**Soil Chemical Properties as Influenced by Long-term Glyphosate-resistant Corn and
Soybean Production in the Central Great Plains, USA**

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Abstract

Glyphosate-resistant (GR) corn (*Zea mays* L) and soybean (*Glycine max* (L.) Merr.) production and glyphosate application could affect soil chemistry due to indirect effects on soil organic matter (SOM) and nutrient cycling. We determined soil chemical properties after 15-yr of glyphosate application, continuous GR corn (CC) and GR corn-soybean rotation (CS) under no-tillage. Herbicide treatments were 1) glyphosate at 0.42 and 2) 0.84 kg a.e. ha⁻¹ each applied twice in all years, 3) an alternating treatment of glyphosate at 0.84 kg ha⁻¹ applied twice followed the next year by a non-glyphosate herbicide treatment, and 4) a non-glyphosate herbicide treatment in all years. Results showed that crop rotation and soil sampling depth affected SOM, pH, and nutrient concentrations. Soil pH, exchangeable Ca and K concentrations at the soil surface were reduced with CC production. In contrast, SOM, Fe, Mn, NO₃-N, SO₄-S and soil P concentrations in the top 7.5 cm of the soil increased with CC production. Glyphosate application affected concentrations of P, NO₃-N, SO₄-S and Fe but not pH, Ca, Mg, Mn or Zn. Findings of this long-term study showed that crop management practices including herbicide program and crop rotation could affect soil chemical properties but cropping systems with CC increased soil surface fertility compared to CS production.

Keywords: Glyphosate-resistant crops; Soil chemical properties; Nutrient cycling; Soil organic matter

1. Introduction

Glyphosate [(N-phosphonomethyl) glycine] currently is the predominant herbicide used for weed control worldwide, largely due to widespread adoption of glyphosate-resistant (GR) crops. Rapid adoption of GR crops is partly due to simplified weed management with GR technology, excellent weed control, and reduced herbicide cost (Gianessi, 2005; Green, 2012). In the USA, GR soybeans acreage went from 17% in 1997 shortly after GR soybeans became commercially available to 94% in 2014. Similarly, planted acreage of GR cotton (*Gossypium hirsutum* L.) and corn in 2014 were 91 and 89%, respectively (USDA, 2015). The use of GR crops is credited with the dramatic increase in the adoption of reduced and no-tillage practices in the USA (Price et al., 2011). Reduction in tillage intensity provides more soil cover and reduced runoff and soil erosion (Dick et al., 1991; Baveye et al., 2011; Singh et al., 2012), increased soil aggregation and water infiltration (Zibilske and Bradford, 2007; Blanco-Canqui et al., 2010), and enhanced soil organic matter accumulation (Blanco-Canqui and Lal, 2008; Kumar et al., 2012; Presley et al., 2012).

Growing GR crops could also have direct and indirect consequences on soil quality through changes in the quantity and quality of crop and weed residues returned to the soil (Motavalli et al., 2004; Weidenhamer and Callaway, 2010). For instance, increased reliance on glyphosate for weed control in GR crops has resulted in weed species shifts (Westra et al., 2008; Wilson et al., 2007; Johnson et al., 2009). Litter and root exudates from these new weed species could alter nutrient pools that can influence soil chemistry and plant-microbe interactions (Weidenhamer and Callaway, 2010). To date, much of the literature on the effects of GR crops and herbicide application on soil have focused on soil microorganisms and plant litter decomposition. The findings of most studies have generally shown little to no effects of GR

crops or glyphosate application on soil microbial biomass and diversity (Hart and Brookes, 1996; Wardle and Parkinson, 1991; Haney et al. 2000; Busse et al., 2001; Liphadzi et al., 2005; Ratcliff et al., 2006; Weaver et al., 2007; Nakatani et al., 2014). Yet others have reported transient or significant effects of glyphosate –based herbicide application on soil microbial community (Hart et al., 2009; Lupwayi and Blackshaw, 2012). Some formulations of glyphosate are reported to cause significant negative effects on earthworm activity (Piola et al., 2013). In a greenhouse study, Gaupp-Berghausen et al. (2015) found a significant reduction in earthworm activity (casting) and reproduction following glyphosate application. If glyphosate use can modify the microbial communities and activity of soil organisms, this will have significant impact on soil chemistry because the soil biota is directly involved in organic matter decomposition and nutrient cycling.

Glyphosate applied to soil can act as a direct source of organic carbon (C) and stimulate microbial activity through increased C mineralization rates. In a laboratory experiment using Weswood silt loam soil, Haney et al. (2000) showed that glyphosate stimulated soil microbial activity resulting in higher C and N mineralization rates. The authors observed linear relationships between increasing glyphosate use rate and amounts of C and N mineralized. Similarly, microbial respiration was greater in glyphosate-treated soils but glyphosate treatment had no effect on microbial community structure and microbial biomass potassium (K) but exchangeable K concentration increased with glyphosate application (Lane et al., 2012). On a silty loam soil in southern Mississippi, USA, Locke et al. (2008) reported greater soil organic carbon (SOC) and N concentration with continuous GR corn compared with non-GR corn production. The authors speculated that weed biomass in plots with GR corn was greater than that of non-GR corn during the fall and winter months, and could have contributed to the

significant SOC measured in the GR corn plots. These changes in C storage associated with GR crop production can affect nutrient cycling and availability in agroecosystems.

