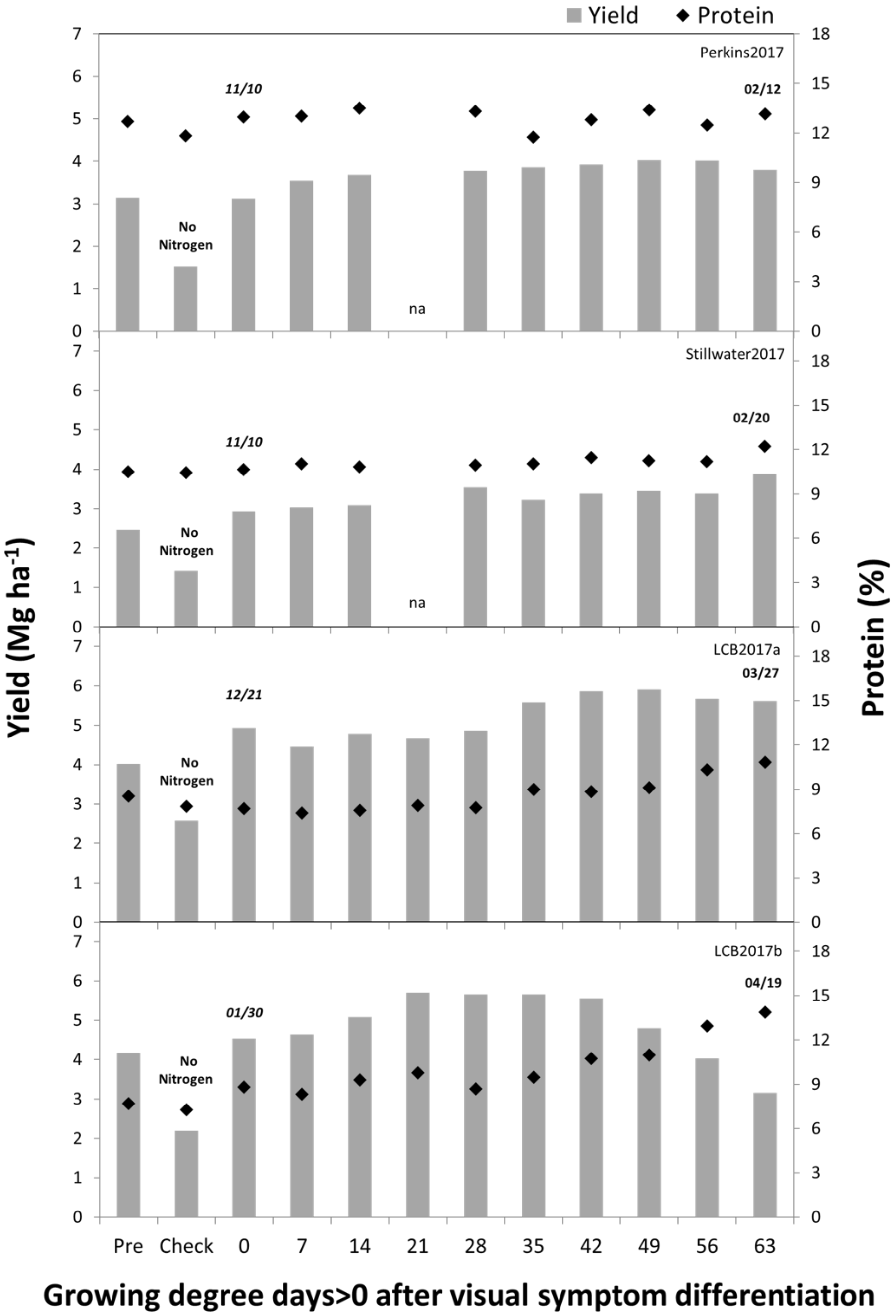


Figure 2 Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Perkins, Lahoma, Lake Carl Blackwell (a) and Lake Carl Blackwell (b) at Oklahoma in 2017-2018.

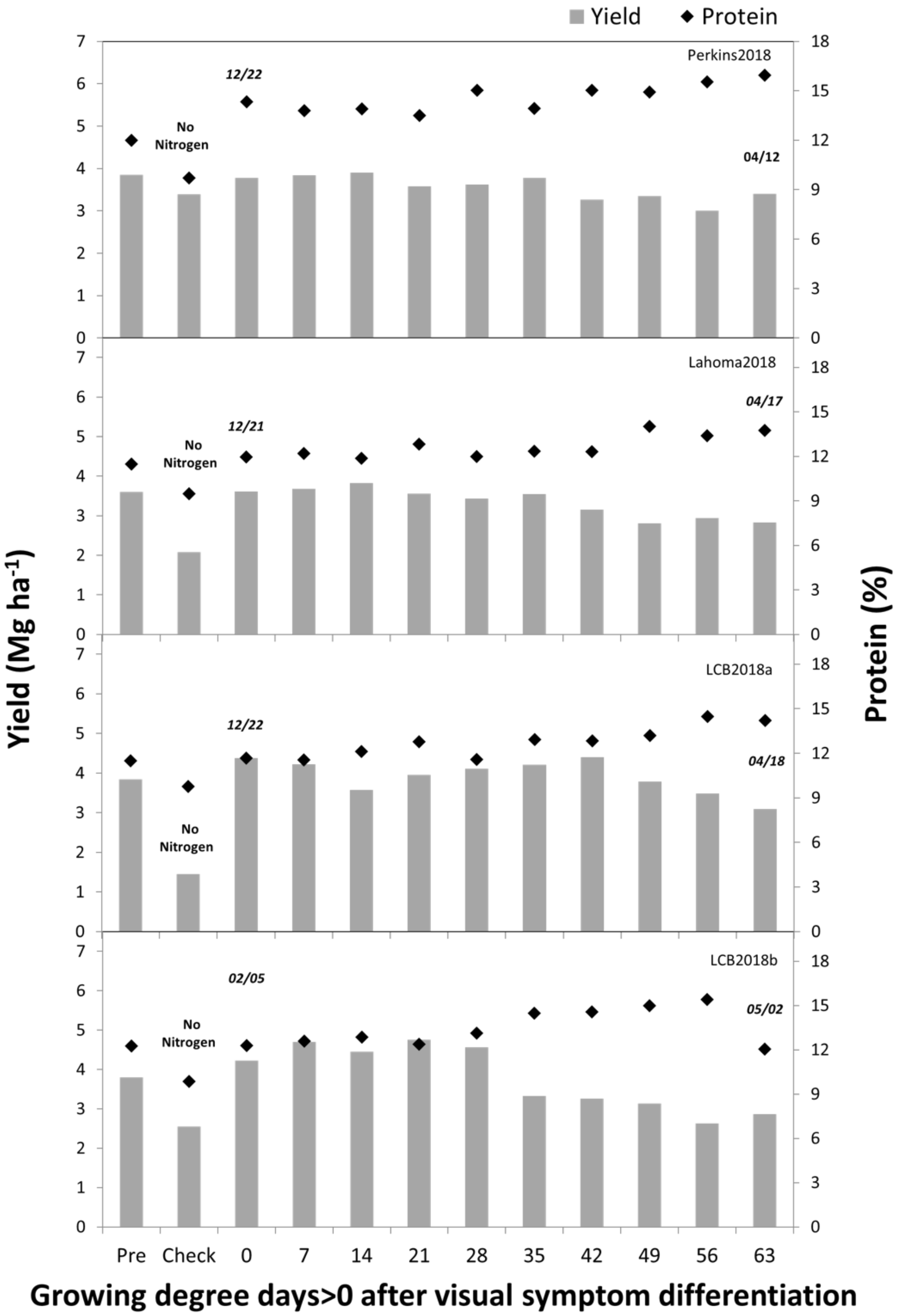
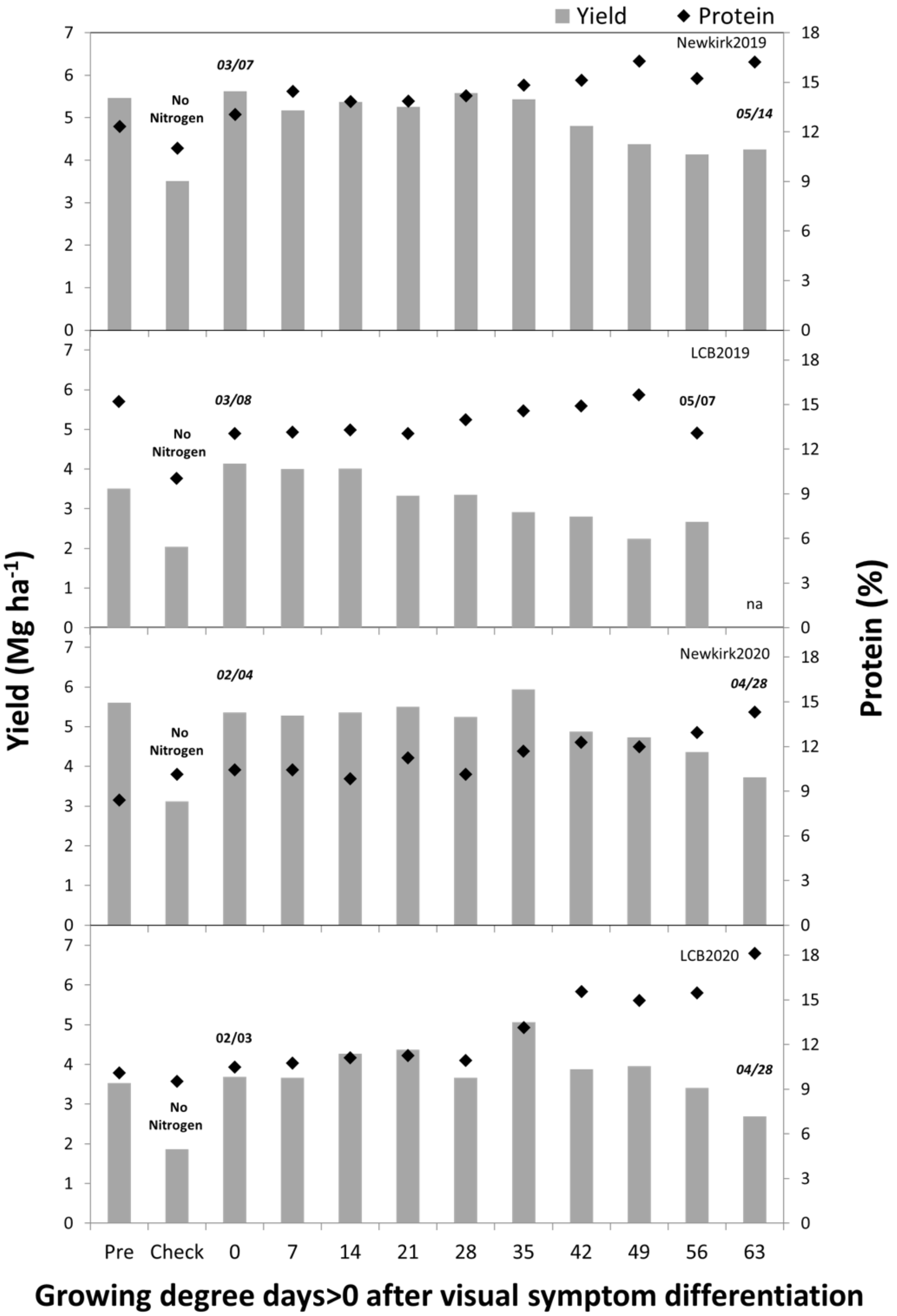


Figure 3 Winter wheat grain yield and protein response to the application of 100 kg N ha^{-1} as affected by the timing of application at Newkirk and Lake Carl Blackwell in Oklahoma in 2018-2019 and 2019-2020.



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CHAPTER II

IMPACT OF LONG-TERM FERTILIZER MANAGEMENT ON THE STRATIFICATION OF SOIL ATTRIBUTES UNDER NO-TILL

Abstract

The increasing cost of fuel and machinery used in conventional tillage systems makes no-till management (NT) an enticing alternative for small-grain producers. However, the addition of soil amendments to the soil surface under NT management has been shown to lead to the stratification of pH and nutrients near the soil surface. Therefore, our objective was to quantify the nutrient stratification across differing levels of nutrient management in three continuous no-till wheat long-term dry-land studies: Perkins, OK (NT since 2005), Stillwater, OK (NT since 2010) and Lahoma, OK (NT since 2010). Fields were sampled after the winter wheat (*Triticum aestivum* L.) harvest in the summer of 2019 at sampling layers of 0-2.54, 2.54-5.08, 5.08-7.62, 7.62-10.16, 10.16-12.7, 12.7-15.24 cm. Not all treatments of the studies were sampled but treatments which were sampled included an unfertilized check and combination of rates of nitrogen (N), phosphorus (P) and potassium (K) for each long-term trial location. Available P, and K, TN, OC, pH and exchangeable aluminum (Al_{KCl}) were quantified for each soil layer. It was found that stratification occurred for all soil attributes tested in the study, with P, K, TN, OC and Al_{kcl} being greater near the soil surface and pH being lower. Patterns of soil pH, TN, and OC stratification were generally related to the rate of annual N fertilization. The long-term addition of P and K fertilizers on the topsoil increases stratification and nutrient availability when compared to the non-applied plot. Although not at the same magnitude, non-fertilized plots were likewise stratified for the tested parameters, which indicates an isolated effect of the NT on the stratification rather than the fertilizer addition. Organic carbon and TN were highly stratified in the first 5 cm and little or no impact was noticed in deeper soil layers. Attention regarding sampling depth in NT areas is essential for accurate soil attributes monitoring and fertilizer recommendation.

