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Impact of long-term fertilization in no-till on the stratification of soil acidity and related parameters

Joao Luis Bigatao Souza^a, Joao Arthur Antonangelo^b, Hailin Zhang^a, Vaughn Reed^c, Bronc Finch^a, Brian Arnall^a,

^a Oklahoma State University, Stillwater, OK, USA

Austin Peay State University, Clarksville, TN, USA

^c Mississippi State University, Starkville, MS, USA

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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 application of fertilizers to the soil surface under NT has been shown to lead to the stratification of pH and other soil acidity parameters near the soil surface. Despite that, only a few works have reported the soil acidity stratification of the arable layer and were restricted to highly weathered acid soils of the tropics. Therefore, our objective was to quantify soil pH, exchangeable aluminum (Al_{KCl}), aluminum saturation (Al%), soil organic carbon (OC), and cation exchange capacity (CEC) by depth in the topsoils across different levels of nutrient management in three long-term continuous NT wheat studies in the southern Plains of US. Plots were sampled after the winter wheat (Triticum aestivum L.) harvest in the summer of 2019 at sampling depths of 0-15 cm, and 0-2.5, 2.5-5, 5-7.5, 7.5-10, 10-12.5, and 12.5-15 cm. Treatments included an unfertilized check and a combination of increased rates of nitrogen (N) in addition to phosphorus (P) and potassium (K) application across all study locations. It was found that stratification occurred for all soil acidity parameters tested in the study, with higher OC and Al_{KCL} and lower pH near the soil surface. Patterns of soil pH and OC stratification were generally related to the rate of annual N fertilization. The long-term N fertilization significantly reduced soil pH in the topsoil (0-15 cm) and stratified soil layers, especially at the higher application rates. Regardless of the soil sampling depth, pH decrease was significantly related to the amount of N applied. Also, N fertilization significantly increased AlKCI and Al% but decreased the sum of $Ca^{2+}+Mg^{2+}+K^++Na^+$ both in the whole topsoil or layers. Al_{KCl} and Al% were increased exponentially and linearly with increasing N rate and were for 0-15 cm and stratified layers, respectively. Al_{KCl} was exponentially (inversely) related to soil pH for both sampling methods. Nitrate (NO_3^-) was inversely related to soil pH until up to 21.9 ± 2.6 mg NO₃—N kg⁻¹. Although not at the same magnitude, non-fertilized plots were likewise stratified for the tested parameters, which indicates other effects of NT on the stratification rather than the fertilizer addition. Overall, the buffer index (BI) was lower if the soil is sampled from 0 to 2.5 cm than 0-15 cm. Attention regarding sampling depth in NT areas is essential for accurate soil attributes monitoring.

1. 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 (Triticum aestivum) managed under NT increased from 19.9 % in 2004 to 44.6 % in 2018 in the US. The NT can be part of a sustainable solution for the increasing demand for food production and fertilizer efficiency given its ability for soil nutrient cycling and organic matter (OM) enhancement in the long term (Bot and Benites, 2005). When compared to the conventional tillage

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Abbreviations: Al%, aluminum saturation; Al_{KCI}, exchangeable aluminum; BI, buffer index; ECEC, effective cation exchange capacity; ICP-AES, inductively coupled plasma — atomic emission spectrophotometry; NO₃—N, nitrate; NT, no-till; SB, sum of base.

Correspondence to: Oklahoma State University, Department of Plant and Soil Sciences, 371 Agricultural Hall, Stillwater, OK 74078-6028, USA.

E-mail addresses: bigatao@okstate.edu (J.L.B. Souza), antonangeloj@apsu.edu (J.A. Antonangelo), hailin.zhang@okstate.edu (H. Zhang), vr401@msstate.edu (V. Reed), bronc.finch@okstate.edu (B. Finch), b.arnall@okstate.edu (B. Arnall).

(CT), NT offers savings on labor, time, and fuel, thus it is a more profitable farming practice (DeLaune and Sij, 2012; Derpsch et al., 2010). The reduction or elimination of tillage can also reduce runoff and sediment loss (Carretta et al., 2021; Merten et al., 2015).

The length of time of a production system under NT, climatic characteristics, and management conditions are recognized to influence the 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 were found in locations with ten or more years under NT management (Abreu et al., 2011). Other studies found similar patterns of carbon accumulation in long-term trials, greater than ten years of management (Alvarez and Steinbach, 2009; de Moraes, 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.

The surface nutrient application associated with the no fertilizer incorporation under NT leads to OC, nutrients, and pH stratification in the soil (Crozier et al., 1999; Lupwayi et al., 2006). However, typical soil depths for soil fertility characterization range from 0 to 10 or 0–20 cm with 0–15 most often across soil testing labs in the US, which corresponds to the soil arable layer (Baker et al., 2007; Barth et al., 2018), or the topsoil. These sampling depths have been used to help farmers to determine fertilizer and lime recommendations based on crop demand, bioavailability of nutrients, and soil pH, even under NT. Notwithstanding, this sampling technique may fail to account for stratification in soil chemical attributes in NT 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 stratification (topsoil stratification). For the arable layer (the 0-15 cm topsoil), nitrogen (N) fertilization is well known for its effects on soil acidification and biomass accumulation (Alvarez, 2005; Barak et al., 1997; Wallace, 1994; Zhou et al., 2014). Commercial N fertilizers are widely used for crop production making soils more acidic due to intense crop production and continuous N application (Schroder et al., 2011). One of the major causes of soil acidity is the nitrification process (Kariuki et al., 2007). Inputs of N fertilizer result in increased acidification via the oxidation of NH_4^+ to NO_3^- , which generates H^+ and lowers the soil pH (Hao et al., 2020; Tang et al., 2002). Thus, although these N fertilizers are not acidic, their inputs to soil are acid-forming (Pierre, 1928). Unsurprisingly, numerous researchers have shown that the long-term application of commercial N fertilizers lowers the soil pH (Rasmussen and Rohde, 1989; Bouman et al., 1995; Chien et al., 2008). The increased soil acidification increases the solubility of Al, which is the primary source of toxicity to plants at pH< 5.5 (Parker et al., 1989; Ernani et al., 2002). Despite the known effects of soil acidity in the arable layer little is known about such effects when soil is sampled from stratified layers within the arable layer, especially in long-term NT systems receiving the ongoing application of commercial fertilizers.