Glyphosate is a chelator of divalent metals and can form complexes with macro and micronutrient cations (Bernards et al., 2005; Barrett and McBride, 2006). The presence of metal cations such as calcium (Ca), iron (Fe), and zinc (Zn) in spray water has been shown to reduce glyphosate activity (Stahlman and Phillips, 1979). Chelation of metal cations by glyphosate in the soil could affect nutrient availability and uptake by crops. Seed and leaf concentrations of Ca, magnesium (Mg), manganese (Mn), and Fe in non-GR soybean were reduced following glyphosate application (Cakmak et al., 2009). There is evidence that glyphosate application induce Fe, Mn and Zn deficiency in glyphosate based crop production systems (Jolley et al., 2004; Eker et al., 2006; Bott et al., 2008; Zobiolo et al., 2010). In addition, glyphosate sorption to the soil is by ligand exchange through the phosphonate group similar to phosphorus (P) sorption. Glyphosate therefore competes for the same sorption sites on the soil exchange complex as P (de Jonge et al., 2001; Gimsing and Borggaard, 2002). This property of glyphosate could affect P cycling in soils with long-term glyphosate usage. Increased P status in the soil can cause desorption and remobilization of previously sorbed glyphosate (Gimsing et al., 2007; Cáceres-Jensen et al., 2009). The reverse effect is possible in which long-term glyphosate application can affect P retention and availability in the soil.

Despite the potential effect of GR crop production on soil chemical properties through nutrient transformation from SOM decomposition and indirect effect of glyphosate on soil nutrients, limited research has been done to elucidate the impacts of glyphosate application on soil chemical properties. The objectives of this study were to determine (I) crop yield and residue

quality and SOM content, and (II) soil chemical properties after long-term glyphosate use and GR crop production.

2. Materials and Methods

2.1. Site Description

This study was conducted at the Kansas State University Northwest Agricultural Research-Extension Center near Colby, Kansas (39°37' N, 101°69' W; 1114 m in elevation) on Keith silt loam soil (Fine-silty, mixed, superactive, mesic Aridic Argiustolls). The Keith series consists of deep, well-drained soils formed in calcareous loess, and has slopes of 0-6% (Soil Survey Staff, 2015). Long-term average annual precipitation at the experimental site is 463 mm, of which more than 75% (356 mm) is received during the growing season from April through September. Mean annual temperature is 11°C. The last killing frost in spring usually occurs around May 1, and the first killing frost in the fall occurs between late-Septembers to the second week of October. The average frost-free growing season is 163-d.

Soil fertility analysis conducted at the beginning of the study in 1998 did not differ among the preassigned crop rotation and herbicide treatment combinations plots. Averaged across the four experimental blocks, soil pH in the top 15 cm of the soil was 7.9, available P was 68.2 mg kg⁻¹, and exchangeable K was 1037 mg kg⁻¹. Similarly, SOM and soil NO₃-N concentrations were 23.0 g kg⁻¹ and 6.6 mg kg⁻¹, respectively. The soil at the study site contains 21% sand, 59% silt and 20% clay, with a textural class of silt loam. The average cation exchange capacity (CEC) was 24.5 cmol_c kg⁻¹ of soil. The relatively high CEC and the medium textured soil at the study site means the soil is productive and that the major limiting factors for crop production are soil moisture and N.

2.2. Study Set-up and Treatments

The study was initiated in 1998 to determine the effects of glyphosate rate and use patterns on changes in weed species composition and population dynamics in GR corn and GR soybean production under dryland no-till practices in northwestern Kansas. Prior to this study, the plot area was managed as annual sorghum-forage production system where conventional tillage and herbicides were used to manage weeds during fallow period before forage sorghum planting. The experiment was a two-factorial split-plot set in a randomized complete block design with four replications. Main plots were continuous corn (CC), and corn and soybean in rotation with both phases of the corn-soybean rotations [corn-soybean (CS) and soybean-corn (SC)] grown each year. These crop systems were evaluated because corn and soybean are the major GR crops grown in the USA and are grown either as a monoculture or as rotation systems (corn-soybean system). Sub-plots were four herbicide treatments consisting of glyphosate only herbicide management and a combination of glyphosate and other herbicide chemistry. Brief descriptions of the treatments are:

- (1) Glyphosate at $0.42 \text{ kg a.e ha}^{-1}$ + ammonium sulfate (AMS) at 2% w/v applied in-crop twice each year (TRT 1);
- (2) Glyphosate at 0.84 kg ha^{-1} + AMS at 2% w/v applied twice in-crop each year (TRT 2);
- (3) Glyphosate at 0.84 kg ha^{-1} + AMS at 2% w/v applied twice in-crop every other year alternated with non-glyphosate herbicide treatment in both crops (TRT 3); and
- (4) Non-glyphosate herbicide treatment for each crop in all years (TRT 4).