Introduction

No-till management (NT) has increased in agricultural systems over the last 30 years. At the end of the last century, areas under NT represented 45 million ha worldwide, while in 2009 this number was estimated to be approximately 111 million ha (Derpsch et al., 2010). According to Claassen et al. (2018), wheat (T*riticum aestivum)* areas managed under no-till systems increased from 19.9% in 2004 to 44.6% in 2018 in US. The increased adoption of the conservational tillage system in agricultural areas brought attention of researchers to the subject and their impact in the soil system. No-till (planting with minimum mechanical soil disturbance) practiced together with organic mulch cover and crop rotation are known to be the main principles of conservation agriculture (Friedrich et al., 2012; Merten et al., 2015). The NT can be part of a sustainable solution for the increasing demand of food production and fertilizer efficiency giving its ability of soil nutrient cycling and organic matter (OM) enhancement in the long-term (Bot and Benites, 2005).

Among the advantages of NT when compared to the conventional tillage (CT) system, savings on labor, time, and fuel, thus being a more profitable economic activity (DeLaune and Sij, 2012; Derpsch et al., 2010). The reduction or elimination of tillage can also favor runoff and sediments loss reduction (Carretta et al., 2021; Merten et al., 2015). The effects of NT systems in farmlands also have been extensively investigated regarding soil physical proprieties. In the long-term, NT system adoption can increase soil aggregate stability and organic carbon (OC) accumulation in the aggregates when compared to CT systems in the arable layer (DU et al., 2013; Sithole et al., 2019). Increased soil aggregation due to OM enhancement favors the water infiltration and retention, thus, decreasing erosion potential in production areas (Komissarov and Klik, 2020; Langdale et al., 1979). Nonetheless, the OM distribution in NT systems may affect soil microbial community's vertical distribution, influencing the mineralization of nutrients, as well as their distribution and availability for plants (Schlatter et al., 2020). Conservation of soil water has been shown also to improve with adoption of NT under dry conditions compared to CT practices (Jin et al., 2009).

The surface nutrient application associated with the lack of incorporation of soil under NT also is a factor that lead to organic carbon (OC), nutrients and pH stratification in the soil (Crozier et al., 1999; Lupwayi et al., 2006). Other long-term field studies also reported significant stratification of P and K due to accumulation of these nutrients close to the soil surface in NT systems (Deubel et al., 2011; Houx Iii et al., 2011; Martínez et al., 2016; Noack et al., 2014).

Differences in soil attributes between tillage managements (NT vs CT) are variable across different environments. The length of time of a production system under no-till management, climatic characteristics and management conditions are recognized to influence in soil attributes (Abreu et al., 2011; Dang et al., 2015). In an experiment performed across eight locations in Oklahoma and Texas, six locations have shown increased OC accumulation within the first five years of NT management. However, greater differences in OC and TN were found in locations with ten or more years of NT management (Abreu et al., 2011). Others found similar patterns of carbon and nitrogen accumulation in long-term trials, with greater than ten years of management (Alvarez and Steinbach, 2009; de Moraes Sa and Lal, 2009). According to Soane et al. (2012) short-term or monoculture field experiment can produce misleading results. Thus, long-term data under different crop rotations and climates contribute continuously for the better understanding of the NT effects in the soil system and its sustainability.

Typical soil depths for characterization of soil chemistry range from 0-15 or 0-20 cm across most of the extension labs in US for agronomic crops, which corresponds to the soil arable layer (Baker et al., 2007; Barth et al., 2018). These sampling depths have been used to help farmers to determine fertilizer recommendation based on crop demand and bioavailability of nutrients. However, this sampling technique may fail to account for stratification in soil chemical attributes in no-till areas due to its effect on nutrient and pH distributions (Barth et al., 2018).

Although the effect of stratification was noticed in previous works (Grove et al., 2007; Sithole et al., 2019), there is still a lack of study related to the long-term nutrient management effects on the arable layer and its relationship with and soil nutrient distribution under NT. Nitrogen fertilization is well known to its effects on soil acidification and biomass accumulation (Alvarez, 2005; Barak et al., 1997; Wallace, 1994; Zhou et al., 2014). Although stratification of soil chemical proprieties in no-till and natural systems are well documented, different long-term experiments continue to allow for the evaluation of nutrient management effects on system stability and soil fertility. Nevertheless, the evaluation of nutrient management effect on these soil proprieties in a long-term perspective makes it possible to evaluate the fertilization impact in no-till systems. Thus, a better understanding of these impacts would help to maintain/increase yields, reduce production costs and improve fertilizer efficiency in production areas.

The objective of this study was to evaluate the long-term superficial fertilization effects on nutrient stratification and other soil chemical attributes of the arable layer in three different locations under NT across Oklahoma, US.

Materials and Methods

Study area

Our study was conducted during 2019 at three long-term NT trial location in Oklahoma (OK): Perkins (35°59'41.3"N, 97°02'32.7"W), established in 1996 and converted to NT in 2004; Lahoma (36°23'18.2"N, 98°06'27.3"W), established in 1970 and converted to NT in 2010; and Stillwater (36°07'19"N, 97°05'28"W), established in 1969 and converted to NT in 2010. Soil classification, average and range of rainfall, and average air temperature of each location are described in Table 8. Since their establishment, the long-term trials have been used for the conceptual development of the yield prediction and N, P and K responsiveness, and for the impact of continuous winter wheat changes in the soil system over time (Aula et al., 2016; Fornah et al., 2020; Raun et al., 1998; Raun et al., 2011). Although several treatments are used at each site, for this study not all were selected to evaluate the long-term effect on soil chemical attributes (Table 9).

Study design

In all locations, treatments were arranged in a randomized complete block design (RCBD) with three replications (n=3). Fertilizers were broadcast at pre-plant every year. In all locations N was applied as urea (46-0-0), phosphorus as triple super phosphate (0-46-0), and potassium as potassium chloride (0-0-60), respectively. Plots were 3.05 by 6.07 m with 3.05 m alleys between the replications.

Soil samples were collected from each plot after wheat harvest (June 2019) using a tubular probe (1.27 cm diameter by 30 cm length), and 25 cores from 0 to 15.24 cm depth was collected from each plot. Soil cores were separated into layers of 0-2.54, 2.54-5.08, 5.08-7.62, 7.62-10.16, 10.16-12.7, 12.7-15.24 cm using a knife and mixed to make a plot-composite sample. Soils samples were air dried until constant mass, and then ground with a hand roller to pass through a 2 mm sieve. Soil pH was determined with 1:1 soil to deionized water ratio by using an ion-selective (H+) glass electrode (McLean, 1983). Plant available phosphorus (P) and potassium (K) were extracted using Mehlich 3 (M3) (Mehlich, 1984) solution and determined using an inductively coupled plasma by atomic emission spectroscopy (ICP-AES) (SPECTRO Analytical Instruments Kleve, Germany). Soil organic carbon (OC) and total nitrogen (TN) were determined using a dry combustion carbon/nitrogen analyzer (CN 628, LECO Corporation, St. Joseph, MI, USA). Exchangeable Aluminum (Al_{KCl}) was determined by ICP-AES after extraction of 5 g of soil with potassium chloride (1.0 M KCl) at the ratio of 1:5 (Bertsch and Bloom, 1996).

Statistical analysis

Statistical analyses were performed using JMP, Version 16 (SAS Institute Inc., Carry, NC,1989-2022).(SAS_Institute, 2015). Treatments were differentiated at each stratified layer for all the analyzed soil attributes. Each site was treated as fixed effect and analyzed separately. Data were differentiated using ANOVA and least square difference to identify differences in means (Tukey test, $\alpha = 0.05$).

Results

The increased adoption of no-till in the last 20 years raises questions about the stability of such production system regarding nutrient management. Although stratification of soil chemical proprieties under no-till is well documented, long-term NPK fertility experiments allow the evaluation of the nutrient management effects on the system stability and soil fertility.

Due to the difference in treatments (fertilizer and rates) among locations, statistical analyses were performed by location. As shown in Table 10, fertilizer management was significant in all locations for all soil attributes tested in this study except for OC levels in Lahoma. The ANOVA also showed that soil layer had a significant effect on all soil chemical attributes with exception of Al_{KCL} in Lahoma. The non-significant effect in Lahoma could be explained by the high variance in the samples. Across all long-term locations, the stratification effect is present indicating the NT management resulted in the vertical stratification.

Lahoma was the only location where interaction between the nutrient management and soil layer was not significant for any of the soil chemical attributes. Interaction between the fertilizer management and soil layer was found for all attributes tested in Stillwater and Perkins with exception of K. These results demonstrate the influence of nutrient management on soil stratification of pH, OC, TN, P and K in the arable soil layer (0 to 15.24 cm) sampled in this study along the highly resolved (2.52 cm) depth increments.

Soil pH

Soil pH ranges of the Lahoma (pH = 5.0-7.0), Stillwater (4.5-6.5), and Perkins (4.6-7.3) locations differed in the points at where a stratification was observed (Figure 4). In all locations, treatments that received nitrogen fertilizer resulted in lower pH than the unfertilized check in the surface layers.

In Lahoma, the treatment 90-20-56 was the most acidified in all the soil layers. This treatment presented lower pH in all depths when compared to treatments without N application or with 22 kg N ha^{-1}. No significant differences were found in pH levels between 67 and 90 kg N ha^{-1} treatments at any depth. Both treatments with N rate of 67 kg ha^{-1} significantly acidified the soil in relation to the unfertilized control in the 0-7.62 cm soil layer. In the 7.62-10.16 soil layer, the treatment 67-00-56 presented lower pH than treatments with 0 N applied, while the 67-20-56 was not significantly different. When comparing the rates of 22 and 67 kg ha^{-1}, the treatment 67-20-56 presented lower pH in the 0-2.54 cm soil layer. Both treatments with rate of 67 kg ha^{-1} of N had lower pH in the 2.54-5.08 cm soil layer than the treatment 22-20-56. Lastly, differences were found for treatment 67-00-00 and 22-20-56 kg N ha^{-1} in the 5.08-7.62 cm soil layer.

In Stillwater, two levels of N fertilization (0 and 90 kg ha^{-1}) were evaluated (0-0-0, 0-29-37, 90-0-37, and 90-29-37). Both treatments with 90 kg N ha^{-1} demonstrated lower pH in all the soil layers when compared to the unfertilized control. When comparing treatments receiving 90 kg ha^{-1} of N with the 00-29-37, differences were found in the layers within the 0-10.16 cm. Even though numerical differences were observed in Figure 4 between treatments 00-00-00 and 00-29-37, no statistically significant difference was detected.

In Perkins, treatments receiving annual N application of 168 kg ha^{-1} had the pH significantly lower in all soil layers when compared to the unfertilized check and the 56-29-0 treatment. Same was found for treatments receiving 112 kg ha^{-1} with exception of the layer of 12.7-15.2 cm. In case of the N rate of 56 kg ha^{-1}, differences were found in the soil layer from 0-7.62 cm when compared to the unfertilized control. Nonetheless, rates of 168 kg N ha^{-1} also significantly decreased the pH in all soil layers when compared to the rate of 56 kg N ha^{-1}.

Evaluating treatments containing rates of 56 and 112 kg N ha^{-1} demonstrated some inconsistence across the soil layers. In the 0-2.54 soil layer treatments 56-29-00 and 56-00-00 were significantly higher in pH than the treatment 112-00-00. However, no difference in this layer was found between treatment 112-29-00 and treatments receiving 56 kg ha^{-1} of N. In the layer of 2.54-5.08 cm, treatments receiving 112 kg ha^{-1} of N had significantly lower pH than the treatment 56-29-00 but not when compared to the treatment 56-00-00. Both treatments receiving 56 hg N ha^{-1} differentiated from treatments receiving 112 kg ha^{-1} considering the soil layer of 5.08-7.62 cm. In the 7.62-12.7 soil layer, differences were found between treatments receiving 56 kg ha^{-1} of N and the treatment receiving 112-29-00. No difference between the treatments rates of 56 and 112 kg ha^{-1} were found in the 12.7-15.24 cm soil layer. Differences in pH were not likely influenced by the application of P at this location as no significant difference within the same N rate with exception of the 56-00-00 and 56-29-00 treatments within the 0-2.54 cm soil layer.

As expected, the stratification is more evident when comparing higher N application rates opposed to those receiving less or no N fertilizer. This highlights that N fertilization induces soil acidification. After applied on the soil, NH_4 from urea hydrolyses is oxidized to NO_3^- through nitrification, which releases 1 H^+ (Hao et al., 2020; Schroder et al., 2011). Soil acidification can then have negative effects on base saturation, aluminum saturation and microbial community (Barth et al., 2018; Crozier et al., 1999). The acidification potential of N fertilizers depends on rate and source. It is evident based on our results that surface broadcasting N fertilizer acidifies areas closer to placement. Our results and other studies demonstrates that this effect is still evident in long-term trials. Schroder et al. (2011) noticed that over a 30-year dataset, N rate was more influential towards the soil acidification than the N source. In our case, as it has been previously mentioned, greater rates applied across the years are increasing the soil acidification more than others. The effect on pH stratification is also present in the control treatments. This provides evidence on the effect of the crop residual accumulation and eventual N mineralization in the soil surface. When N, C and Sulfur (S) are mineralized from organic matter, there is a release of H^+ due the oxidation of organic matter, resulting in soil acidification (Bolan et al., 1991).