While stratification of soil chemical proprieties under NT and natural systems is well documented, 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 NT systems. Thus, a better understanding of these impacts would help to improve yields, reduce production costs and improve fertilizer and lime efficiency. Although stratification of soil chemical proprieties under NT is well-documented studies so far have focused on stratifying the topsoil into only two to three layers at maximum, except for the recent studies of Rheinheimer et al. (2018a); b), working with a high-resolution stratified soil sampling (every 1 cm) from very acidic soils in the tropics. Thus, we believe that

long-term experiments in non-tropical lands receiving NPK allow the evaluation of the nutrient management effects on the NT system stability and soil fertility in the topsoil. The objective of this study was to evaluate the long-term superficial fertilization effects on soil acidity stratification of the topsoil (0–15 cm) and its stratification in soil layers with 2.5 cm depth increment (0–2.5, 2.5–5, 5–7.5, 7.5–10, 10–12.5, and 12.5–15 cm) in three long-term NT experiments across Oklahoma, US.

2. Material and methods

2.1. Study locations (field trials)

Our study was carried out in 2019 at three long-term NT trials in Oklahoma (OK): Perkins $(35^{\circ}59'41.3"N, 97^{\circ}02'32.7"W)$, established in 1996 (26-y) and converted to NT in 2004; Lahoma $(36^{\circ}23'18.2"N, 98^{\circ}06'27.3"W)$, established in 1970 (52-y) and converted to NT in 2010; and Stillwater $(36^{\circ}07'19"N, 97^{\circ}05'28"W)$, established in 1969 (53-y) and converted to NT in 2010. Soil classification (Web soil survey), average and range of rainfall, and average air temperature of each location are described in Table 1.

Since their establishment, the long-term trials have been used for the conceptual development of the yield prediction and NPK responsiveness, and for the impact of continuous winter wheat on changes in the soil system over time (Aula et al., 2016; Fornah et al., 2020; Raun et al., 1998, 2011). The treatments used at each site are presented in Table 2 and were selected to evaluate the long-term effect on soil chemical attributes for this study.

2.2. 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. Nitrogen was applied as urea (46 % N), phosphorus as triple superphosphate (20 % P), and potassium as potassium chloride (50 % K), respectively (Table 2). 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 (1.905 cm diameter by 30.48 cm length), and 25 cores from 0 to 15 cm depth were sampled from each plot. Additional 25 soil cores from 0 to 15 cm were sampled and separated into layers of 0–2.5, 2.5–5, 5–7.5, 7.5–10, 10–12.5, and 12.5–15 cm using a knife and mixed to make a plot-composite sample. Soil samples were air-dried until constant weight, and then ground with a hand roller to pass through a 2 mm sieve.

2.3. Soil analysis

Soil pH was determined with 1:1 soil to deionized water ratio by using an ion-selective (H⁺) glass electrode. Soil organic carbon (OC) was determined using a dry combustion carbon/nitrogen analyzer (CN 628, LECO Corporation, St. Joseph, MI, USA). Exchangeable Aluminum (Al_{KCl}) was determined by an inductively coupled plasma atomic emission spectroscopy (ICP-AES) (SPECTRO Analytical Instruments Kleve, Germany) after extraction of 5 g of soil with 25 mL potassium chloride (1 M KCl) (Bertsch and Bloom, 1996). Aluminum saturation (Al%) was calculated as a measure of Al toxicity: [Al% = (Al_{KCl}/ECEC) \times 100], where Al_{KCl} is the exchangeable Al (cmol kg⁻¹) and ECEC is the effective cation exchange capacity, which is the sum of exchangeable Na⁺, K⁺, Ca^{2+} , and Mg^{2+} extracted by 1 M NH₄OAc at pH 7.0 (cmol kg⁻¹) and determined by ICP-AES plus Al_{KCl} (cmol kg⁻¹) (Schroder et al., 2011). Soil nitrate (NO₃-N) was extracted with 1 M KCl solution and quantified by a Flow Injection Autoanalyzer (LACHAT, 1994 - QuickChem Method 12-107-04-1-B - LACHAT Instrument, Milwaukee, WI). The soil buffer index (BI) was measured for samples with pH< 5.5 (minimum pH required for a continuous winter wheat production system) after adding the Sikora buffer solution to the same suspension previously used

Table 1

Trial location (Oklahoma), soil classification and description, year of establishment, average rainfall and range, and average temperature for three study sites used in this study. Averages retrieved from 1994 to 2018 (Oklahoma Mesonet).