Glyphosate in its isopropylamine salt form was used each of the first 4-yr of the study and the K-salt form of glyphosate was used in each of the later years. The non-glyphosate treatments for

corn consisted of recommended use rates of acetolachlor plus isoxaflutole and atrazine applied pre-emergence to crop and weeds followed in most years by fluroxypyr or mesotrione plus nicosulfuron with appropriate adjuvants applied post-emergence. The non-glyphosate treatments for soybean during the first three years of the study consisted of recommended use rates of clomazone plus alachlor applied pre-emergence followed by clethodim with crop oil concentrate applied post-emergence. During the next 11-yr, the non-glyphosate soybean treatment was changed to sulfentrazone plus pendimethalin applied pre-emergence followed by imazamox plus encapsulated alachlor and quizalofop with appropriate adjuvants applied post-emergence. Details on the type and amounts of herbicides applied for each year of the study is provided in the supplementary Table.

Prior to crop planting, the entire experimental area received a pre-plant burn-down application of glyphosate at 0.84 kg ha^{-1} in each of the first 10-yr, glyphosate at 1.12 kg ha^{-1} in each of the next 5 yr, and paraquat at 0.84 kg ha^{-1} in the final year. All treatments were fixed in space for the duration of the study. Use rates of individual herbicides for the non-glyphosate treatments for both crops were changed infrequently in an effort to improve weed control. The selected herbicide treatments though without a true control (non-herbicide treatment) allows for the comparison of glyphosate only based herbicide management program with an alternative herbicide management regime where glyphosate is applied in conjunction with other herbicide chemistry. The goal is to evaluate weed selection pressure and population shifts and crop productivity as influenced by long-term glyphosate use in GR crop production systems. Nonetheless, the study also provides a real opportunity to determine the long-term effects of GR crop production and concomitant glyphosate and other herbicide use on soil chemistry.

Plot sizes were 48.8 m × 30.5 m for the crop the main plots, and 12.2 m × 30.5 m for the herbicide management treatments. Corn and soybean hybrids used changed occasionally over the course of the study as Seed Company's replaced discontinued hybrids with newer, better-adapted hybrids. However, in each instance only locally adapted hybrids were used. In each year of the study, GR corn hybrids were planted in 0.8-m row spacing with a John Deere 7300 no-till planter at average seeding rate of 42,500 seeds ha⁻¹ in the later part of spring (April 20 through May 15). Nitrogen fertilizer as urea ammonium nitrate was applied at 112 kg ha⁻¹ prior to planting corn in each crop rotation. Additionally, 93 L ha⁻¹ of starter fertilizer consisting of 13 kg ha⁻¹ N and 45 kg ha⁻¹ P₂O₅ was banded 5 cm to the side and 5 cm below the seed zone of each corn row during planting.

During the first 4-yr of the study, GR soybeans were planted Mid-May through the first week in June (May 10-June 6) at a seeding rate of 380,000 seeds ha⁻¹ in the same manner as corn except no starter fertilizer was applied to soybeans in the rotation. In all subsequent years, soybean planting was done with a Sunflower 9411 double disc drill in 0.4-m row spacing at the same planting window and seeding rate of 380,000 seeds ha⁻¹. Narrowing of row spacing was done in an attempt to improve weed control in non-glyphosate herbicide treatments. Grain yields were determined by harvesting an area of 3 m × 6.1 m from each plot with a plot combine. Corn was usually harvested in late September (~ 110-d growth cycle) while soybean was harvested in October (~112-d growth cycle) in each year of the study. Yield data were adjusted to 13.5 g kg⁻¹ grain moisture content.

2.3. Soil Sampling and Analysis

Soil samples were collected at the beginning of the study in spring of 1998. Four soil cores (2.5 cm diam.) per plot were randomly collected at 0 to 15 cm soil depth. The samples

were air-dried and used for initial physical and chemical characterization of the study site. Additional soil samples were taken in November 2013 after corn and soybean harvest to determine changes in soil chemical properties after 15-yr of GR corn and soybean production. In the second sampling period, nine soil cores (2.5 cm diam.) were randomly collected in each plot from the 0 to 15 cm soil depth at 7.5 cm increments. The collected samples were composited per depth ($n = 9$) for each plot, air-dried, crushed, and sieved to pass through a 2-mm stainless steel screen. The sieved soil samples were then analyzed for pH and soil extractable nutrients at the KSU soil testing laboratory using standard soil testing procedures (Leikam et al., 2003). Briefly, pH and electrical conductivity were determined potentiometrically by an electrode (Thomas, 1996). Soil nitrate-N ($\text{NO}_3\text{-N}$) concentration in samples were determined colorimetrically after the soil samples were extracted with 2 M KCl. Soil test P was determined by Mehlich-3 extraction method (Mehlich, 1984), exchangeable Ca, Mg, Na, and K by NH_4OAc extraction (Knudsen et al., 1982), Fe, Mn and Zn were extracted with the DTPA extraction (Lindsay and Norvell, 1978). Soil organic matter was determined by the modified Walkley-Black procedure with a correction factor of 0.68 (Walkley, 1947).