Winter wheat grain yield at the locations was increased with increased N fertilization (data not shown). Nitrogen was proven as the most limiting nutrient in all locations as the addition of N increased yield at a greater rate than other nutrients added (P for Perkins; K and P for Lahoma and Stillwater) (Eickhoff et al., 2019; Omara et al., 2019a). The increase in biomass and residues accumulation/decomposition due to N fertilization could also cause a greater stratification of soil as a consequence of the rapid organic matter decomposition. As N was the most limiting nutrient in the study locations, higher rates have a direct effect on biomass production and accumulation. Thus, resulting in greater stratification when N is applied. Crop residue accumulation on the topsoil and its decomposition appears to influence the pH stratification since unfertilized plots do not present such stratification patterns to the same extent. Results from Perkins also illustrates the crop residual accumulation role in acidification. When comparing treatments with no N fertilization or where the same rate of N was applied, the effect of P fertilizer addition increased yield and biomass accumulation, thus making the stratification and acidification more evident across different depths.

Phosphorus

The distribution of M3 P concentration in the soil profiles of the long-term studies in Lahoma, Perkins, and Stillwater is presented in Figure 5. In Lahoma, except for the 0-2.5cm depth the 00-20-56 treatment had the greatest concentration of P throughout the soil profile. In the surface layer the 67-20-56 treatment had the greatest P concentration and both of the previous mentioned treatments contained P levels which were significantly greater than all other treatments. In the 0-5.08 cm soil layer 67-20-56 and 00-20-56 161 and 137 mg kg^{-1} respectively while the 0-0-0 check had a P concentration of 56 mg kg^{-1}. At this site the 67-0-56 treatment had the lowest concentration at all depths analyzed. In the surface 2.5cm this treatment's P concentration was 50 mg kg^{-1}. For all depths below the surface the 0-20-56 treatment maintained significantly greater P concentration than the 67-0-56. From 2.5 to 12.7 depth the 0-20-56 P concentration was significantly greater than 0-0-0, 67-0-56, and 90-20-56. In the 5.08-7.62 cm soil layer, the treatment 00-20-56 was greater than all other treatments with the exception of 67-20-56. At this depth the 67-20-56 treatment P concentration was significantly greater than 0-0-0, 67-0-56, and 90-20-56. In the same layer, differences were found between the treatment 22-20-56 and the 67-00-56. In the 7.62-10.16 soil layer the treatment 67-20-56 was higher when compared to the treatments 90-20-56 and 67-00-56.

In Stillwater, treatments receiving P fertilization had significantly higher P levels in all soil layers with exception for the 12.7-15.24 cm. Thus, the P fertilization increased P soil levels when compared to other treatments. In the 12.7-15.24 cm soil layer, the treatment 00-29-37 had significantly higher P than the treatments 90-00-37 and unfertilized check, however, the treatment 90-29-37 did not differentiate from any other treatment in this soil layer. The data also makes possible to observe that although only numerically, higher N fertilizer rates resulted in overall decreased soil P levels.

In Perkins, P fertilization also had an impact on soil P. The highest P levels were found in the treatments 112-29-00 in the top 10.15 cm of the soil profile while 56-29-00 had the highest P level from 10.15-15.24. At no point was there a significant difference between the P values of these two treatments. At all depths all three treatments which received fertilizer P were statistically greater than those that did not. At all depths there was no significant difference in the soil test P levels of the treatments which did not receive P fertilizer, with the exception that the 00-00-00 check and 168-29-00 where not statistically different at a depth of 12.7-15.24. In the 0-2.54 cm layer 56-29-00 and 112-29-00 had significantly greater P levels than 168-29-0. In the

2.54-5.08 cm soil layer, no differences were found between treatments 112-29-00 and 56-29-00 treatments nor the 56-29-00 and 168-29-00. However, 168-29-00 soil test P values were significantly greater than the 168-29-00. From 5.08-12.7 cm differences were not found among treatments 168-29-00, 56-29-00 and 168-29-00. Lastly, in the 12.7-15.24 cm soil layer, treatments 56-29-00 and 112-29-00 had significantly higher soil P than the 168-29-00 treatment.

The results show that M3 P was stratified in all study locations (Table 10). Interactions for P were found for the locations of Stillwater and Perkins. Treatments with the highest M3 extractable P in each location were 110, 173 and 121 mg kg^{-1} greater in the 0-2.54 cm than in the 12.7-15.24 cm soil layers at Lahoma, Stillwater and Perkins, respectively (Figure 5). This demonstrates that broadcasting the P fertilizer can significantly stratify the extractable P in the soil under NT. Treatments without any P addition also demonstrate a numerical stratification pattern in all locations. Non-fertilized controls (00-00-00) P levels ranged from 61, 66 and 44 mg kg^{-1} in the 0 to 2.54 cm soil layer to 17, 3 and 12 mg kg^{-1} in the 12.7 to 15.24 cm soil layer in Lahoma, Stillwater and Perkins, respectively. This indicates that the lack of incorporation of crop residues have influence in the NT stratification effect. The accumulation and further mineralization of crop residues from previous years as well as the increasing of OC in the soil surface also had an impact on P stratification, not only the broadcasted fertilization.

Potassium

In Lahoma, the highest K values were found for the treatment where K fertilization was performed every year and no N was applied, 00-20-56 (Figure 6). This treatment was significantly greater than the un-fertilized check at all depths. The 00-00-00 treatment had the least amount of soil test K at all layers. In the 0-2.52 cm soil layer, treatments 00-20-56, 67-20-56 and 67-00-56 had significantly higher K values than the unfertilized check. No differences in that layer were found between the unfertilized check and treatments 90-20-56, and 22-20-56. In the

2.52-5.08 cm, treatment 00-20-56 presented higher K levels than treatments 22-20-56, 90-20-56 and the unfertilized check. In the 5.08-7.62 cm soil layer, the treatment 00-20-56 presented greater means than all the other treatments. Treatments 67-00-56, 67-20-56 and 22-20-56 also presented higher means than treatments 90-20-56 and the unfertilized check. In the 7.62-10.16 cm soil layer treatments 00-20-56 and 67-00-56 also presented significantly K levels than the unfertilized check and treatment 90-20-56. In this layer, the treatment 67-20-56 also presented significantly higher K levels than the treatment 90-20-56. In the 10.16-12.7 cm, treatment 00-20-56 have higher K values than treatments 90-20-56, 22-20-56 and unfertilized control. Treatment 67-00-56 also presented higher K levels than the treatment 22-20-56 and the unfertilized control. The treatment 67-20-56 also presented significantly higher K levels than the unfertilized control. In the layer 12.7-15.24 cm, treatments 00-20-56 and 67-00-56 significantly presented higher K levels than the unfertilized control. No other significantly differences were found in this layer.

In Stillwater, all treatments follow similar trends across the soil profile. The highest K values were found for treatments 00-29-37 and 90-0-37, respectively, and the lowest, unfertilized check and 90-29-37, respectively. The 00-29-37 was the only treatment to be statistically greater than the 00-00-00 at all depths. In the 2.54-5.08 cm soil layer, all other treatments (90-00-37 and 90-29-37) were significantly higher in K than the unfertilized check. For the soil layers from 5.08 to 15.24 these treatments where not statistically greater than the 00-00-00.

In Perkins, no K fertilization was performed in any of the treatments. The only difference found across all soil layers was in the 0-2.54 cm soil layer where the unfertilized control presented higher K levels than the treatment 56-00-00. It is important to emphasize the noticeable N fertilization had a numerically impact on the soil K levels since the unfertilized control presented the highest K levels in the soil layers found between 0-12.7 cm.

As was observed for P, K was stratified in the study locations and the long-term nutrient management had a significant effect on K distribution. Figure 6 demonstrates that M3 extractable K was significantly greater in the uppermost sampled layers for the same treatments at each location. However, differences among the treatments for the same soil layer were not always significant. Perkins was the only location where treatments differentiated in K soil contents in the 0-2.54 cm soil layer. Since none of the treatments in Perkins had K applications and the unfertilized check had higher levels of K, it is possible to assume that the N and P fertilization had an effect on K soil levels in the long-term. Apparently, the increase of N and P fertilization increases crop demand for K and further K grain exportation, decreasing soil test K levels in the long-term.

In Stillwater, although a numerical difference is noticed no significant effects of the treatments was observed in any of sampled soil layers. Numerically, the levels of K increased in comparison to the unfertilized check demonstrating a build-up effect of the continuous long-term K fertilization. It is also important to notice that the fertilization was broadcasted in the soil surface and K^+ cation in the soil is relatively immobile. However, after 9 years of K surface application, it is possible to see that K is numerically increasing in all the stratified depths investigated, demonstrating a certain mobility. Nonetheless, treatments that received N and K fertilization increased the K levels above the unfertilized check. However, K levels are lower in treatments that received N. As mentioned before, this illustrates that N can increase the crop demand for K.

In Lahoma, all treatments received a higher K rate than other locations (56 kg ha^{-1}) with exception of the unfertilized control. This illustrates numerical differences in K levels across all locations (Figure 6) and entices the build-up effect. None of the treatments in Perkins (lowest K soil levels) received K fertilization and, in Stillwater, the applied rates were 0 and 37 kg ha^{-1}. Since the Figure 6 is displayed with the same range of K values (*y-axis*), it is possible to observe that higher K rates are increasing K soil test levels.

Lahoma presented a lower soil K content when there was N and K fertilization than only K. The difference in the K contents between treatments is apparent in all layers although they are not always significant. For the treatments used in this study, there was still a difference in the deepest layer studied for Lahoma, which indicates a K stratification is possible at lower soil depths. These results suggest that in the long term higher N rates can lead to a higher need of K rate to maintain initial (or desired/adequate) soil levels.

Since the establishment of the trials in Lahoma, Stillwater, and Perkins, the successive application of P and K fertilizer increased their bioavailable contents in soils. This indicates that rates applied were higher than the crop uptake and removal. The data also suggest a reduction in soil P and K when N is applied at higher rates demonstrating that the N:P:K relationship might influence plant uptake.

According to Grove et al. (2007), P and K fertilizer placement has been much more studied in the literature than liming. Results suggest that K is strongly stratified in most of the NT production systems areas due to organic matter surface deposition. As shown in our study, there is an increase in K contents and K stratification when K fertilizer is applied. A more pressing concern may be related to the fact that N fertilization decreases bioavailable P and K due to the higher biomass accumulation thus increasing the P and K uptake and reducing their soil testing values (Wright et al., 2007). It is also possible that due to higher uptake and further grain exportation, the effect of N fertilization on soil organic matter and pH is indirectly reducing the available contents of those nutrients in the soil.

Organic Carbon and Total Nitrogen

According to Figures 7 and 8, the impact of the nutrient management on TN and OC was mostly found in the first soil layer (0-2.54 cm) regardless of the location. The differences across treatments are primarily influenced by N application followed by P and K rates. The observable increase in OC and TN values was correlated to surface application of N fertilizer. It is also hypothesized that the stratification is increased with increasing N rate because of N being the most limiting nutrient in these studies. It is assumed that higher N rates increase the response to crop biomass production and further accumulation in the soil surface through the years, not impacting the deeper layers the same degree as the surface.

In Lahoma, the highest levels of TN and OC levels in the 0-2.54 cm layer were found in the treatments 67-20-56 and 90-20-56, (1.54 and 1.38 % OC; 0.15 and 0.14 % TN, respectively). However, the OC/TN levels for treatment 67-20-56 was only greater than the treatments not receiving N, the unfertilized control and 00-20-56 (0.99 and 1.01 % OC; 0.11 and 0.11 % TN, respectively).

From 2.54-10.16 cm and 12.7-15.4 no statistical difference was found in the OC values of any of the treatments. In the 10.16-12.07 cm layer, in the OC value of 00-20-56 (0.69 % OC) was less than that of 67-20-56 and 67-00-56 (0.76 and 0.75 % OC, respectively). While there was little statistical difference below the surface 2.54 cm the trend of the 67 kg N ha^{-1} treatments having the highest levels of OC was maintained to depth. Much like OC the TN of 67-00-56 (0.11 %TN) within the 2.54-5.08 cm soil layer, was statistically higher than the 00-00-00. In the 5.08-7.62 soil layer, treatments receiving 67-00-56 and 67-20-56 (0.10 %TN) were statically higher than all the others (0.09 % TN or less). No difference was found in the 7.62-12.7 cm considering TN. However, in the layer 12.7-15.24 cm, the treatment 67-00-56 (0.11 % TN) was higher than the unfertilized check (0.09 % TN) and the 90-20-56 (0.08 %TN). For the treatment 90-20-56 the TN was also lower than the 67-20-56 (0.10 % TN) in the deepest sampled layer.

In Stillwater, highest levels of TN and OC were also associated with increased N fertilization. Treatments containing N application presented numerically higher TN and OC than treatments without N fertilization. Among the four treatments, differences were only found between the unfertilized check (00-00-00, 1.07% OC), and the 90-29-37 (1.41 % OC), and 90-00-37 (1.37 %

OC) in the 0-2.54 cm soil layer. The treatment 00-29-37 was not different from any other treatment. This particular treatment that have only the P and K addition, and the biomass increase numerically above check but not enough to be significantly different. In the 5.08-7.62 and the 10.16-12.7 cm soil layers, OC was significantly higher for the 90-29-37 treatment (0.83 and 0.78 % OC, respectively) than the unfertilized check (0.76 and 0.72 % OC, respectively).