Location (Trial)	Soil series and Description	Establishment (Year)	No-till Establishment (Year)	Annual mean rainfall (mm)	Range (mm)	Annual mean temp. (°C)
Lahoma	Pulaski; (Coarse-loamy, mixed, superactive, nonacid, thermic Udic Ustifluvents	1970	2010	771	503–1314	15.6
Stillwater	Kirkland; (Fine, mixed, superactive, thermic Udertic Paleustolls)	1969	2010	922	606-1493	15
Perkins	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

Table 2

Fertilizer treatments used in the study: nitrogen (N, as urea), phosphorus (P, as triple superphosphate), and potassium (K, as potassium chloride).

Location	kg ha ⁻¹ of N-	kg ha ⁻¹ of N-P-K								
Lahoma	0-0-0	0-20-56	22-20-56	67-0-56	67-20-56	90-20-56				
Stillwater	0-0-0	0-29-37	90-0-37	90-29-37						
Perkins	0-0-0	56-0-0	56-29-0	112-0-0	112-29-0	168-0-0	168–29–0			

for the first pH determination and then the pH was determined again by a glass electrode (Sikora, 2006).

2.4. Statistical analysis

Statistical analyses were performed using JMP, Version 16 (SAS Institute Inc., Carry, NC,1989–2022). For comparison among all NPK fertilizers (treatments) in each location (Table 2), treatments were differentiated at each stratified layer for all the analyzed soil acidity parameters. For this, each site was treated as a fixed effect and analyzed separately. Data were differentiated using ANOVA and Honest Significant Difference (HSD) to identify differences in means (Tukey at $\alpha = 0.05$).

Simple linear regressions of pH, Al_{KCl}, and Al% against N rates were performed. The increased N rates comprising all locations were used because the 'effect test' showed that all response variables were significant for the isolated effect '*N* rate' (Table 3). This allowed us to compare the behavior of soil acidity parameters against N rates for soils sampled from the topsoil (0–15 cm) and the stratified topsoil samples. Since all

exchangeable cations (except Al_{KCl}) were significant at least at p < 0.05 (Table 3), K+Na+Ca+Mg were summed up to comprise the sum of base (SB), and then SB was regressed against the N rates as well. Nonlinear regressions were performed to describe the relationship of pH to Al_{KCl} , Al%, and NO₃—N; and of N rates to Al_{KCl} and Al% only for soils sampled from the arable layer (0–15 cm). All models were performed using the whole dataset of measurements. Particularly, not all arable layer samples (0–15 cm) were able to determine NO₃—N. In that case, we provided results comprising all replicated data from the stratified topsoil layers only, thus ensuring enough observations to compose a reliable model.

For BI, it was evaluated only in the arable layer (0–15 cm) and the first stratified soil layer within the arable layer (0–2.5 cm), where a considerable amount of soil samples presented pH< 5.5 (Table 3 and Supplementary Table S1). The standard error (SE) of the mean was used to compare both methods of soil sampling at each N rate since the interaction '*Depth*×*N* rate' was significant (Supplementary Table S1). Thus, as in the case of the linear regressions, locations were treated as a random effect for comparison goals of agronomic importance. The SE

Table 3

Analysis of variance for the effect of soil layer (depth), location (trial), and nitrogen application rates on soil chemical attributes.

Source	pН	BI	TC	NO ₃ -	Al _{KCl}	Al%	K	Ca	Mg	Na	ECEC
				Ν							
						p-value					
	Stratified topsoil										
Depth	<.0001	—	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001
Trial	<.0001	—	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Depth imes Trial	0.0044	—	0.0064	<.0001	<.0001	0.0068	0.1815	<.0001	<.0001	<.0001	<.0001
N rate	<.0001	—	<.0001	<.0001	<.0001	<.0001	0.0266	<.0001	<.0001	<.0001	<.0001
Depth imes N rate	0.0014	—	0.0029	<.0001	<.0001	<.0001	0.9188	0.0034	0.6228	0.0141	0.4619
Trial imes N rate	<.0001	—	0.1863	0.515	<.0001	0.0006	0.1512	0.2527	0.0465	<.0001	0.3046
Depth imes Trial imes N	0.7117	_	0.8394	0.3423	<.0001	0.0661	0.9975	0.7421	0.7661	0.1204	0.8968
rate	_						.		.	.	
	Topsoil (0-15 cm)										
Trial	<.0001	<.0001	<.0001	0.3027	<.0001	0.0265	<.0001	<.0001	<.0001	<.0001	<.0001
N rate	<.0001	0.816	0.0059	0.0968	<.0001	0.0008	0.204	<.0001	<.0001	0.0132	<.0001
Trial imes N rate	0.0438	<.0001	0.689	0.2773	0.0001	0.034	0.453	0.0279	0.3956	0.0181	0.0108

BI: buffer index. TC: Total carbon (%). AI_{KCI} : Exchangeable—Al. Al%: Aluminum saturation = (AI_{KCI} /ECEC) × 100. ECEC: Effective cation exchange capacity, which is the sum of exchangeable Na⁺, K⁺, Ca²⁺, and Mg²⁺ measured in 1 mol L⁻¹ NH₄OAc at pH 7.0 (cmol kg⁻¹) plus Exchangeable—Al (cmol kg⁻¹). Blue, green, yellow, red, and uncolored numbers are, respectively, p < 0.0001, p < 0.001, p < 0.001, p < 0.05, and non-significant (p > 0.05). "—": not applicable.

was chosen because it estimates the variability across samples of a given population, which was different for each N rate since the need for BI measurement was also variable across samples.