2.4. Crop Residue Sampling and Analysis

After crop harvest in 2013, soil surface residue samples were randomly collected from four 1-m^2 quadrats in the central rows and composited for each plot. Residue samples were washed with deionized water to remove soil, and oven dried at 60°C for 48 h for dry matter determination. The samples were then ground to pass a 1-mm screen in a Wiley mill (Model 4, Thomas-Wiley Laboratory Mill, Thomas Scientific, and Swedesboro, NJ) and analyzed for total C and N concentration using Leco C/N analyzer (Leco Corp., St Joseph, MI).

2.5. Statistical Analysis

Data were subjected to ANOVA as a split-plot design using the PROC MIXED procedure of SAS ver. 9.2 (SAS Institute, 2006). Crop rotations, herbicide treatments and soil sampling depth were the fixed effects while replications and their interactions were considered random effects. Because there was no significant differences in soil chemical properties between the two phases of corn-soybean rotation, data were averaged across the two phases and the mean values used for all statistical tests. The LSMEANS procedure of PROC MIXED along with adjusted Tukey was used for mean comparisons. Interactions and treatment effects were considered significant when F test P values were ≤ 0.05 . Regression analyses were conducted with the Proc Reg procedure in SAS to determine the relationship between soil pH and micronutrient availability.

3. Results

3.1. Grain yield and crop residue

Year $\times$ herbicide treatments $\times$ crop rotation interaction had a significant ($P < 0.0001$) effect on corn grain yields at the early (first two years) and toward the end of the study (2010 and 2011). Compared to the first 2-yr of the study, glyphosate-based herbicide treatments resulted in significantly lower corn yields toward the end of the study. For example in 2011, grain yields with the alternative herbicide treatments (TRT3 and TRT 4) were on the average 1.6-fold greater than yields of the glyphosate-based treatments (TRT1 and TRT2) with both CC and CS production (Fig. 1).

Similarly, year $\times$ herbicide treatment interaction had a significant ($P < 0.0001$) effect on soybean grain yields. The application of glyphosate at either 0.42 or 0.84 kg a.e ha⁻¹ did increase soybean yields in the early years of the study but yields were significantly reduced towards the end of the study, particularly at the lowest glyphosate use rate (Fig. 2). Soybean yield in 2011 with alternative herbicide treatments were twice as much as yields produced when glyphosate was the sole herbicide used.

Herbicide treatment had no effect on quantity or quality of crop residue collected on the soil surface in 2013. Averaged across crop rotations, residue amounts were 5364, 5416, 5589, and 4674 kg ha⁻¹ for TRT1, TRT2, TRT3, and TRT4, respectively. Residue amounts and quality differed between CC and CS crop rotations. Total surface residue amounts measured in 2013 was 5836 kg ha⁻¹ for CC and 3727 kg ha⁻¹ for CS (Table 1). Greater crop residue in the CC crop production was expected because aboveground biomass production of corn is greater than soybean. Total residue C concentration was not different between the cropping systems (Table 1) and C: N was lower in CS compared to CC production system.

3.2. Soil organic matter and pH

After 14-yr of the study, herbicide treatment had no effect ($P = 0.104$; Table 2) on SOM. However, sampling depth $\times$ crop rotation interaction ($P = 0.048$; Table2) effect on SOM was significant. In the top 7.5 cm of soil, SOM concentration for CC production was 32 g kg⁻¹, greater than that of CS (27 g kg⁻¹) when averaged across herbicide treatments. Below 7.5 cm, SOM was not different between crop rotations (Table3).

Soil pH was not affected by herbicide application ($P = 0.103$) or crop rotation ($P = 0.093$, Table 2). However, pH was affected by sampling depth ($P < 0.0001$) and sampling depth $\times$ crop rotation interaction ($P < 0.0001$). Soil pH differed between the two crop rotations only in the

upper 7.5 cm soil depth. The CS crop rotation had greater soil pH than CC in the top 7.5 cm but at deeper soil depths, pH did not differ between the crop rotations (Table 3).

3.3. Available phosphorus (P), nitrate-N and sulfate-S

Available P differed among the herbicide treatments. Averaged across crop rotations and soil depth, soil P concentration increased with glyphosate only treatments (TRT 1 & TRT 2). Soil P was not different between the alternative herbicide treatments (Fig. 3a). Available soil P concentration was affected by sampling depth and crop rotation interaction ($P = 0.014$). Averaged across herbicide treatments, soil P in the top 7.5 cm with CC production (97 mg kg^{-1}) was 26% greater than that of CS (77 g kg^{-1} , Table 4). The difference in available P between the crop rotations was not significant below 7.5 cm.