Although for most soil layers TN was higher on treatments receiving N applications, differences were only found in the 0-2.54 where the treatment 90-29-37 (0.17 %TN) was higher than the 00-29-37 (0.14 % TN) and the unfertilized check (0.12 % TN). The treatment 90-00-37 (0.15 % TN) was also higher than the unfertilized check but not from the 00-29-37 treatment.

In Perkins, the addition of N and P had a significant impact on the soil OC in the 0-2.54 cm soil layer. The highest OC was found when N and P was applied at the highest rate (168-29-00, 1.93% OC) and this treatment was significantly higher than the others with exception of the treatment 112-29-00 (1.74 % OC). These two treatments also presented the highest OC across all locations. This effect might be due to the higher N and P rates applied in Perkins. The treatment 112-29-00 also presented higher OC than the 56-00-00 (1.24 % OC), 112-00-00 (1.2 % OC) and the unfertilized check (1.13 % OC). Phosphorus fertilization appears to increase the biomass production above the N limitation in this area. The treatments 168-29-00 and 112-29-00 were statistically higher in OC than all treatments that did not received P application, illustrating the P fertilization response in OC. Nonetheless, the 112-29-00 treatment was numerically higher in OC than the treatment 168-00-00 (1.41 % OC) which presented a higher rate of N. No statistical difference in OC was found in the other soil layers.

Although numerical differences were evident in TN in Perkins in the 0-2.54 cm soil layer, the only significant difference was found between the treatment 112-29-00 (0.17% TN) and the 168-00-00 treatment (0.11 % TN). In the 2.54 to 5.08 cm soil layer, the lowest TN was found for the treatments that received the highest N rates (168 kg ha^{-1}). These two treatments presented lower TN than all other treatments with exception of the 56-00-00 (0.07 % TN). In all other layers, these two treatments had also the lowest TN and the unfertilized control the highest. We assume that these higher N rates produce higher root growth and consequently increased N uptake, thus depleting TN in depth due to the higher residue translocation to the surface. Higher N rates are accumulating OC and TN in the surface and depleting TN in the deeper layers. The fertilization in Perkins, differently than the other areas, did not demonstrate an increase in TN, when fertilization was performed, for the soil layer of 5.08 to 15.24 cm.

Nonetheless, the treatments with rate of 168 kg ha^{-1} have the lowest pH (Figure 4) in the 0-10.16 cm soil layer followed by the same rate with P fertilization. Thus, these treatments present highest Al_{KCl} concentration, as will be discussed later. Briefly, the high rate of N fertilization resulted in higher Al concentrations than the other treatments. Lastly, as displayed in Figure 6, high N fertilization is decreasing bioavailable K in the soil. Since K fertilization is not performed in Perkins, K depletion could be a factor interfering in the TN in this area.

Exchangeable Aluminum

As described in Figure 9, Al_{KCl} was analyzed in all location for the studied soil layers. In Lahoma, Al_{KCl} was numerically greater in the treatment receiving 90 kg ha^{-1} of N. However, no significant differences were found between the treatments across the studied soil layers. Nonetheless, patterns of Al_{KCl} stratification appear to differ among the locations. In Lahoma, the highest Al_{KCl} concentration was found in the 7.62-10.16 cm soil layer, differently from the other locations of the study where the highest concentration was found in the 2.54-5.08 cm soil layer. The increase on Al_{KCl} at depth can be explained by Schroder et al. (2011) in a neighboring study in the same experiment station. The authors demonstrated that N rates that were not in excess of the crop uptake (68 kg ha^{-1} or less) took much longer to acidify the soil.

Our results agree well with this concept in that the two N rates that were less than the agronomic rate for wheat (i.e., the 34 and 68 kg N ha^{-1} rates) resulted in soil pH that was less acidic because these rates were not in excess of the crop uptake rates.

In Stillwater, the rate 90 kg ha^{-1} (highest N rate treatment analyzed) also presented greater Al_{KCl} values than other treatments. It was also noted that P fertilization numerically increase Al_{KCl}. However, the effect did not produce statistical significance within any of the soil layers when comparing treatments with the same N rate. In the soil layers within 0-7.62 cm, significant differences were found between the treatment 90-29-37 and treatments not receiving N fertilization (00-00-00 and 00-29-37). In the 7.62-10.16 cm soil layer, the treatment 90-29-37 was greater than the check but not from the 00-29-37 treatment. However, when comparing the treatment 90-00-37 with treatments without N fertilization, significant differences were only found in the 2.54-5.08 and 5.08-7.62 cm soil layers.

In Perkins, the relation between N rate and Al_{KCl} was evident and the impact is more aggravated in the surface layers. Rates of 168 kg ha^{-1} presented higher Al_{KCl} than the unfertilized control in the 0-2.54 and 2.54-5.08 cm. The treatment 168-00-00 also recorded greater Al_{KCl} in the 5.08-7.62 cm soil layer than the unfertilized control. Treatment 168-00-00 also demonstrated higher rates of Al_{KCl} than treatments receiving 56 kg ha^{-1} in the 0-2.52 and 2.52-5.08 cm soil layer. The 168-00-00 treatment had the greatest Al_{KCl} from the surface to the depth of 12.7 cm. This treatment was significantly greater than the 00-00-00 to the depth of 7.62 cm, then beyond that layer there was no impact of treatment upon Al_{KCl} values. In the surface 2.52 cm the 168-29-00 and 112-00-00 were also significantly higher than the check; however, below this point there was not statistical difference between these treatments. It is important to notice that in this location the addition of P fertilizer within the same N rate did not have a significant effect in any of the soil layers.

Discussion

Phosphorus and Potassium fertilization effect on soil Phosphorus and Potassium

It is noticeable on Figure 5 that the M3 extractable P is highly influenced by the rate of P application. Across all locations, treatments that received annual fertilization of P presented greater P levels than the treatments that did not receive any P fertilization. Similarly, locations that received K fertilization (Lahoma and Stillwater) had greater soil K levels than treatments not receiving K fertilization. This increases the build-up effect in production systems due to annual over applications of P and K fertilization (Buresh et al., 1997; Liu et al., 2010). However, whenever fertilization is surface applied and no soil mechanical mixing is performed, stratification of nutrients becomes more evident in the upper layers (Figure 5 and 6) (Scheiner and Lavado, 1998).

Nitrogen effect on soil Phosphorus and Potassium levels

The influence of N fertilization in the soil P and K levels is also noticeable (Figures 5 and 6). Treatments that received greater N rates generally present lower P and K soil concentrations indicating an antagonist effect of N fertilization. In the case of P, this can be due to the soil acidification caused by N fertilization and higher crop P utilization and further exportation. Potassium soil levels are unlikely to be influenced by the soil pH but is likely to be influenced by crop grain exportation and soil surface residue deposition across the years. Nitrogen has been demonstrated as the most limiting nutrient among the nutrients tested (N, P and K) (Eickhoff et al., 2019; Lollato et al., 2019). Increased crop biomass and yield is expected to happen whenever N is applied. The hypothesis is that the N application rate positively affected the uptake and removal of P and K (Fornari et al., 2020; Thiraporn et al., 1992), decreasing P and K soil levels in the long-term.

Soil pH as affected by Nitrogen fertilization

Surface layers (0 to 5.08 cm) documented the lowest pH at all locations (Figure 4), with values ranging from 4.4 to 6.5 which often fell below the optimum of 6.5 to 7 for most essential nutrient's availability (Penn and Camberato, 2019). Organic matter mineralization, rainfall and N surface fertilization with urea are hypothesized to be the cause of surface acidification (Helyar and Porter, 1989). However, our results demonstrate that N fertilization affecting soil acidification is more evident. Although treatments not receiving N fertilization presented pH stratification at some degree, soil acidification increased proportionally to the N rate across all locations (Schroder et al., 2011). Yet, while the impact of soil pH on P availability is well documented, the highest M3 P levels were also found in those surface layers. This demonstrated that surface applications of P fertilizers overcome P fixation by aluminum and iron in acid soil conditions (Duiker and Beegle, 2006).

Organic matter influence on Aluminum and Phosphorus

As observed in Figure 9, Al_{kcl} levels rapidly increase when soil pH drops below around 5.3(Gillespie et al., 2021). High Al_{KCl} can fix inorganic available forms of P in the soil consequently decreasing its availability P to the crop (Ch'ng et al., 2014). Nonetheless, aluminum toxicity is a recurrent cause of crop failure in extreme acid soil in Oklahoma (Schroder et al., 2011) due to its effect on root growth and above ground biomass. At the same time that N fertilization acidifies the soil, it also contributes to the highest increments on yields and crop biomass production and accumulation, increasing the organic matter in the surface soil (Reicosky et al., 1995). Our data evidence the increase in OC, however, the effect is mostly noticed in the 0-5 cm soil layer (Figures 7 and 8). Our results also demonstrated that Al_{KCl} concentrations are lower in the surface soil layer (0-2.54 cm) than in the layer below soil surface (2.54-5.08 cm) (Figure 9). The hypothesis is that high OC and P content in the 0-5 cm layer could contribute to lowering Al_{KCl} concentration in the 0-2.54 cm soil layer. Greater OC, as a consequence of greater organic matter concentration, are additional sources of negative charges in the soil (CEC) contributing to the Al_{KCl} decrease in the uppermost soil layer. Thus, greater organic matter content and relatively increased microbial activity could have higher P mineralization rates, increasing P availability in this soil layer (Moro et al., 2021) even at low pH that are known to be detrimental to P availability to the crop.

OSU soil sample depth recommendation

In Oklahoma crop P requirements and fertilizer recommendations are based in soil test values combined with a sufficiency level rather than yield goals due to its relative immobility in the soil. For small grains, P level of 100% sufficiency is equivalent to 32.5 mg kg^{-1} or more ; and 45% sufficient is equivalent to 20 mg kg^{-1} (Zhang et al., 2017) . Oklahoma State University recommends soil sample depth of 15 cm for small grains. Due to the high stratification caused by NT and surface fertilization, little or no fertilization (100 to 90 % sufficiency index) would be required in the 0-2.54 soil layers for four of the six treatments in Lahoma, three of the four in Stillwater, and three of the seven in Perkins. However, at the deepest soil layer analyzed (12.7-15.2 cm), which is still within the OSU soil depth recommendation, P fertilizer would be necessary for all the treatments. At this layer, from the 17 treatments analyzed across all locations, nine would be below 45% sufficiency (20 mg kg^{-1}) and all treatments would require fertilization. Similarly for P, soil K concentration and pH levels would also be impacted by the soil sampling depth changing lime and K fertilizer requirements in NT areas. Failing to ensure proper soil sampling depth may affect the P and K fertilizer, as well as, lime requirement in NT increasing the production cost significantly, if soil sample depth were deeper, or decreasing productivity and efficiency if shallower.

Conclusion

Nutrient and pH stratification were observed in all locations and it is mainly related to N being the most limiting nutrient. With the adoption of NT, stratification becomes more pronounced due to less soil mixing, surface application of fertilizers and surface residue deposition. Our data suggests that soil nutrients stratification is still evident after 9 years of NT (Lahoma and Stillwater) and 14 years of NT (Perkins). Nitrogen fertilization is acidifying and decreasing soil available P and K proportionally to the annual applied N rate. Greater OC and TN were also mostly dependent on the N fertilization. Exchangeable Aluminum is mostly influenced by the N fertilization effect on soil pH, however, greater OC contents in the soil surface decreased Al_{KCl} concentrations even at lower pH. The annual application of P and K fertilizers increased the soil P and K availability. However, soil P and K levels increased more close to the fertilizer placement.

The soil sampling to determine fertilizer recommendations under NT should be evaluated as the stratification in the arable area is well pronounced as showed in this study. This study demonstrates the importance of monitoring long-term soil nutrient changes for the better soil fertility management practices. For most of the soil chemical attributes tested, the sampling depth would highly influence the fertilizer recommendations. This study demonstrates that NT fields require even more attention to sampling depth to optimize fertilizer efficiency.

TABLES

Table 8 Trial location, soil series classification, year of establishment, rainfall and temperature for three study sites used in this study. Averages retrieved from 1994 to 2018.

Location	Soil series and Description	Establishment	No-till Establishment	Annual Avg. Rainfall (mm)	Range (mm)	Mean Annual temperature (°C)
Lahoma, OK	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents	1970	2010	771	503-1314	15.6
Stillwater, OK	Kirkland; (Fine, mixed, superactive, thermic Udertic Paleustolls)	1969	2010	922	606-1493	15
Perkins, OK	Konawa fine sandy loam; (Fine-loamy, mixed, thermic, Ultic Haplustalfs) Teller; (fine-loamy, mixed, active, thermic Udic Agriustoll)	1996	2005	879	563-1301	15.9

Rainfall and temperature data retrieved from the Oklahoma Mesonet.

Table 9 Treatments (nutrient management) used in the study.

Location	**Treatmets**						
	kg ha^{-1} of N-P-K						
Lahoma	0-0-0	00-20-56	22-20-56	67-00-56	67-20-56	90-20-56	
Stillwater	00-00-00	00-29-37	90-0-37	90-29-37			
Perkins	00-00-00	56-00-00	56-29-00	112-00-00	112-29-00	168-00-00	168-29-00

Treatments are described as kg ha^{-1} of applied nitrogen (as urea), phosphorus (as triple super phosphate), and potassium (as potassium chloride), respectively.

Table 10 Analyses of variance test of main effects and interaction of nutrient management (treatment) and soil layer on soil chemical attributes in three long-term no-till following winter wheat studies in Oklahoma, US. Statistical significance demonstrates stratification effect in each of the soil attributes evaluated.