3. Results and discussion

3.1. Soil pH

In all locations, treatments that received N fertilizer resulted in lower pH than the unfertilized check in the first layers sampled (Fig. 1). More specifically, soil pH ranges in Lahoma (5.0–7.0), Stillwater (4.5–6.5), and Perkins (4.6–7.3) differed in the layers at where a stratification was observed.

In Lahoma, the treatment 90–20–56 (N-P-K in kg N ha⁻¹) was the most acidified in all soil layers (Fig. 1). 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 layer. Both treatments with N rate of 67 kg ha⁻¹ significantly acidified the soil in relation to the unfertilized control in the 0–7.5 cm soil layer. In the 7.5–10 cm soil layer, treatment 67–0–56 presented lower pH than treatments containing no N. When comparing the rates of 22 and 67 kg N ha⁻¹, treatment 67–20–56 presented lower pH in the 0–2.5 cm soil layer. Both treatments with a rate of 67 kg ha⁻¹ of N had lower pH in the 2.5–5 cm soil layer than the treatment 22–20–56. Lastly, differences were found between treatments 67–0–56 and 22–20–56 kg N ha⁻¹ in the 5–7.5 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 (Fig. 1). When comparing treatments receiving 90 kg ha⁻¹ of N with the 0–29–37, differences were found in the layers within the 0–10 cm. Even though numerical differences were observed in Fig. 1 between treatments 0–0–0 and 0–29–37, 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 (Fig. 1). Same was found for treatments receiving 112 kg N ha⁻¹ except for the 12.5–15 cm layer. In case of the N rate of 56 kg ha⁻¹, differences were found in the soil

layer from 0–7.5 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.

Overall, treatments containing rates of 56 and 112 kg N ha⁻¹ demonstrated some inconsistence across the soil layers. In the 0-2.5 cm soil layer, treatments 56-29-0 and 56-0-0 were significantly higher in pH than the treatment 112–0–0. However, no difference in this layer was found between treatment 112-29-0 and treatments receiving 56 kg N ha^{-1} . In the 2.5–5 cm layer, treatments receiving 112 kg N ha^{-1} had significantly lower pH than the treatment 56-29-0 except for when compared to the 56–0–0 treatment. Both treatments receiving 56 hg N ha^{-1} differentiated from treatments receiving 112 kg ha^{-1} in the 5-7.5 cm soil layer. In the 7.5-12.5 cm soil layer, differences were found between treatments receiving 56 kg N ha⁻¹ and the treatment 112-29-0. No difference between the treatments rates of 56 and 112 kg N ha^{-1} were found in the 12.5–15 cm soil layer. Differences in pH were not likely influenced by the P application at this location as no significant differences within the same N rate were observed, except for the 56–0–0 and 56–29–0 treatments within the 0–2.5 cm soil layer.

As expected, the pH stratification is more evident when comparing higher N application rates as opposed to those receiving less or no N fertilizer. This is further evidenced by the significant negative relationships (p < 0.001, $R^2 \ge 0.38$) that existed between soil pH and increased N application rate across all locations regardless of the soil sampling method (topsoil and stratified topsoil) (Fig. 2a and b). Similar results were found by Schroder et al. (2011), who also noticed that over a 30-year dataset the N rate was more influential towards soil acidification. In our case, as previously mentioned, higher N rates applied across locations resulted in more soil acidification (Figs. 2a and b).

The effect on pH stratification is also present in the control treatments. This provides evidence of the effect of the crop residual accumulation and eventual N mineralization on the soil surface. When N, C, and Sulfur (S) are mineralized from organic matter, there is a release of H^+ due to 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 N addition increased wheat 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., 2019).



Fig. 1. Soil pH (left) and Exchangeable aluminum (Al_{KCl}) (right) from stratified soil samples as a function of fertilizer application (N-P-K in kg ha⁻¹) in three long-term NT trials. Colored lines are treatments. Horizontal bars indicate the Honest Significant Difference (HSD), (p \leq 0.05). Stratification of pH is mostly related to the nitrogen rate. Useful conversion factor: Al (mg kg⁻¹) / 90 = Al, in cmol_c kg⁻¹.



Fig. 2. The linear relationship between soil pH (a and b) or sum of base (SB) (c and d) and N rate of soils sampled from 0 to 15 cm (a and c) and stratified layers (b and d). Stratified layers comprise all locations. Each layer is n = 51. *** : p < 0.001. SB = Na⁺, K⁺, Ca^{2+,} and Mg²⁺ measured in 1 mol L⁻¹ NH₄OAc at pH 7.0 (cmol kg⁻¹).

The increase in biomass and residue accumulation due to N fertilization could also cause a greater stratification of soil properties 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 (high responsiveness to N application) thus resulting in greater stratification when N is applied. Crop residue accumulation on the soil surface and its decomposition appears to influence the pH stratification since unfertilized plots also present such stratification patterns although not to the same extent. Results from Perkins also illustrates the crop residue accumulation or where the same rate of N was applied, the effect of P fertilizer addition increased yield and biomass accumulation (*data not shown*), thus making the stratification and acidification more evident across different depths.