Soil $\text{NO}_3\text{-N}$ concentration was affected by crop rotation and herbicide management (Table 2). Residual soil $\text{NO}_3\text{-N}$ was significantly reduced in the glyphosate-based treatments compared to the other herbicide treatments (Fig. 3). Averaged across sampling depth and herbicide treatments, residual soil $\text{NO}_3\text{-N}$ with CC was 18.5 mg kg^{-1} , greater than CS (12.7 mg kg^{-1}). This represents three-fold increase in soil $\text{NO}_3\text{-N}$ concentration compared to initial levels. Sulfate-S ($\text{SO}_4\text{-S}$) was significantly ($P = 0.001$) affected by herbicide $\times$ sampling depth interaction. In the top 7.5 cm of the soil, $\text{SO}_4\text{-S}$ concentration was greater in the glyphosate only treatments (Fig. 4a). However, $\text{SO}_4\text{-S}$ concentration was not different among the herbicide treatments below 7.5 cm. Crop rotation $\times$ sampling interaction effects on $\text{SO}_4\text{-S}$ was significant (Fig.4b). $\text{SO}_4\text{-S}$ was not different between the crop rotations below 7.5 cm. However, at the surface 7.5 cm, CC production resulted in greater $\text{SO}_4\text{-S}$ concentration.

3.4. Exchangeable Cations

Herbicide management had a significant effect on soil exchangeable K but not Ca, Mg and Na (Table 2). Soil K was greatest with the lower glyphosate application (TRT 1) but no differences were observed in K concentration between the highest glyphosate application rate and the remaining herbicide treatments (Fig.1c). Soil K concentration differed among crop rotation systems at each sampling depth. In the top 7.5 cm, soil K with CC was lower than CS. However, at 7.5 to 15 cm soil depth, K concentration under CC was greater than that of CS (Table 3). Similar to K, soil Ca concentration was affected by crop rotation $\times$ depth interaction ($P < 0.0001$; Table 2). At the soil surface, Ca concentration for CC production (2789 mg kg^{-1}) was significantly lower than CS (4116 mg kg^{-1}) (Table 4). Below 7.5 cm however, Ca concentration did not differ between the cropping systems (Table 4). Exchangeable Mg concentration was affected by soil depth $\times$ crop rotation interaction ($P < 0.0001$; Table 2). With CC production, soil Mg was similar at both sampling depths. However, with CS, Mg concentration in the top 7.5 cm was greater than Mg concentration at lower soil depths. Neither herbicide management nor crop rotation had any effect on soil Na concentration. Averaged across herbicide management and crop rotations, exchangeable Na concentration in the top 7.5 cm was 26.7 mg kg^{-1} and that below 7.5 cm was 24 mg kg^{-1} .

3.5. Micronutrients (Fe, Mn and Zn)

Herbicide treatments had no effect on soil Mn or Zn concentrations. However, Fe concentration differed among the herbicide treatments (Table 2). Averaged across crop rotation and sampling depth, glyphosate only treatments did increase soil Fe concentration compared to the alternative herbicide treatments (Fig. 3d). Sampling depth and crop rotation interaction

affected Fe and Mn concentrations in the soil but not Zn (Table 2). Averaged across herbicide treatments, Fe concentration with CC was 2.7-fold greater than that of CS at the soil surface. Similarly, Mn concentration with CC was 55 mg kg⁻¹, greater than 31 mg kg⁻¹ for CS (Table 3). Conversely, Fe and Mn concentrations below 7.5 cm soil depth were not different between the crop rotations.

4. Discussion

4.1. Herbicide effects

Long-term glyphosate use could have direct and indirect effects on soil chemistry due to potential activity with soil nutrients and effects on microorganisms that are directly involved in residue decomposition and nutrient cycling. Herbicide management affected available P, Fe, K, NO₃-N and SO₄-S concentrations but had no significant effect on soil pH, SOM, exchangeable Ca, Mg, Mn or Zn concentrations in our current study. Glyphosate is known to compete with P for binding sites on the soil exchange complex (de Jonge et al., 2001; Gimsing and Borggaard, 2002), and this can affect P availability in the soil. It is plausible that the presence of glyphosate caused increased retention of P on the soil exchange sites and explains greater P concentration observed in the glyphosate only treated plots compared to other herbicide treatments. Our findings also showed that herbicide application had no significant effect on Mn availability but soil Fe concentration was greater in the glyphosate only treatments.

These observed differences in Fe concentration among the herbicide treatments could be due to formation of glyphosate-Fe complex that can potentially reduce Fe bioavailability for plant uptake. Ability of glyphosate to form strong glyphosate-metal complexes has been well documented (Bernards et al., 2005; Huber, 2007). Chelation of metal cations with glyphosate use is known to reduce micronutrient availability, particularly Mn and Fe uptake by GR crops (Eker

et al., 2006; Bott et al., 2008; Cakmak et al., 2009). Although herbicide management affected exchangeable K concentration, this effect could be masked by crop removal. The K-salt form of glyphosate was the formulation used in the 10 most recent years of the study and could have added K to the soil. However, the amount of K added to the soil due to glyphosate application is insignificant compared to the inherent greater K levels in the soil at the study site and crop removal. Both corn and soybeans yields tended to be significantly reduced at the lower glyphosate application rate (TRT1) over the study period. It is possible that the reduced crops yield in TRT1 resulted in reduced nutrient removal and relatively greater soil K levels but not K additions from glyphosate application.