Factor	pH	TC	TN	P	K	Al_{KCL}
	---------------------Lahoma---------------------					
Nutrient management	***	ns	**	***	***	***
Soil layer	***	***	***	***	***	ns
Nutrient management x soil layer	ns	ns	ns	ns	ns	ns
	---------------------Stillwater---------------------					
Nutrient management	***	***	***	***	***	***
Soil layer	***	***	***	***	***	***
Nutrient management x soil layer	ns	***	**	***	ns	***
	---------------------Perkins---------------------					
Nutrient management	***	***	***	***	***	***
Soil layer	***	***	***	***	***	***
Nutrient management x soil layer	*	***	***	***	ns	*

* Significance at $p \leq 0.05$ level; ** Significance at $p \leq 0.01$ level; Significance at $p \leq 0.001$ level; ns: not significant at 0.05 level.

FIGURES

Figure 4 pH from stratified soil samples as a function of fertilizer application in three long-term no-till trials. Colored lines are treatments. Horizontal bars indicate the Honest Significant Difference (HSD), ($p \leq 0.05$). Stratification of pH is mostly related with the nitrogen rate.

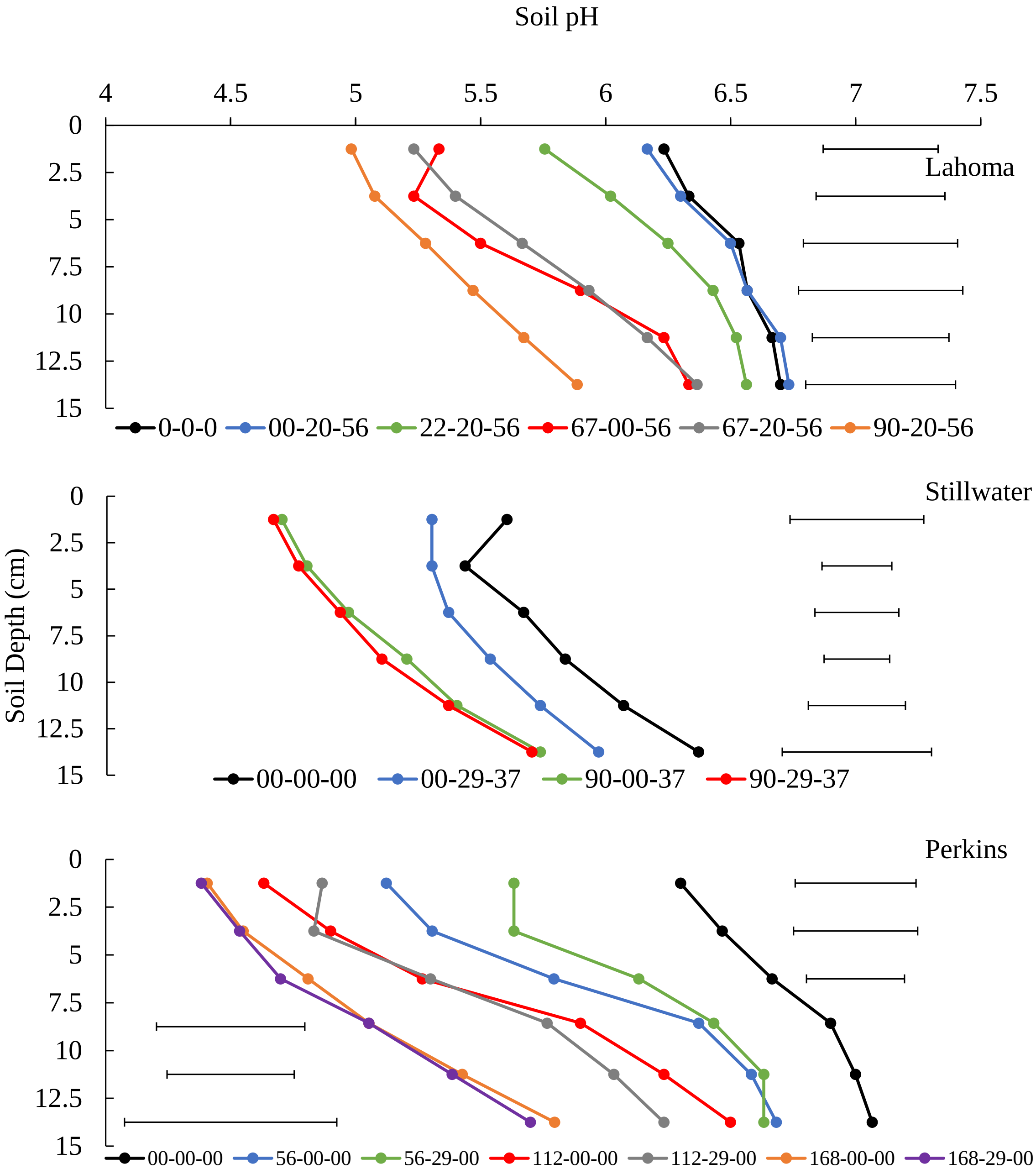


Figure 5 Mehlich 3 extractable phosphorus from stratified soil samples as a function of fertilizer application in 3 long-term no-till trials. Colored lines are treatments. Horizontal bars indicate the Honest Significant Difference (HSD), ($p \leq 0.05$). Distribution patterns of Mehlich 3 phosphorus are highly stratified including the phosphorus non-fertilized plots.

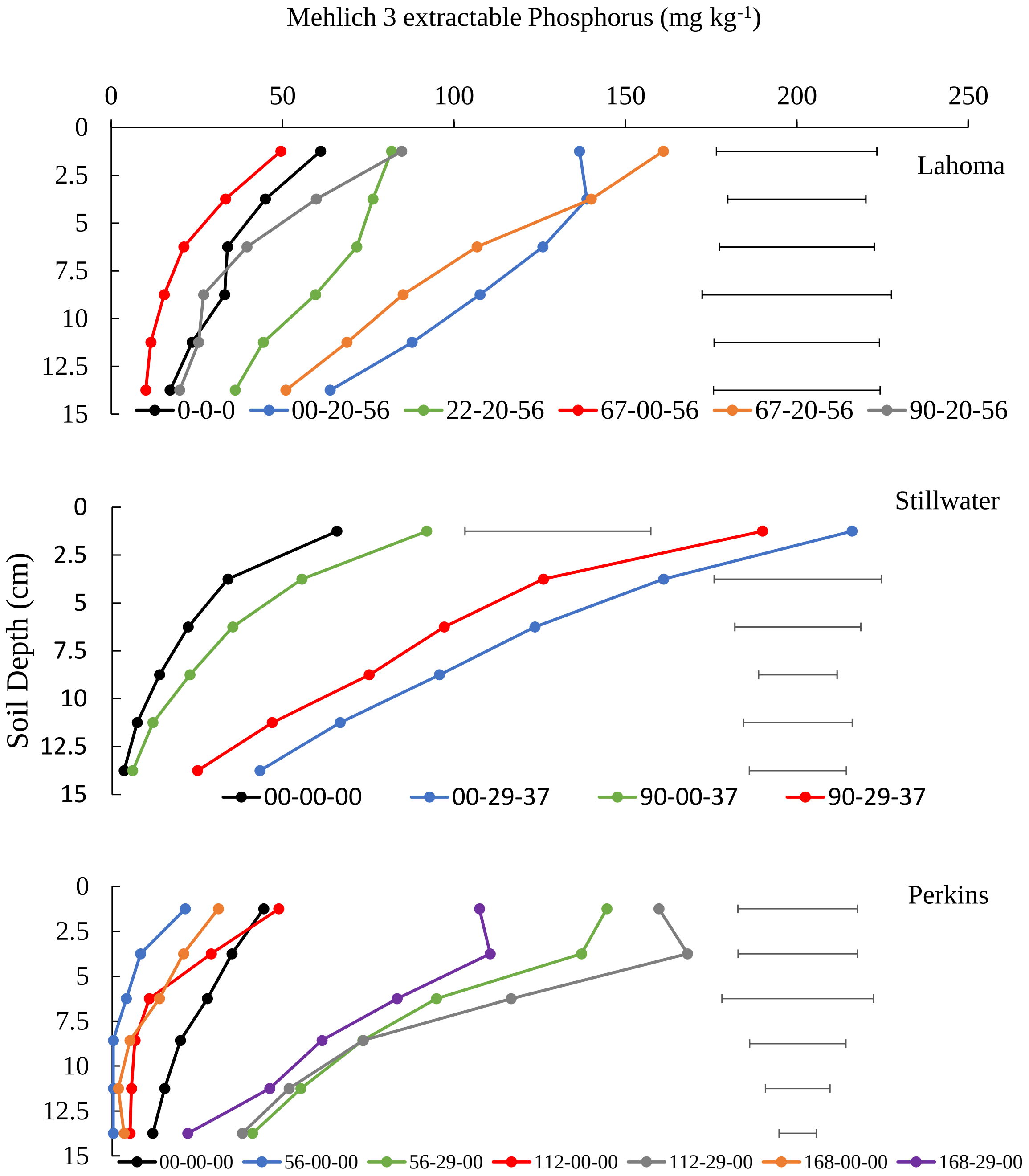


Figure 6 Mehlich 3 extractable Potassium (K) from stratified soil samples as a function of fertilizer application in 3 long-term no-till trials. Colored lines are treatments. Horizontal bars indicate the Honest Significant Difference (HSD), (p ≤ 0.05). Distribution patterns of Mehlich 3 potassium are stratified including the phosphorus non-fertilized plots.

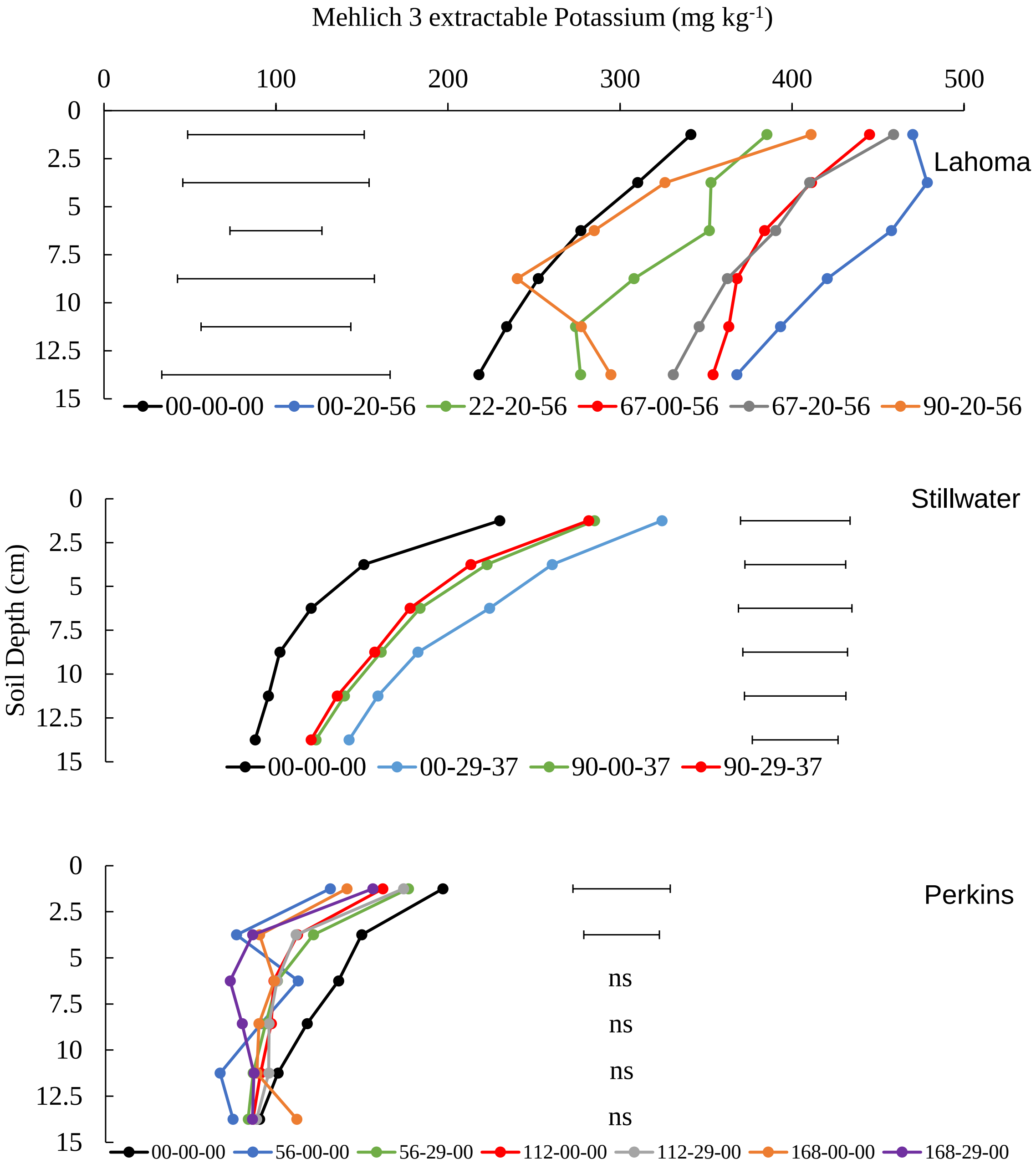


Figure 7 Total nitrogen from stratified soil samples as a function of fertilizer application in 3 long-term no-till trials. Colored lines are treatments. Horizontal bars indicate the Honest Significant Difference (HSD), ($p \leq 0.05$). Differences in total nitrogen were found only in the first 0-5 cm regardless the location and it appear to be mostly driven by N application.