3.2. Sum of exchangeable base cations (sum of base)

The long-term application of increased rates of N fertilizer reduced the sum of exchangeable K+Ca+Mg+Na linearly regardless of the sampling method (Fig. 2c and d). At the greatest application rate of 168 kg N ha⁻¹, SB was decreased by approximately 62.8 % for the topsoil sampling, similarly by 57 % for the stratified topsoil sampling (respectively Figs. 2c and d). The results of our study concur with those which have demonstrated that long-term applications of different sources of N, including urea, greatly reduced the levels of exchangeable bases (Schroder et al., 2011; Stone et al., 1991; Bouman et al., 1995; Barak et al., 1997; Russell et al., 2006). Thus, no differences in the levels of exchangeable cations are produced by the same application rate of different N sources (Schroder et al., 2011). Such a behavior is not expected for nitrate-based fertilizers since there is no nitrification and thus no soil acidification.

Overall our results show that rates of N fertilizers have to be properly managed and applied regardless of the commercial source. In the case of our study, urea was utilized and has shown higher acidification as rates of application were increased. Not surprisingly, SB reduction as a function of increased N rates followed the same pattern as soil pH (Figs. 2a, 2b, 2c, and 2d). When evaluating the stratified soil samples, it is noticeable that lower pH ad SB levels are presented in the uppermost soil layers given the surface application of N fertilizers under NT. This also agrees with the results presented in Fig. 1.

3.3. Exchangeable aluminum and aluminum saturation

In Lahoma, Al_{KCl} was numerically greater in the treatment receiving 90 kg ha⁻¹ of N (Fig. 1). However, no differences were found between the treatments across the studied soil layers. Nonetheless, patterns of Al_{KCl} stratification appear to differ among the locations. Still for this location, the highest Al_{KCl} was found in the 7.5–10 cm soil layer, differently from the other locations of the study where the highest concentration was found in the 2.5–5 cm soil layer. Overall, our results are in agreement with Schroder et al. (2011) since the two low N rates in their study (34 and 68 kg N ha⁻¹) did not meet the agronomic rate required for the optimum wheat yield resulting in soil pH values that indicate a less acidic medium.

In Stillwater, the N rate of 90 kg ha⁻¹ (highest N rate) also presented greater Al_{KCl} values than other treatments (Fig. 1). In the soil layers within 0–7.5 cm, significant differences were found between the treatment 90–29–37 and treatments not receiving N fertilization (0–0–0 and 0–29–37). In the 7.5–10 cm soil layer, the treatment 90–29–37 was greater than the check although not greater than the 0–29–37 treatment. However, when comparing the treatment 90–0–37 with treatments containing no N rates, significant differences were found in the 2.5–5. and 5–7.5 cm soil layers.

In Perkins, the relationship between N rate and Al_{KCl} was evident and the impact is more aggravated in the surface layers. Rates of 168 kg N ha⁻¹ presented higher Al_{KCl} than the unfertilized control in the 0–2.5 and 2.5–5 cm (Fig. 1). The treatment 168–0–0 also resulted in greater Al_{KCl} in the 5–7.5 cm soil layer than the unfertilized control. The same treatment (168–0–0) also demonstrated higher levels of Al_{KCl} than treatments receiving 56 kg ha⁻¹ in the 0–2.5 and 2.5–5 cm soil layers. Overall, the 168–0–0 treatment had the greatest Al_{KCl} from the surface to the depth of 12.5 cm. This treatment was significantly greater than the 0–0–0 to the depth of 7.5 cm, then no impact of treatments upon Al_{KCl} values was observed beyond that layer. In the first 2.5 cm, the 168–29–0 and 112–0–0 were also significantly higher in Al_{KCl} than the check; however, below this point, there was no 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 on Al_{KCl}

in any of the soil layers. Similar results were also observed in Stillwater and Lahoma.

The long-term application of increased rates of N fertilizer significantly increased exchangeable Al (Al_{KCl}) compared with the control (Fig. 3a and b), except in Lahoma. Exchangeable Al increased exponentially and linearly with increasing N application rate for the topsoil and stratified topsoil, respectively (Fig. 3a and b). Our results are somewhat similar to those reported elsewhere in the literature. Moore and Edwards (2005), after sampling soils from the arable layer, reported that long-term N application at rates ranging from 65 to 260 kg $\rm N~ha^{-1}$ exponentially increased exchangeable Al from $< 1.0 \mbox{ mg Al kg}^{-1}$ at the beginning of their study to $> 100 \text{ mg Al kg}^{-1}$ in the subsequent years. Schroder et al. (2011) observed Al_{KCl} linearly increase as N rates increased, which agrees with our results presented in Fig. 3b when soils were sampled from stratified layers. Noticeably, besides the different models fitted to explain Al_{KCl} increments with increased N rates for both sampling methods, there was also a different range of Al_{KCl} for those methods, where arable layer sampling showed a range of 0 to ~ 100 mg Al kg⁻¹ while stratified sampling provided Al_{KCl} contents ranging from 0 to \sim 200 mg Al kg⁻¹ (Figs. 3a and 3b). Therefore, there is a dilution effect when soil is sampled from 0 to 15 cm, which might underestimate toxic Al at specific depths that might impact germination and other plant performances.

Aluminum saturation (Al%), which is the percentage of the effective cation exchange capacity (ECEC) occupied by exchangeable Al (Al_{KCl}), has been used as an indicator of Al toxicity rather than Al_{KCl} in soils and as an index for the lime requirement (Fox, 1979; Farina et al., 1980). The Al% increased with the N application rate (p < 0.001, $R^2 \ge 0.28$) and exhibited the same patterns as Al_{KCl} (exponential and linear) for the two soil sampling methods (Figs. 3c and 3d). Those results are also similar to the findings of Schroder et al. (2011).