Sulfate and $\text{NO}_3\text{-N}$ accumulation are transient events in the soil but is worth noting that herbicide management can have indirect effect on these nutrients in the soil. Adjuvants and other additives apply with herbicides could potentially affect soil chemistry. Greater $\text{SO}_4\text{-S}$ concentration in the top soil of the glyphosate-based treatments (Fig. 4a) could be due to ammonium sulfate (AMS) applied with glyphosate. To increase effectiveness of weed control, glyphosate tank mixes usually contains 1 to 2% by weight of AMS as a conditioner in the spray solution. Over time, this $\text{SO}_4\text{-S}$ can accumulate in the soil surface particularly in a semi-arid region like western Kansas that receives about 460 mm annually. Similarly, TRT3 had AMS and urea ammonium nitrate as additives with the herbicide application and possibly explain greater residual $\text{NO}_3\text{-N}$ with this treatment. A lack of a “true control” treatment (herbicide-free plots) is a drawback of our current study especially when glyphosate was applied to the entire experiment in a pre-plant burn-down. This could confound the effects measured in the non-glyphosate based treatments. Notwithstanding, this long-term field experiment provides a unique opportunity to determine soil chemical properties as affected by glyphosate-based herbicide management

program compared to alternative herbicide management that combines glyphosate and other herbicides. Besides, our experimental design mimics a typical crop production situation where growers will change herbicide chemistries and formulations infrequently over several growing seasons to provide good weed control.

4.2. Crop rotation effects

Long-term cropping systems experiments are needed to provide information on the interaction effects of crop management (tillage, crop rotations, fertilization and herbicide) on soil nutrient availability and crop yields. The intent of this study was to determine soil chemistry after 14-yr of corn monoculture or corn produced in rotation with soybean. Most of the differences in soil chemistry between CC and CS were mainly confined to the top 0-to7.5 cm of the soil profile. This is expected because the study was conducted under no-till conditions that tend to increase SOM content and nutrient availability near the soil surface compared to tilled systems (Edwards et al., 1992; Howard et al., 1999; Houx III et al., 2011). Eliminating tillage reduced soil disturbance resulting in greater soil carbon storage from above- and below-ground crop biomass (Franzluebbers and Hons, 1996). This explains the significant SOM and nutrient accumulation in the top 7.5 cm in the present study. SOM accumulation in the surface of no-till systems has been widely reported by several authors (Ismail et al., 1994; Reicosky et al., 1995; Sainju et al., 2007; Wright et al., 2007; Kumar et al., 2012; Liu et al., 2013). In a long-term tillage and cropping systems study, Kumar et al. (2012) showed that soil organic carbon (SOC) content was greater with no-till than reduced-till and moldboard-plowed treatments in the top 0 to 20 cm of the soil, but below 20 cm tillage effects were not significant.

The greater concentration of SOM observed in CC compared to CS may be due to differences in the magnitude of crop residue returned to the soil and the C:N ratio between the two crop production systems. Corn grain yield with CS rotation was slightly greater than corn monoculture (CC) but crop residue in CS system was lower (as shown in Table 3 for residue measured in 2013) because corn is produced once in every 2-yr while corn is grown every year in the CC system. Corn is a high biomass-producing crop and can contribute approximately 8.0 Mg ha⁻¹ in crop residue (USDA-NASS, 2012) annually to the soil surface. In contrast, soybeans are a low residue-producing crop and contribute about 2.2 Mg ha⁻¹ yr⁻¹ (USDA-NASS, 2012) residues to the CS cropping system. In addition, crop residue C: N ratio measured in 2013 in CS was less than that in CC cropping system, probably due to higher N concentration of the soybean residue in the CS rotation. Because C: N ratio of the crop residue in CS is < 30, the threshold C: N ratio for residue decomposition and net N mineralization to occur (Alexander, 1977), it is plausible that residue decomposition and mineralization will be greater in CS than CC cropping system.

Similar to SOM, the soil pH measured after 14-yr of the study was significantly lower in CC production system. Relative to the initial soil pH (7.9), CC production caused 0.5 units drop in soil pH when averaged across the two sampling depths. However, rotations with corn and soybean production (CS) resulted in a 0.3 units drop in pH relative to the initial pH levels. The significant decrease in soil pH in the CC production system is due in part to inorganic N fertilizer application to corn. The CC production system had fourteen annual applications of 112 kg N ha⁻¹ as urea ammonium nitrate. Rotations with corn and soybean received N application only in the corn phase of the production systems. Therefore, the CS system had half the total amount of N applied to CC. It is not surprising that the observed decrease in pH is associated with