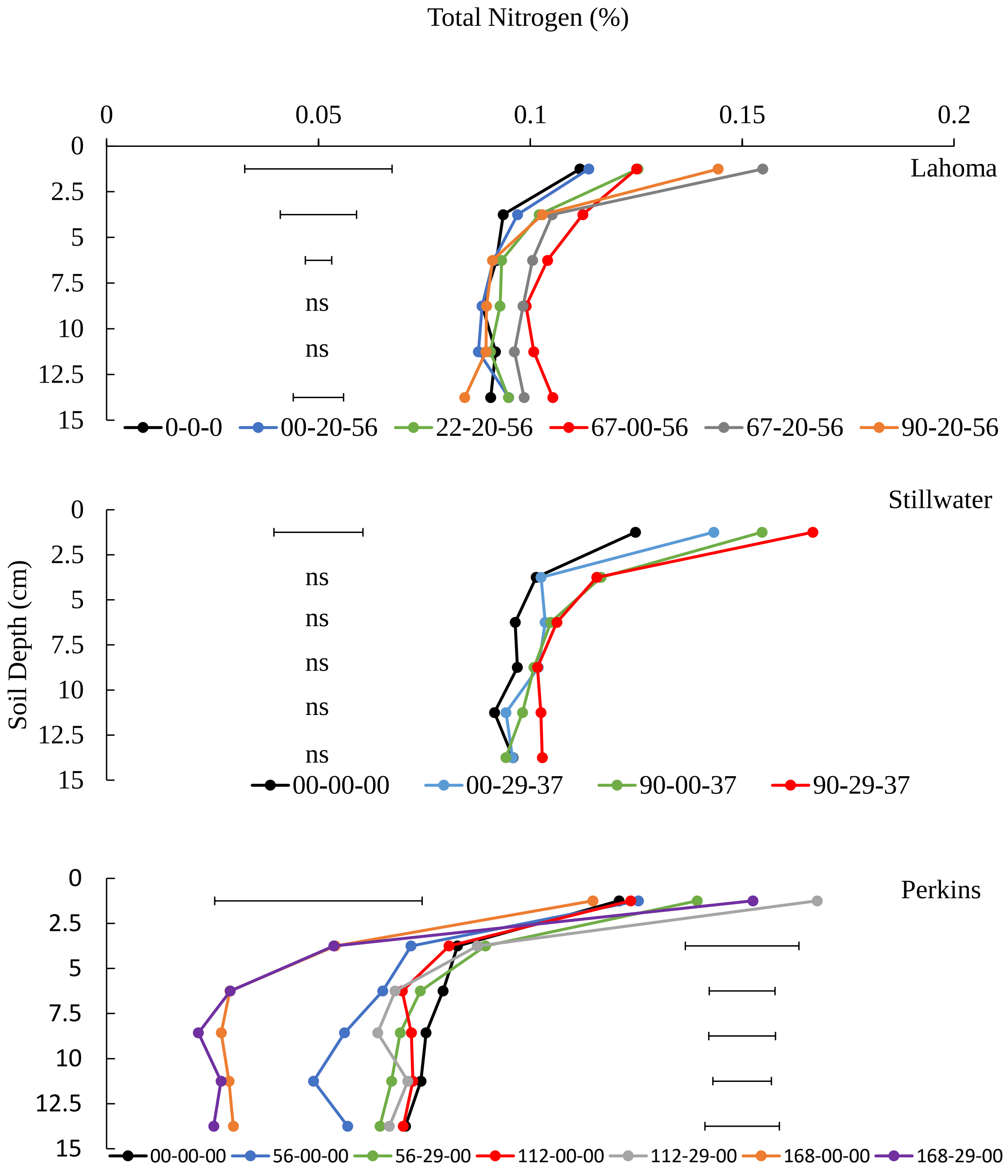


Figure 8 Organic carbon from stratified soil samples as a function of fertilizer application in 3 long-term no-till trials. Colored lines are treatments. Horizontal bars indicate the Honest Significant Difference (HSD), ($p \leq 0.05$). Differences in organic carbon were found only in the first 0-5 cm regardless the location and it appear to be mostly driven by N application.

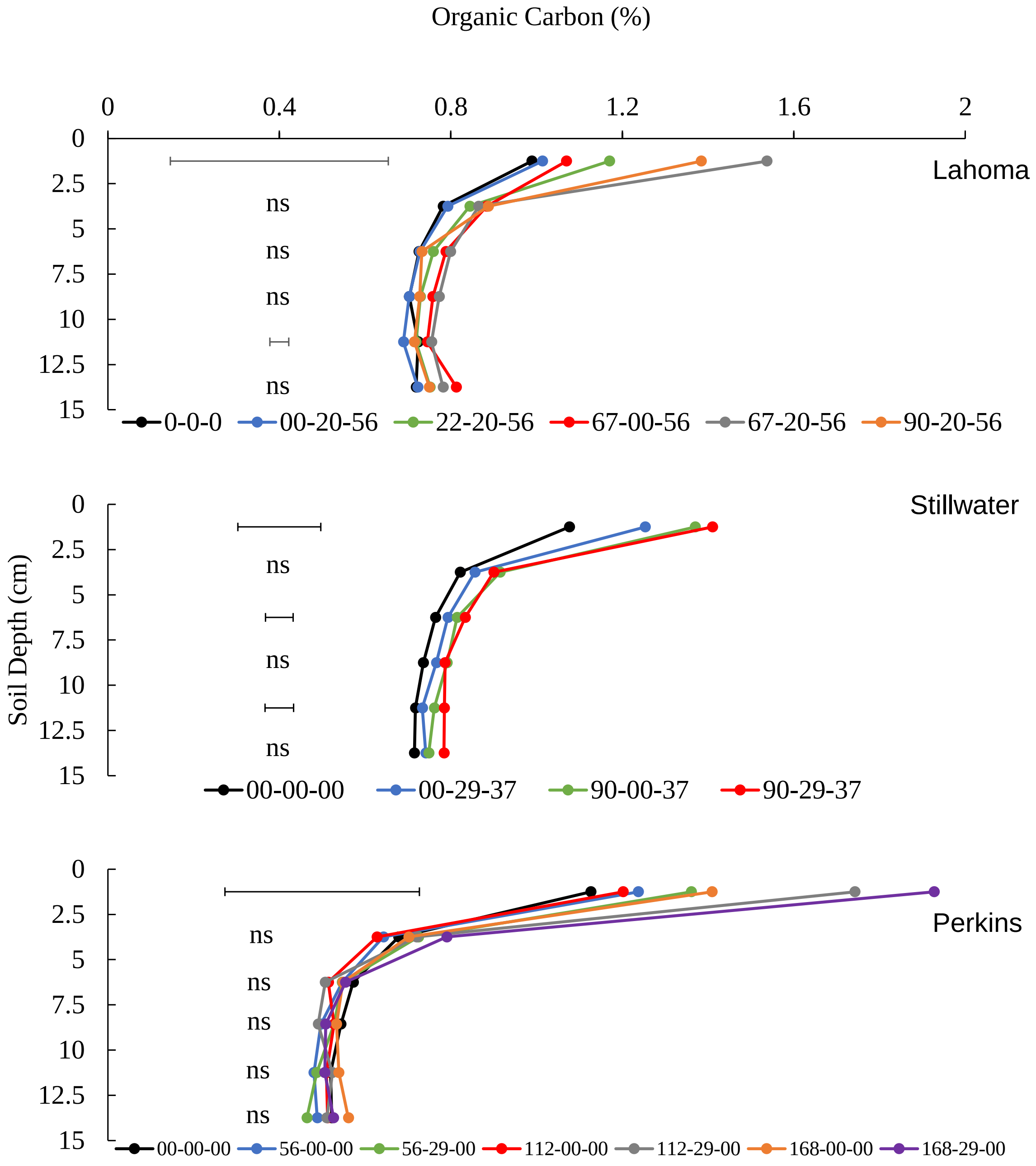
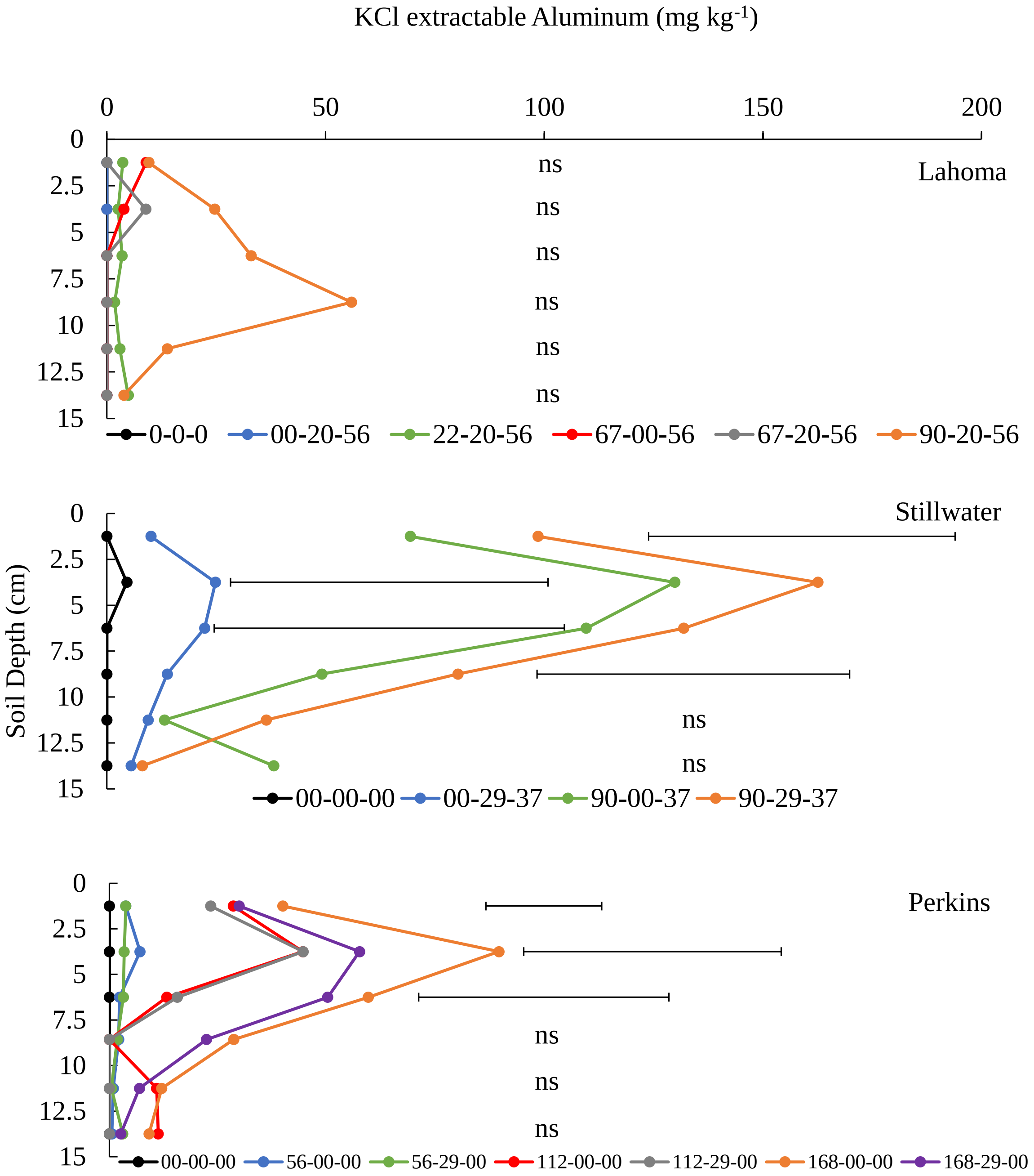


Figure 9 KCl exchangeable Aluminum (Al) from stratified soil samples as a function of fertilizer application in 3 long-term no-till trials. Colored lines are treatments. Horizontal bars indicate the Honest Significant Difference (HSD), ($p \leq 0.05$).