As observed for Al_{KCl}, the upper-end in the Al% range of stratified topsoil was twice as much as in the topsoil, presenting respectively 0 to ~50 % and 0 to ~25 % (Fig. 3c and d). This will become a problem at the threshold of Al% (~10 %), from 0 – 10 cm, when crop yields might be already impacted (Martins et al., 2014). At specific depths within the topsoil, where root systems might be active, such a value would be diluted if the soil is sampled from 0 to 15 cm. In the case of our study, both highest upper-ends of Al_{KCl} and Al% were found in the first 5 cm of the topsoil, more specifically from 2.5 to 5 cm, where roots are generally very active. At the same soil layer, Al% was already > 15 % with

112 kg N ha⁻¹ applied while it was < 10 % for the sampled topsoil (Fig. 3c and d). Kamprath (1984) indicated that Al%< 10 % was needed for maximum yields of wheat on Oxisols and Ultisols. For the same crop, Gillespie et al. (2020) referred to Oklahoma soils with Al%< 16 % as being at the lower level of potential Al phytotoxicity. Aluminum saturation ranging from 15 % to 30 % was found to cause up to a 98 % loss in winter wheat forage yield and a grain-crop failure (Wise, 2002).

A significant inverse exponential relationship was observed between Al% and soil pH and it was found for both topsoil sampling and stratified topsoil sampling (p < 0.001, and R^2 raged from 0.31 to 0.79) (Fig. 4). The results of our study are similar to those of other researchers who have reported that soil pH and Al% are inversely related (Schroder et al., 2011; Pintro et al., 2005; Kariuki et al., 2007). The same was found for the relationship between Al_{KCl} and pH (Supplementary Fig. S1).

3.4. Organic carbon

According to Fig. 5, the impact of the nutrient management on OC was mostly found in the first soil layer (0–2.5 cm) regardless of the location. The differences across treatments are primarily influenced by N application. It is hypothesized that the stratification is intensified as N rate increases because N is the most limiting nutrient in these studies, as previously mentioned. Thus, 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 to the same extent as the uppermost surface layers.

In Lahoma, the highest levels of OC from 0 to 2.5 cm were found in the treatments 67–20–56 and 90–20–56, (1.54 % and 1.38 % OC, respectively) (Fig. 5). However, the OC levels for treatment 67–20–56 was only greater than the treatments not receiving N, the unfertilized control, and 0–20–56 (0.99 % and 1.01 % OC, respectively). From 2.5–10 and 12.5–15 cm, no difference was found in the OC values for any treatment. In the 10.0–12.5 cm layer, the OC value of 0–20–56 (0.69 % OC) was lower than that of 67–20–56 and 67–0–56 (0.76 % and 0.75 % OC, respectively). While there was little statistical difference below 2.5 cm, the trend of the 67 kg N ha⁻¹ treatments having the highest levels of OC was maintained to up to 15 cm.

In Stillwater, the highest levels of OC were also associated with increased N fertilization. Treatments containing N application presented numerically higher OC than treatments receiving no N (Fig. 5). Among the four treatments, differences were found only between the



Fig. 3. The linear and exponential relationship between Exchangeable-Al (Al_{KCl}) (a and b) or aluminum saturation (Al) (c and d) and N rate of soils sampled from 0 to 15 cm (a and c: exponential) and stratified layers (b and d: linear). Stratified layers comprise all locations. Each layer is n = 51. ***: p < 0.001. Al% $= (Al_{KCl}/ECEC) \times 100$, where: ECEC is the effective cation exchange capacity, which is the sum of exchangeable Na⁺, K⁺, Ca^{2+,} and Mg²⁺ measured in 1 mol L⁻¹ NH₄OAc at pH 7.0 (cmol kg⁻¹) plus Exchangeable—Al (cmol kg⁻¹). Useful conversion factor: Al (mg kg⁻¹) / 90 = Al, in cmol_c kg⁻¹.



Fig. 4. The exponential relationship between aluminum saturation (Al%) and pH of soils sampled from 0 to 15 cm (*a*) and stratified layers (*b*). Each layer is n = 51 and *combined* has all trials. *** : p < 0.001. Al% = (Al_{KCI}/ECEC) × 100, where: ECEC is the effective cation exchange capacity, which is the sum of exchangeable Na⁺, K⁺, Ca²⁺, and Mg²⁺ measured in 1 mol L⁻¹ NH₄OAc at pH 7.0 (cmol kg⁻¹) plus Al_{KCI} (cmol kg⁻¹).

unfertilized check (0–0–0, 1.07 % OC), and the 90–29–37 (1.41 % OC), and 90–0–37 (1.37 % OC) in the 0–2.5 cm soil layer. The treatment 0–29–37 was not different from any other treatment. In the 5–7.5 and the 10–12.5 cm soil layers, OC was significantly greater for the

90–29–37 treatment (0.83 % and 0.78 % OC, respectively) than for the unfertilized check (0.76 % and 0.72 % OC, respectively).