CC was nearly 50% greater than that of the CS production. Our findings agree with Reddy et al. (2013) who showed that soil surface (5 cm) pH in CC was lower than continuous soybean or CS rotation after 4-yr of corn and soybean production. Others (Bouman et al., 1995; Barak et al., 1997; Fageria et al., 2010; Schroder et al., 2011) have reported decreasing soil surface pH as a consequent of long-term N fertilizer application. This occurs because during nitrification of NH_4^+ into NO_3^- , excess H^+ are produced which are responsible for decreasing soil pH when urea or ammonium containing N fertilizers are applied. Available P was significant greater in the surface soil of the CC production compared to CS rotation. The difference occurred because fertilizer inputs occurred differently in the two-crop production systems. Fertilizer was applied only in the corn phase of CS cropping system while both N and P were applied every year to CC production. The differences are also likely due to crop removal differences between crops in the rotation. Typical grain P concentrations are 0.35-0.59% in soybean and 0.20-0.32% in corn (Leikam et al., 2003), suggesting the amount of P removed will be greater in the CS than CC production. This finding is significant because cropping system management could have potential implications on nutrient availability and accumulation in the soil. Accumulation of P in the surface soil is not surprising because P is relatively immobile within the soil and tends to accumulate on the soil surface in no-till systems in the absence of tillage to redistribute P to lower soil depths. We do not anticipate runoff from our study site because of the relatively lower annual precipitation amounts (460 mm) but high soil test P levels from long-term crop management practices could create environmental quality challenge in areas prone to significant surface runoff.

The greater soil $\text{NO}_3\text{-N}$ concentration for the CC rotation is due to accumulation of N from annual fertilizer application compared with CS crop production that received N application

only in the corn phase of the rotation. In addition, SOM has greater influence on the capacity of the soil to supply nutrients including N, P, sulfur (S) and micronutrients. Therefore, mineralization of SOM can contribute significantly to the overall increase in residual N. Soil S mostly originates from mineralization of SOM and cropping systems that accumulates high SOM will tend to have greater content. This explains the observed differences in $\text{SO}_4\text{-S}$ concentration between CC and CS crop rotation systems. Soil K concentration differed between the crop production systems. The findings were due to nutrient uptake patterns of corn and soybean that occurred at different depths in the soil profile because of differences in rooting system. Corn has a fibrous root system with roots concentrated near the surface soil. In contrast, soybean has a tap root system with less dense roots and may draw nutrients from lower portions of the soil profile. This explains why K concentration in CS cropping system was greater than CC in the top 7.5 cm of the soil. Similarly, greater Mg concentrations in the surface 7.5 cm in the CS plots may be due to limited Mg uptake from the upper layers of the soil because most of the roots of soybean are located in the sub-soil.

Differences Ca concentration between the cropping systems could be explained by increased in soil acidity observed in the top 7.5 cm with CC treatments. Regression analysis showing soil Ca concentration as a function of pH indicates a linear relationship between pH and soil Ca level with an r^2 of 0.77 (Fig. 5a). These results agree with the findings of Ismail et al. (1994) who showed a significant decline in exchangeable Ca and Mg concentrations in a long-term (20-yr) no-till continuous corn production system because of decreased in soil pH with N fertilization. Similarly, soil acidification from urea and anhydrous ammonia application caused significant depletion of exchangeable Ca and Mg from the soil exchange complex (Bouman et al., 1995; Schroder et al., 2011).

Micronutrient availability is closely associated with changes in soil pH and SOM content. Greater Fe and Mn concentrations with CC may be due to relatively lower pH measured at the soil surface in CC compared with CS. Regression analysis showed an inverse relationship between pH and soil micronutrient concentration. Iron and Mn availability is increased when soil pH is slightly acidic to neutral conditions (Fig. 5b and c). As soil pH increases, micronutrient availability to plants are reduced because of the change in ionic form of the cations into metal oxides or hydroxides that are relatively insoluble (Brady and Weil, 2008). Soil conditions such as high pH, free calcium carbonate in the soil and low SOM favor development of micronutrient deficiencies (Rashid and Ryan, 2004). Micronutrient deficiencies can cause significant yield losses. For example, soybeans yield losses caused by Fe deficiency chlorosis in the US northern Great Plains is estimated to be \$120 million per annum (Hansen et al., 2004). Besides pH, differences in SOM content between the cropping systems can influence nutrient availability. Mineralization from SOM can contribute significant amounts of micronutrients particularly Fe to the CC production system that has greater SOM content.

4.3. Summary and conclusion

Crop rotation and soil sampling depth affected pH, $\text{NO}_3\text{-N}$, exchangeable Ca, K, Mg, Fe, Mn, Zn, and P concentrations. Continuous corn production reduced soil pH, and exchangeable Ca concentrations in the soil surface. Crop residue, SOM, and plant nutrient concentrations in the top 7.5 cm of the soil profile increased with CC production. Herbicide treatments had no effect on soil pH, SOM, exchangeable Ca, Mg, Mn or Zn concentrations. However, $\text{NO}_3\text{-N}$, $\text{SO}_4\text{-S}$, available P and Fe concentrations were affected by herbicide application. Our findings showed that herbicide application and crop rotation could affect soil chemical properties in GR crop

production systems. Overall, cropping systems with CC increased soil surface fertility compared to CS production. Further investigation is needed to determine if nutrient uptake of major dryland crops in the US Great Plains are affected by long-term glyphosate use.

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Figure Caption

Fig 1. Herbicide management effects on corn yield in continuous corn (a), and corn-soybean (b) cropping system over the study period.