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APPENDICES

Figure 10 – Soil pH relationship between stratified and composite soil sampling methods. A) Stratified soil pH averaged based on the proteomic activity of hydrogen ([H+]) (y-axes) and composite sample soil pH (0-15.24 cm) (x-axes). B) Stratified soil pH arithmetically averaged (y-axes) and (y-axes) and composite sample soil pH (0-15.24 cm) (x-axes). C) Stratified soil pH

arithmetically averaged (y-axes) and composite sample soil pH (0-15.24 cm) (x-axes) Stratified soil pH averaged based on the proteomic activity of hydrogen ([H+]) (y-axes)

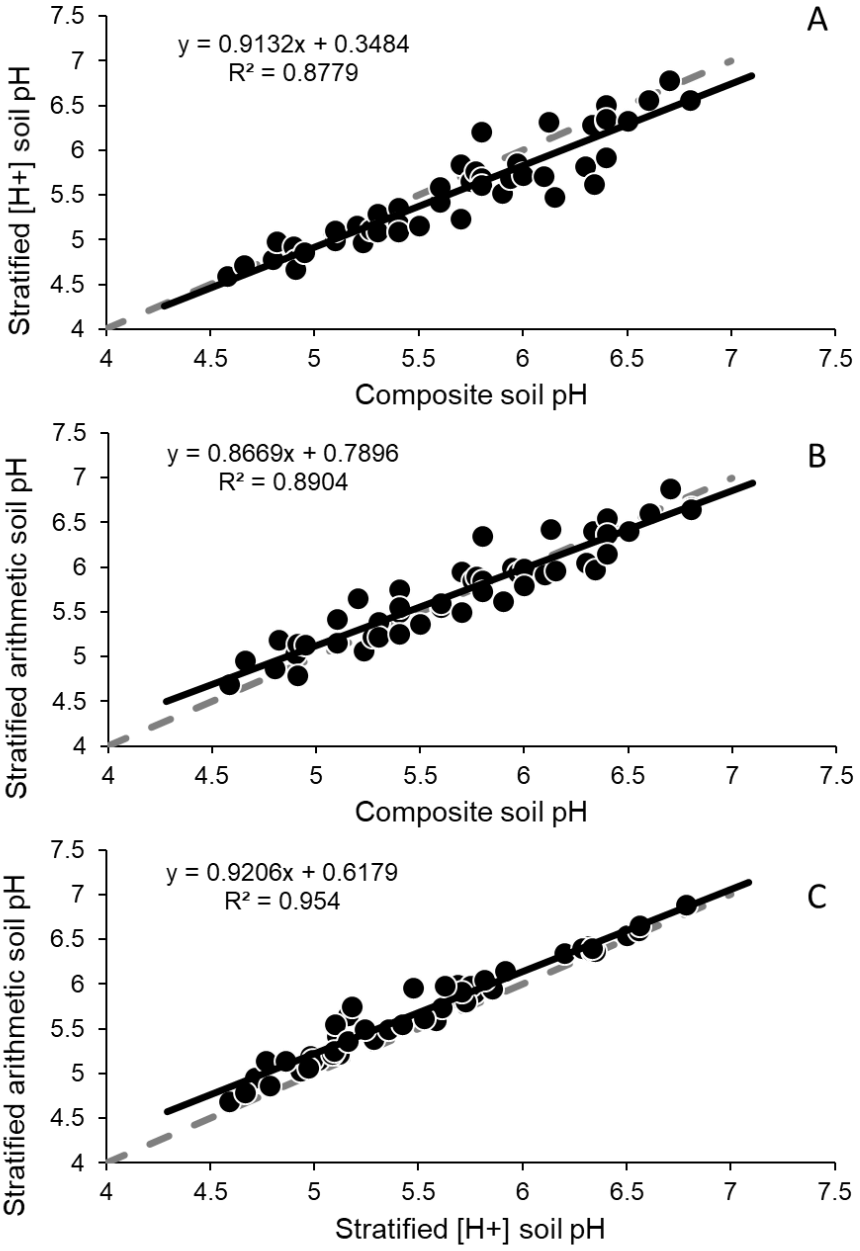

Table 11 – Effect of nutrient management on soil pH by treatment and depth across three continuous wheat long-term locations in Oklahoma.

	Lahoma		**Stillwater**		**Perkins**	
Depth (cm)	**Treatment**	**pH**	**Treatment**	**pH**	**Treatment**	**pH**
0-2.54	00-00-00	6.23 a	00-00-00	5.60 a	00-00-00	6.30 a
	00-20-56	6.17 ab	00-29-37	5.30 a	56-29-00	5.63 b
	22-20-56	5.76 bc	90-00-37	4.70 b	56-00-00	5.12 c
	67-00-56	5.33 cd	90-29-37	4.67 b	112-29-00	4.87 cd
	67-20-56	5.23 d			112-00-00	4.63 d
	90-20-56	4.98 d			168-00-00	4.41 d
					168-29-00	4.38 d
2.54-5.08	00-00-00	6.33 a	00-00-00	5.43 a	00-00-00	6.47 a
	00-20-56	6.30 a	00-29-37	5.30 a	56-29-00	5.63 b
	22-20-56	6.02 a	90-00-37	4.80 b	56-00-00	5.31 bc
	67-20-56	5.40 b	90-29-37	4.77 b	112-00-00	4.90 cd
	67-00-56	5.23 b			112-29-00	4.83 cd
	90-20-56	5.08 b			168-00-00	4.55 d
					168-29-00	4.54 d
5.08-7.62	00-00-00	6.53 a	00-00-00	5.67 a	00-00-00	6.67 a
	00-20-56	6.50 a	00-29-37	5.37 a	56-29-00	6.13 b
	22-20-56	6.25 ab	90-00-37	4.97 b	56-00-00	5.79 b
	67-20-56	5.67 bc	90-29-37	4.93 b	112-29-00	5.30 c
	67-00-56	5.50 c			112-00-00	5.27 c
	90-20-56	5.28 c			168-00-00	4.81 d
					168-29-00	4.70 d
7.62-10.16	00-20-56	6.57 a	00-00-00	5.67 a	00-00-00	6.90 a
	00-00-00	6.57 a	00-29-37	5.37 a	56-29-00	6.43 ab
	22-20-56	6.43 ab	90-00-37	4.97 b	56-00-00	6.37 ab
	67-20-56	5.93 abc	90-29-37	4.93 b	112-00-00	5.90 bc
	67-00-56	5.90 bc			112-29-00	5.77 c
	90-20-56	5.47 c			168-00-00	5.05 d
					168-29-00	5.05 d
10.16-12.7	00-20-56	6.70 a	00-00-00	6.07 a	00-00-00	7.00 a
	00-00-00	6.67 a	00-29-37	5.73 ab	56-29-00	6.63 ab
	22-20-56	6.52 a	90-00-37	5.40 b	56-00-00	6.58 ab
	67-00-56	6.23 a	90-29-37	5.37 b	112-00-00	6.23 bc
	67-20-56	6.17 ab			112-29-00	6.03 c
	90-20-56	5.67 b			168-00-00	5.43 d
					168-29-00	5.39 d
12.7-15.24	00-20-56	6.73 a	00-00-00	6.37 a	00-00-00	7.07 a
	00-00-00	6.70 a	00-29-37	5.97 ab	56-00-00	6.68 a
	22-20-56	6.56 a	90-00-37	5.73 b	56-29-00	6.63 ab
	67-20-56	6.37 ab	90-29-37	5.70 b	112-00-00	6.50 abc
	67-00-56	6.33 ab			112-29-00	6.23 abc
	90-20-56	5.89 b			168-00-00	5.80 bc
					168-29-00	5.70 c

Treatments values are expressed in kg ha^{-1} *of N-P-K applied annually as urea, triple super phosphate and potassium chloride. Different letters demonstrate significantly differences in treatment effect in each soil layer within each location (Tukey test, p<0.05).*

Table 12 – Effect of nutrient management on soil Mehlich 3 extractable phosphorus by treatment and depth across three continuous wheat long-term locations in Oklahoma.

	Lahoma		**Stillwater**		**Perkins**	
Depth (cm)	**Treatment**	**P (mg kg^{-1})**	**Treatment**	**P (mg kg^{-1})**	**Treatment**	**P (mg kg^{-1})**
0-2.54	67-20-56	161 a	00-29-37	216 a	112-29-00	160 a
	00-20-56	137 a	90-29-37	190 a	56-29-00	144 a
	90-20-56	85 b	90-00-37	92 b	168-29-00	107 b
	22-20-56	82 b	00-00-00	66 b	112-00-00	49 c
	00-00-00	61 b			00-00-00	44 c
	67-00-56	49 b			168-00-00	31 c
					56-00-00	21 c
2.54-5.08	67-20-56	140 a	00-29-37	161 a	112-29-00	168 a
	00-20-56	139 a	90-29-37	126 a	56-29-00	137 ab
	22-20-56	76 b	90-00-37	55 b	168-29-00	110 b
	90-20-56	60 bc	00-00-00	34 b	00-00-00	35 c
	00-00-00	45 bc			112-00-00	29 c
	67-00-56	33 c			168-00-00	21 c
					56-00-00	8 c
5.08-7.62	00-20-56	126 a	00-29-37	123 a	112-29-00	116 a
	67-20-56	107 ab	90-29-37	97 a	56-29-00	95 a
	22-20-56	72 bc	90-00-37	35 b	168-29-00	83 a
	90-20-56	40 cd	00-00-00	22 b	00-00-00	28 b
	00-00-00	34 cd			168-00-00	14 b
	67-00-56	21 d			112-00-00	11 b
					56-00-00	4 b
7.62-10.16	00-20-56	108 a	00-29-37	95 a	112-29-00	73 a
	67-20-56	85 ab	90-29-37	75 a	56-29-00	73 a
	22-20-56	60 abc	90-00-37	23 b	168-29-00	61 a
	00-00-00	33 bc	00-00-00	14 b	00-00-00	20 b
	90-20-56	27 c			112-00-00	7 b
	67-00-56	15 c			168-00-00	5 b
					56-00-00	0 b
10.16-12.7	00-20-56	88 a	00-29-37	66 a	56-29-00	55 a
	67-20-56	69 ab	90-29-37	47 a	112-29-00	52 a
	22-20-56	44 abc	90-00-37	12 b	168-29-00	46 a
	90-20-56	26 bc	00-00-00	7 b	00-00-00	15 b
	00-00-00	24 bc			112-00-00	6 b
	67-00-56	12 c			168-00-00	2 b
					56-00-00	0 b
12.7-15.24	00-20-56	64 a	00-29-37	43 a	56-29-00	41 a
	67-20-56	51 ab	90-29-37	25 ab	112-29-00	38 a
	22-20-56	36 ab	90-00-37	6 b	168-29-00	22 b
	90-20-56	20 ab	00-00-00	3 b	00-00-00	12 bc
	00-00-00	17 ab			112-00-00	5 cd
	67-00-56	10 b			168-00-00	4 cd
					56-00-00	0 d

Treatments values are expressed in kg ha^{-1} of N-P-K applied annually as urea, triple super phosphate and potassium chloride. Different letters demonstrate significantly differences in treatment effect in each soil layer by location (Tukey test, $p<0.05$).

Table 13 – Effect of nutrient management on soil Mehlich 3 extractable potassium by treatment and depth across three continuous wheat long-term locations in Oklahoma.

	Lahoma		**Stillwater**		**Perkins**	
Soil depth	**Treatment**	**K (mg kg^{-1})**	**Treatment**	**K (mg kg^{-1})**	**Treatment**	**K (mg kg^{-1})**
0-2.54	00-20-56	470 a	00-29-37	323 a	00-00-00	196 a
	67-20-56	459 a	90-00-37	284 ab	56-29-00	176 ab
	67-00-56	445 a	90-29-37	280 ab	112-29-00	173 ab
	90-20-56	411 ab	00-00-00	229 b	112-00-00	161 ab
	22-20-56	385 ab			168-29-00	155 ab
	00-00-00	341 b			168-00-00	140 ab
					56-00-00	131 b
2.54-5.08	00-20-56	479 a	00-29-37	259 a	00-00-00	149 a
	67-00-56	411 ab	90-00-37	221 a	56-29-00	121 ab
	67-20-56	410 ab	90-29-37	212 a	112-00-00	112 abc
	22-20-56	330 b	00-00-00	150 b	112-29-00	111 abc
	90-20-56	326 b			168-00-00	90 bc
	00-00-00	310 b			168-29-00	86 bc
					56-00-00	76 c
5.08-7.62	00-20-56	458 a	00-29-37	223 a	00-00-00	135 a
	67-20-56	390 b	90-00-37	182 ab	56-00-00	112 a
	67-00-56	384 b	90-29-37	177 ab	56-29-00	100 a
	22-20-56	352 b	00-00-00	119 b	112-29-00	100 a
	90-20-56	285 c			168-00-00	98 a
	00-00-00	277 c			112-00-00	98 a
					168-29-00	72 a
7.62-10.16	00-20-56	420 a	00-29-37	181 a	00-00-00	117 a
	67-00-56	368 a	90-00-37	160 ab	112-00-00	96 a
	67-20-56	362 ab	90-29-37	156 ab	112-29-00	95 a
	22-20-56	308 abc	00-00-00	101 b	56-29-00	93 a
	00-00-00	252 bc			56-00-00	90 a
	90-20-56	240 c			168-00-00	89 a
					168-29-00	79 a
10.16-12.7	00-20-56	393 a	00-29-37	158 a	00-00-00	100 a
	67-00-56	363 ab	90-00-37	139 ab	112-29-00	95 a
	67-20-56	346 abc	90-29-37	134 ab	112-00-00	90 a
	90-20-56	277 bcd	00-00-00	94 b	168-00-00	88 a
	22-20-56	274 cd			168-29-00	86 a
	00-00-00	234 d			56-29-00	86 a
					56-00-00	67 a
12.7-15.24	00-20-56	368 a	00-29-37	141 a	168-00-00	111 a
	67-00-56	354 a	90-00-37	122 ab	00-00-00	90 a
	67-20-56	331 ab	90-29-37	119 ab	112-29-00	88 a
	90-20-56	295 ab	00-00-00	87 b	112-00-00	86 a
	22-20-56	277 ab			168-29-00	85 a
	00-00-00	218 b			56-29-00	83 a
					56-00-00	74 a

Treatments values are expressed in kg ha^{-1} of N-P-K applied annually as urea, triple super phosphate and potassium chloride. Different letters demonstrate significantly differences in treatment effect in each soil layer by location (Tukey test, $p<0.05$).

Table 14 – Effect of nutrient management on soil total nitrogen by treatment and depth across three continuous wheat long-term locations in Oklahoma.