In Perkins, the addition of N and P had a significant impact on the soil OC in the 0-2.5 cm soil layer (Fig. 5). The greatest OC level was found when N and P were applied at the highest rate (168-29-0, 1.93 % OC) and this treatment was significantly greater than the others with exception of the treatment 112-29-0 (1.74 % OC). These two treatments also presented the greatest OC across all locations. This effect might be due to the higher N and P rates applied in Perkins. The treatment 112-29-0 also presented greater OC than the 56-0-0 (1.24 % OC), 112-0-0 (1.2 % OC) and the unfertilized check (1.13 % OC). Phosphorus fertilization appears to increase biomass production above the N limitation in this area. The treatments 168-29-0 and 112-29-0 were statistically higher in OC than all treatments not receiving P, illustrating the P fertilization response in the OC levels. Nonetheless, the 112-29-0 treatment was numerically higher in OC than the treatment 168-0-0 (1.41 % OC) which presented a higher rate of N. No statistical difference in OC was found in the other soil layers.

3.5. Effective cation exchange capacity

The overall results of ECEC across all locations show a stratification pattern where ECEC increases as the stratified soil layer increases up to 15 cm in the topsoil (Table 3 and Fig. 5). Only in Perkins, the stratification was not as evident as in the other two locations (Fig. 5). As shown in Table 3 and Fig. 5, in addition to the significant ECEC stratification within the topsoil there was also the effect of NPK treatments and N rates, except for Lahoma (Fig. 5).

Although all locations exhibited a pattern of stratification, their overall ECEC values were very contrasting, and not all trials presented significant differences for the NPK applications when comparing each layer separately (Fig. 5). This might rely on the fact that soil types are different (Table 1) and thus they respond differently to the several combinations of NPK applications. In Stillwater and Perkins, it is clear that control treatments are showing greater ECEC than those receiving N application, mainly in the first stratified layers of the topsoil (Fig. 5).

Even though ECEC takes into account the Al_{KCl} , the control treatment presented the greater ECEC, and such treatment received no N, thus Al_{KCl} levels were lower than in other treatments. In that case, it was expected a lower ECEC in comparison to treatments receiving N. However, the Al% was generally much lower than 50 % even at the first layer



Fig. 5. Organic carbon (left) and effective cation exchange capacity (ECEC) (right) from stratified soil samples as a function of fertilizer application (N-P-K in kg ha⁻¹) in 3 long-term NT trials. Colored lines are treatments. Horizontal bars indicate the Honest Significant Difference (HSD), ($p \le 0.05$). Differences in organic carbon were found only in the first 0–5 cm regardless of the location and it appears to be mostly driven by N application. ECEC is the sum of Na⁺, K⁺, Ca²⁺, and Mg²⁺ measured in 1 mol L⁻¹ NH₄OAc at pH 7.0 (cmol kg⁻¹) plus Exchangeable—Al (cmol kg⁻¹). ECEC is in units of mol charge⁻¹ (mol_c).

of the topsoil, and the SB was greater when no N was applied (given the higher pH obtained in such treatment), which likely contributed to the greater ECEC. At the deeper layers in the topsoil, N fertilizers do not affect considerably the soil pH, therefore, the SB increases and Al% decreases contributing to an increase in ECEC. This makes sense because, in the case of our study, the base saturation plays a bigger role in the ECEC (>50 %) than the Al_{KCl}.

The positive linear relationship between soil pH and ECEC is shown in Supplementary Fig. S2. Since ECEC increases as a function of pH then most ECEC is occupied by 'basic cations' instead of H+Al. In this case, it is safe to assume that the upper soil layers with lower pH, thus more H+Al and fewer 'basic cations', will present a lower ECEC. Therefore, for our study, ECEC is more dependent on soil pH than on soil carbon, which is reinforced by the inverse (although weak) relationship between TC and ECEC (Supplementary Fig. S2). In the uppermost soil layer, where the OM content is higher; as a consequence of the long-term NT, is also where the pH is lower (surface N fertilization), so that CEC comprised mostly of 'basic cations' is lower as well. Essentially, even at high levels of TC in the upper layers of the topsoil, it is possible to assume that the negative charges provided by the OM will be less at low soil pH than that at a neutral to high soil pH soil. This is because of the difference between the pKa of organic matter at a low soil pH and when the soil pH is higher.

Additional results considering the BS saturation (BS%), which comprises the percentage saturation of basic cations in the ECEC are shown in Supplementary Table S2. Even though Pearson correlations are significant, the Spearman values are greater. This apprehends because the relationship between those variables is not linear, as previously shown in this study, and the Spearman correlation evaluates the monotonic relationship and it is not restricted to only linear associations (Supplementary Table S2).

3.6. The pH and nitrate relationship

Since the conversion of ammonium—N (NH₄—N) to nitrate—N (NO₃—N) acidifies soil by releasing protons—H⁺ we have assessed the $NO_3N \times pH$ relationship and presented the results in Fig. 6. The urea fertilizer contains N in amide form which is swiftly converted by soil microorganisms into ammoniacal form and then into nitrate form. This, aligned with the fact that nitrification rates from urea are greater than other N sources (International Plant Nutrition Institute, I. P. N. I, 2019), makes it safe to assume that the presence of NO₃—N is an indication of soil acidification. The inverse relationship between NO₃—N and pH converged a quadratic—with—plateau model where a threshold indicated that soil pH was reduced from ~7.2–4.9 ± 0.6 when the NO₃—N contents reached 21.9 ± 2.6 mg kg⁻¹, and no further pH reduction was observed beyond that point (Fig. 6). These findings agree with the fact



that the degree of acidification depends on the ability of the soil to resist change (buffer capacity), and the amount of N applied (International Plant Nutrition Institute, I. P. N. I, 2019).