TRT1= Glyphosate at 0.42 kg a.e ha⁻¹ + ammonium sulfate (AMS); TRT2 = Glyphosate at 0.84 kg ha⁻¹ + AMS at 2% ; TRT3 = Glyphosate at 0.84 kg ha⁻¹ + AMS at 2% w/v applied twice in-crop every other year alternated with non-glyphosate herbicide treatment in both crops; TRT4= Non-glyphosate herbicide treatment for each crop in all years

Fig. 2. Herbicide management effects on soybean yield over the study period.

TRT1= Glyphosate at 0.42 kg a.e ha⁻¹ + ammonium sulfate (AMS); TRT2 = Glyphosate at 0.84 kg ha⁻¹ + AMS at 2% ; TRT3 = Glyphosate at 0.84 kg ha⁻¹ + AMS at 2% w/v applied twice in-crop every other year alternated with non-glyphosate herbicide treatment in both crops; TRT4= Non-glyphosate herbicide

Fig.3. Soil phosphorus, (b) nitrate-N (NO₃-N), (c) potassium, and (d) iron concentrations measured in 2013 as affected by herbicide application. Error bars represent one standard error of the mean. Means followed by the same letter (s) are not significantly different using the least squares means (LSMEANS) and adjusted Tukey multiple comparison procedure ($P > 0.05$).

TRT1= Glyphosate at 0.42 kg a.e ha⁻¹ + ammonium sulfate (AMS); TRT2 = Glyphosate at 0.84 kg ha⁻¹ + AMS at 2% ; TRT3 = Glyphosate at 0.84 kg ha⁻¹ + AMS at 2% w/v applied twice in-crop every other year alternated with non-glyphosate herbicide treatment in both crops; TRT4= Non-glyphosate herbicide

713

714 Fig. 4. Sulfate-sulfur concentration measured in 2013 as affected by (a) herbicide application (b)
715 crop rotation. Error bars represent one standard error of the mean. Means followed by the same
716 letter (s) are not significantly different using the least squares means (LSMEANS) and adjusted
717 Tukey multiple comparison procedure ($P > 0.05$).

718 TRT1= Glyphosate at $0.42 \text{ kg a.e ha}^{-1}$ + ammonium sulfate (AMS); TRT2 = Glyphosate at 0.84
719 kg ha^{-1} + AMS at 2% ; TRT3 = Glyphosate at 0.84 kg ha^{-1} + AMS at 2% w/v applied twice in-
720 crop every other year alternated with non-glyphosate herbicide treatment in both crops; TRT4=
721 Non-glyphosate herbicide.

722 CC = Continuous corn; CS = corn-soybean rotation

723

724 Fig. 5. Relationship of soil PH with Calcium (a), iron (b), Manganese (c), and Zinc (d)
725 availability measured in 2013.

726

727 Table 1. Soil surface residue amounts and quality measured in 2013 as affected crop rotation
 728 system.

Crop rotation	Surface residue	Total N content	Total C Content	C : N ratio
	kg ha ⁻¹	g kg ⁻¹		
Corn-corn	5836a [†]	12.8b	375a	30.2a
Corn-soybean	3727b	15.2a	381a	26.1b
SE [‡]	468	1.0	8.0	1.9

729 [†]Means followed by same letter (s) are not significantly different using the least squares means
 730 (LSMEANS) and adjusted Tukey multiple comparison procedure ($P > 0.05$). Data are averaged
 731 across four herbicide treatments and four replicates ($n = 16$).

732 [‡]SE = Standard error of the mean

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736 Table 2. Analysis of variance summary of the effects of herbicide management, and crop rotation on soil organic matter (SOM), pH,
 737 phosphorus (P), nitrate-N (NO₃-N) exchangeable calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), iron (Fe), manganese
 738 (Mg) and zinc (Zn) in soil samples collected at 0 to 7.5 cm and 7.5 to 15 cm in 2013.

Treatment effect	pH	SOM	NO ₃ -N	K	Ca	Mg	Na	P	Fe	Mn	Zn
Rotation	0.0932	0.1043	0.0025	0.6942	0.2755	0.3108	0.4286	0.2019	0.0270	<.0001	0.8347
Herbicide	0.1027	0.3509	0.0080	0.0289	0.0684	0.6841	0.6212	0.0031	0.0368	0.153	0.1616
Rotation x herbicide	0.5747	0.8759	0.2744	0.0591	0.9031	0.4183	0.4788	0.9856	0.2115	0.6620	0.9625
Depth	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	0.0036	<.0001	<.0001	<.0001	<.0001
Rotation x depth	<.0001	0.0476	0.0953	<.0001	<.0001	<.0001	0.380	0.0141	<.0001	<.0001	0.2587
Herbicide x depth	0.7936	0.5423	0.2251	0.1903	0.7026	0.1649	0.9057	0.9186	0.2099	0.8320	0.3552
Rotation x depth x herbicide	0.5263	0.8497	0.9365	0.3125	0.7491	0.6971	0.4837	0.4938	0.2766	0.9382	0.7556

739 Treatment effects in bold are significant at $P \leq 0.05$.

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Figure 1

Figure 2

Figure 3

Figure 4

Fig. 4.

Figure 5

Fig.5.