	Lahoma		**Stillwater**		**Perkins**	
Depth (cm)	**Treatment**	**TN (%)**	**Treatment**	**TN (%)**	**Treatment**	**TN (%)**
0-2.54	67-20-56	0.15 a	90-29-37	0.17 a	112-29-00	0.17 a
	90-20-56	0.14 ab	90-00-37	0.15 ab	168-29-00	0.15 ab
	22-20-56	0.13 ab	00-29-37	0.14 bc	56-29-00	0.14 ab
	67-00-56	0.13 ab	00-00-00	0.12 c	56-00-00	0.13 ab
	00-20-56	0.11 b			112-00-00	0.12 ab
	00-00-00	0.11 b			00-00-00	0.12 ab
					168-00-00	0.11 b
2.54-5.08	67-00-56	0.11 a	90-00-37	0.12 a	56-29-00	0.09 a
	67-20-56	0.11 ab	90-29-37	0.12 a	112-29-00	0.09 a
	90-20-56	0.10 ab	00-29-37	0.10 a	00-00-00	0.08 a
	22-20-56	0.10 ab	00-00-00	0.10 a	112-00-00	0.08 a
	00-20-56	0.10 ab			56-00-00	0.07 ab
	00-00-00	0.09 b			168-00-00	0.05 b
					168-29-00	0.05 b
5.08-7.62	67-00-56	0.10 a	90-29-37	0.11 a	00-00-00	0.08 a
	67-20-56	0.10 a	90-00-37	0.10 a	56-29-00	0.07 a
	22-20-56	0.09 b	00-29-37	0.10 a	112-00-00	0.07 a
	00-00-00	0.09 b	00-00-00	0.10 a	112-29-00	0.07 a
	00-20-56	0.09 b			56-00-00	0.07 a
	90-20-56	0.09 b			168-29-00	0.03 b
					168-00-00	0.03 b
7.62-10.16	67-00-56	0.10 a	00-29-37	0.10 a	00-00-00	0.08 a
	67-20-56	0.10 a	90-29-37	0.10 a	112-00-00	0.07 ab
	22-20-56	0.09 a	90-00-37	0.10 a	56-29-00	0.07 ab
	90-20-56	0.09 a	00-00-00	0.10 a	112-29-00	0.06 ab
	00-00-00	0.09 a			56-00-00	0.06 b
	00-20-56	0.09 a			168-00-00	0.03 c
					168-29-00	0.02 c
10.16-12.7	67-00-56	0.10 a	90-29-37	0.10 a	00-00-00	0.07 a
	67-20-56	0.10 a	90-00-37	0.10 a	112-00-00	0.07 a
	00-00-00	0.09 a	00-29-37	0.09 a	112-29-00	0.07 a
	22-20-56	0.09 a	00-00-00	0.09 a	56-29-00	0.07 a
	90-20-56	0.09 a			56-00-00	0.05 b
	00-20-56	0.09 a			168-00-00	0.03 c
					168-29-00	0.03 c
12.7-15.24	67-00-56	0.11 a	90-29-37	0.10 a	00-00-00	0.07 a
	67-20-56	0.10 ab	00-00-00	0.10 a	112-00-00	0.07 a
	00-20-56	0.09 abc	00-29-37	0.10 a	112-29-00	0.07 a
	22-20-56	0.09 abc	90-00-37	0.09 a	56-29-00	0.06 a
	00-00-00	0.09 bc			56-00-00	0.06 a
	90-20-56	0.08 c			168-00-00	0.03 b
					168-29-00	0.03 b

Treatments values are expressed in kg ha^{-1} of N-P-K applied annually as urea, triple super phosphate and potassium chloride. Different letters demonstrate significantly differences in treatment effect in each soil layer by location (Tukey test, $p<0.05$).

Table 15 – Effect of nutrient management on soil organic carbon by treatment and depth across three continuous wheat long-term locations in Oklahoma.

	Lahoma		**Stillwater**		**Perkins**	
Depth (cm)	**Treatment**	**OC (%)**	**Treatment**	**OC (%)**	**Treatment**	**OC (%)**
0-2.54	67-20-56	1.54 a	90-29-37	1.41 a	150-60-00	1.93 a
	90-20-56	1.38 ab	90-00-37	1.37 a	112-29-00	1.74 ab
	22-20-56	1.17 ab	00-29-37	1.25 ab	168-00-00	1.41 bc
	67-00-56	1.07 ab	00-00-00	1.08 b	56-29-00	1.36 bc
	00-20-56	1.01 b			56-00-00	1.24 c
	00-00-00	0.99 b			112-00-00	1.20 c
					00-00-00	1.13 c
2.54-5.08	90-20-56	0.89 a	90-00-37	0.91 a	150-60-00	0.79 a
	67-00-56	0.88 a	90-29-37	0.90 a	56-29-00	0.72 a
	67-20-56	0.86 a	00-29-37	0.86 a	112-29-00	0.72 a
	22-20-56	0.84 a	00-00-00	0.82 a	168-00-00	0.70 a
	00-20-56	0.79 a			00-00-00	0.68 a
	0-0-0	0.78 a			56-00-00	0.64 a
					112-00-00	0.63 a
5.08-7.62	67-20-56	0.80 a	90-29-37	0.83 a	00-00-00	0.57 a
	67-00-56	0.79 a	90-00-37	0.81 ab	168-29-00	0.55 a
	22-20-56	0.76 a	00-29-37	0.79 ab	168-00-00	0.55 a
	90-20-56	0.73 a	00-00-00	0.76 b	56-00-00	0.55 a
	00-20-56	0.73 a			56-29-00	0.55 a
	00-00-00	0.73 a			112-00-00	0.51 a
					112-29-00	0.51 a
7.62-10.16	67-20-56	0.77 a	90-00-37	0.79 a	00-00-00	0.54 a
	67-00-56	0.76 a	90-29-37	0.79 a	168-00-00	0.53 a
	22-20-56	0.73 a	00-29-37	0.77 a	112-00-00	0.53 a
	90-20-56	0.73 a	00-00-00	0.74 a	56-29-00	0.53 a
	00-00-00	0.70 a			168-29-00	0.51 a
	00-20-56	0.70 a			56-00-00	0.50 a
					112-29-00	0.49 a
10.16-12.7	67-20-56	0.76 a	90-29-37	0.78 a	168-00-00	0.54 a
	67-00-56	0.75 a	90-00-37	0.76 ab	112-29-00	0.52 a
	00-00-00	0.72 ab	00-29-37	0.73 ab	00-00-00	0.52 a
	22-20-56	0.72 ab	00-00-00	0.72 b	112-00-00	0.51 a
	90-20-56	0.72 ab			168-29-00	0.51 a
	00-20-56	0.69 b			56-29-00	0.49 a
					56-00-00	0.48 a
12.7-15.24	67-00-56	0.81 a	90-29-37	0.78 a	168-00-00	0.56 a
	67-20-56	0.78 a	90-00-37	0.75 a	168-29-00	0.53 a
	22-20-56	0.75 a	00-29-37	0.74 a	00-00-00	0.52 a
	90-20-56	0.75 a	00-00-00	0.71 a	112-00-00	0.51 a
	00-20-56	0.72 a			112-29-00	0.51 a
	00-00-00	0.72 a			56-00-00	0.49 a
					56-29-00	0.46 a

Treatments values are expressed in kg ha^{-1} of N-P-K applied annually as urea, triple super phosphate and potassium chloride. Different letters demonstrate significantly differences in treatment effect in each soil layer by location (Tukey test, $p<0.05$).

Table 16 – Effect of nutrient management on soil KCl exchangeable aluminum by treatment and depth across three continuous wheat long-term locations in Oklahoma.

	Lahoma		**Stillwater**		**Perkins**	
Depth (cm)	**Treatment**	**Al (mg kg^{-1})**	**Treatment**	**Al (mg kg^{-1})**	**Treatment**	**Al (mg kg^{-1})**
0-2.54	90-20-56	10 a	90-29-37	99 a	168-00-00	40 a
	67-00-56	9 a	90-00-37	70 ab	168-29-00	30 ab
	22-20-56	4 a	00-29-37	10 b	112-00-00	29 ab
	00-20-56	0 a	00-00-00	0 b	112-29-00	23 abc
	00-00-00	0 a			56-29-00	4 bc
	67-20-56	0 a			56-00-00	4 bc
					00-00-00	0 c
2.54-5.08	90-20-56	25 a	90-29-37	164 a	168-00-00	90 a
	67-20-56	9 a	90-00-37	131 a	168-29-00	58 ab
	67-00-56	4 a	00-29-37	25 b	112-29-00	45 ab
	22-20-56	3 a	00-00-00	5 b	112-00-00	45 ab
	00-20-56	0 a			56-00-00	7 b
	00-00-00	0 a			56-29-00	3 b
					00-00-00	0 b
5.08-7.62	90-20-56	33 a	90-29-37	133 a	168-00-00	60 a
	22-20-56	3 a	90-00-37	110 a	168-29-00	50 ab
	00-20-56	0 a	00-29-37	22 b	112-29-00	16 ab
	00-00-00	0 a	00-00-00	0 b	112-00-00	13 ab
	67-20-56	0 a			56-29-00	3 ab
	67-00-56	0 a			56-00-00	2 ab
					00-00-00	0 b
7.62-10.16	90-20-56	56 a	90-29-37	81 a	168-00-00	29 a
	22-20-56	2 a	90-00-37	49 ab	168-29-00	22 a
	00-20-56	0 a	00-29-37	14 ab	56-00-00	2 a
	00-00-00	0 a	00-00-00	0 b	56-29-00	2 a
	67-20-56	0 a			00-00-00	0 a
	67-00-56	0 a			112-29-00	0 a
					112-00-00	0 a
10.16-12.7	90-20-56	14 a	90-29-37	37 a	168-00-00	12 a
	22-20-56	3 a	90-00-37	13 a	112-00-00	11 a
	00-20-56	0 a	00-29-37	9 a	168-29-00	7 a
	00-00-00	0 a	00-00-00	0 a	56-00-00	1 a
	67-20-56	0 a			56-29-00	0 a
	67-00-56	0 a			00-00-00	0 a
					112-29-00	0 a
12.7-15.24	22-20-56	5 a	90-00-37	38 a	112-00-00	11 a
	90-20-56	4 a	90-29-37	8 a	168-00-00	9 a
	00-20-56	0 a	00-29-37	6 a	56-29-00	3 a
	00-00-00	0 a	00-00-00	0 a	168-29-00	3 a
	67-20-56	0 a			56-00-00	1 a
	67-00-56	0 a			00-00-00	0 a
					112-29-00	0 a

Treatments values are expressed in kg ha^{-1} of N-P-K applied annually as urea, triple super phosphate and potassium chloride. Different letters demonstrate significantly differences in treatment effect in each soil layer by location (Tukey test, $p<0.05$).

Figure 17 - Comparison between composite (0-15.24 cm) soil samples with averaged stratified sampling method (each 2.54 cm) in three long-term locations in Oklahoma.

		Phosphorus			**Potassium**			**KCl exchangeble aluminum**			**Total nitrogen**			**Organic carbon**		
	Treatment	mg kg $^{-1}$									%					
		Composite	Stratified	|Δ|	Composite	Stratified	|Δ|	Composite	Stratified	|Δ|	Composite	Stratified	|Δ|	Composite	Stratified	|Δ|
Lahoma	00-00-00	33	36	3	264	272	8	8	0	8	0.095	0.095	0.001	0.774	0.774	0.000
	00-20-56	105	110	5	414	431	18	8	0	8	0.099	0.096	0.004	0.832	0.775	0.057
	22-20-56	60	62	2	297	312	15	7	3	4	0.094	0.100	0.006	0.808	0.829	0.021
	67-00-56	25	24	1	390	388	2	7	2	5	0.129	0.120	0.009	1.145	1.003	0.142
	67-20-56	105	102	3	378	383	5	0	1	1	0.105	0.109	0.004	0.897	0.919	0.022
	90-29-37	38	43	5	319	306	13	2	23	22	0.102	0.100	0.002	0.881	0.866	0.014
Stillwater	00-00-00	23	24	2	136	130	6	0	1	1	0.097	0.101	0.004	0.821	0.805	0.016
	00-29-37	105	118	12	197	214	18	12	14	2	0.109	0.107	0.002	0.875	0.857	0.018
	90-00-37	38	37	1	192	185	7	55	64	9	0.112	0.112	0.000	0.911	0.900	0.011
	90-29-37	89	93	4	169	180	11	74	87	13	0.122	0.116	0.006	0.928	0.917	0.011
Perkins	00-00-00	26	26	0	134	131	2	0	0	0	0.085	0.084	0.001	0.652	0.660	0.009
	56-00-00	27	6	21	117	92	25	0	3	3	0.065	0.071	0.005	0.610	0.649	0.039
	56-29-00	61	91	30	117	110	7	1	3	1	0.092	0.084	0.008	0.740	0.685	0.055
	112-00-00	17	18	0	113	107	6	12	18	6	0.083	0.081	0.002	0.657	0.649	0.008
	112-29-00	117	101	16	115	110	4	9	14	5	0.087	0.088	0.001	0.738	0.749	0.011
	168-00-00	13	13	0	108	103	5	34	40	6	0.054	0.047	0.007	0.801	0.715	0.086
	168-29-00	52	72	20	109	94	15	32	28	3	0.059	0.052	0.008	0.838	0.802	0.036

Δ: Difference between averages of stratified and composite samples.

VITA

Joao Luis Bigatao Souza

Candidate for the Degree of

Doctor of Philosophy

Thesis: INFLUENCE OF NITROGEN MANAGEMENT ON WINTER WHEAT GRAIN YIELD AND EVALUATION OF NUTRIENT MANAGEMENT ON THE STRATIFICATION OF SOIL PARAMETERS IN NO-TILL

Major Field: Soil Science

Education:

Completed the requirements for the Doctor of Philosophy in Soil Science at Oklahoma State University, Stillwater, Oklahoma in December, 2021.

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