Therefore, despite the pH reduction as NO₃—N formation increases up to 21.9 ± 2.6 mg kg⁻¹, there was no soil pH change from that point until up to 60 mg NO₃—N kg⁻¹ (Fig. 6). These higher levels of NO₃—N are distributed in the uppermost soil layer (0–2.5 cm) as represented by the squared blue points (Fig. 6). This layer, as previously mentioned, is most affected by the superficial N application under NT, and such accumulation of NO₃—N might be harmful to the environment if the excess N is lost by leaching and/or runoff. Also, the nitrogen use efficiency (NUE) is prone to be reduced if there is no crop response to the excessive N.

3.7. Buffer index

The BI is a method of measuring soil pH after adding the Sikora buffer solution to the same suspension previously used for the first soil pH determination (Sikora, 2006). It is required when the first pH measurement gives values below the desired pH for optimum crop growth. In our study, the desired pH for a continuous winter wheat system in Oklahoma is 5.5.

Overall, Fig. 7 clearly shows that, except for the control (0 kg N ha⁻¹), all N rates provided lower BI values from 0 to 2.5 cm when compared to the 0–15 cm soil layer. As previously elucidated in this study, soil pH was lower in the uppermost soil, which agrees with the lower BI values presented from 0 to 2.5 cm. On the other hand, the no application of N fertilizer (control) showed no difference between the two soil layers used for comparison.

The lower BI from 0 to 2.5 cm indicates a higher reservoir of acidity than the 0-15 cm agreeing with the lower pH found for the uppermost stratified soil layer. This might affect seed germination (Wala et al., 2022) and herbicides' effectiveness (Jamshidi et al., 2021) because the 0-15 cm would dilute the potential acidity of the first layers where seeds and herbicides are placed.

Attention must be paid to the relationship between the BI, an indicator of reserve acidity (RA), and the active acidity (AA) determined by the simple pH measurement. In this sense, soil samples with a BI of 6.5 not necessarily would present the same pH, instead, they could present different pH values, e.g., 4 and 5 (<5.5), which would indicate a weaker acidity reservoir of the former as compared to the latter, however, a higher AA. Therefore, in the case of our study, soils presenting a lower BI from the uppermost soil layer could have the same pH (AA) as those



Fig. 7. Buffer index (BI). Results comprise all locations. Bars are the standard error (SE) of the mean. SE is calculated by dividing the standard deviation of the sample by the square root of the sample size. When different, results are significant at $p \leq 0.001$.

from the topsoil layer (0–15 cm), however, the uppermost soil layer (0–2.5 cm) presented a lower pH, higher Al%, and lower BI than the topsoil layer (0–15 cm). Further, a simple Pearson correlation has shown significant positive and negative relationships between BI and pH, and between BI and Al%, respectively, regardless of the soil sampling layer (Supplementary Table S3). Thus, for this work, it is safe to assume that the higher AA the higher the RA which agrees with other studies found in the literature (van Breemen et al., 1984, Bowman et al., 2008, Gruba et al., 2013, Lu et al., 2015).

4. Final considerations

4.1. Soil pH as affected by nitrogen fertilization

The uppermost soil layers (0–5 cm) documented the lowest pH at all locations (Fig. 1), with values ranging from 4.4 to 6.5 which often fell below the optimum of 6.5–7 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). Our results demonstrated that N fertilization affecting soil acidification is more evident. Although treatments not receiving N fertilization presented pH stratification to some degree, soil acidification increased proportionally to the N rate across all locations.

4.2. Organic matter influence on aluminum

As observed in Fig. 1, Al_{KCl} levels rapidly increase when soil pH drops below around 5.3, which was also found by Gillespie et al. (2021). Nonetheless, aluminum toxicity is a recurrent cause of crop failure in extreme acid soil in Oklahoma (Schroder et al., 2011) due to its negative effect on root growth and above-ground biomass. At the same time that N fertilization acidifies the soil, it also contributes to the highest yield increase and crop biomass production and accumulation, further increasing the organic matter in the topsoil (Reicosky et al., 1995). Our data provided evidence of OC increase; however, the effect is mostly noticeable in the 0-5 cm soil layer (Fig. 5). Our results also demonstrated that Al_{KCl} concentrations are lower in the soil layer (0–2.5 cm) than in the layer below it (2.5–5 cm) (Fig. 1). This is probably due to the fact that high OC content in the 0–5 cm soil layer could contribute to lowering Al_{KCl} concentration in the 0-2.5 cm soil layer thus reducing aluminum toxicity by forming complexes of Al-dissolved organic carbon (Antonangelo et al., 2022; Li et al., 2022).

5. Conclusions

Soil pH stratification was observed in all locations and it is primarily due to N application. With the adoption of NT, stratification becomes more pronounced due to less soil mixing, repeated application of fertilizers without soil plowing, and surface residue deposition. Our data suggest that soil acidity parameter stratification is evident after nine years of NT (Lahoma and Stillwater) and 14 years of NT (Perkins). Greater OC stratification was also mostly dependent on 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_{KCI} concentrations even at lower pH.

The soil sampling depth to determine lime recommendations under NT should be evaluated as the stratification in the active surface soils is well pronounced as shown in this study. For most of the soil acidity parameters tested, the sampling depth would highly influence the lime recommendations since stratification was observed for both Al% and BI. Failing to ensure proper soil sampling depth may affect lime requirement in NT thus increasing the production cost significantly. Future studies are encouraged to relate the crop yields with soil sampled from stratified layers of topsoil under NT aiming to evaluate if sampling depth affects addressing surface acidification.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2022.105624.

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