

Impact of phosphorus placement on corn rooting dynamics under long-term strip-tillage

by

Marcie Renee Sindt

B.S., Concordia University, Nebraska, 2015

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2017

Approved by:

Major Professor
Dr. Colby J. Moorberg

Copyright

© Marcie Renee Sindt 2017.

Abstract

Soil profile phosphorus (P) distribution is known to influence rooting dynamics. However, it's unknown if P placement in long-term no-till management influences root development in high P-testing soils. The research objective was to compare impacts of P placement on corn (*Zea mays*, L.) root development. Replicated field trials were conducted in Manhattan, KS on a long-term, strip-tilled, corn-soybean-wheat rotation. Five P treatments were applied to the corn rotation for 11 years and included a control (0 kg), 22 kg starter and 67 kg broadcast (BC+ST), 90 kg broadcast (BC), 22 kg starter and 67 kg deep band (DB+ST), and 90 kg deep band (DB) of P₂O₅ per hectare. This study was conducted in 2015 and 2016 – years 10 and 11 of the long-term study, respectively. All treatments tested above 20 ppm P, with highest concentrations at 10-15 cm for DB, and 0-5 cm for all other treatments. Root length treatment differences were observed primarily in the upper 35 cm during vegetative growth. In 2015, both total root length (TRL) and root length by depth were significantly less for starter P treatments. Though no TRL differences were observed in 2016, BC+ST exhibited reduced root length compared to BC across multiple depths. The BC and DB treatments had similar root growth in 2015. In 2016, greater TRL and root length across multiple depths were observed for BC compared to DB. Although grain yield was not affected, this study showed that P placement in high testing soils impacts root development, particularly during vegetative growth.

Table of Contents

List of Figures	vi
List of Tables	vii
Acknowledgements	viii
Chapter 1 - Literature Review.....	1
INTRODUCTION	1
PHOSPHORUS	1
Plant Phosphorus.....	1
Soil Phosphorus	1
Phosphorus Uptake	3
Plant Adaptations to Limited Phosphorus.....	4
Phosphorus Deficiency	6
Phosphorus Fertilization	6
Phosphorus Fertilizer Application	7
LONG-TERM STRIP-TILL MANAGEMENT	10
The Tillage Effect	10
Effect of Tillage on Soil Nutrients.....	11
Effect of Tillage on Root Growth	12
METHODS OF ROOT ANALYSIS	13
Minirhizotron Method.....	13
Tube Design and Installation	14
Image Acquisition.....	15
Minirhizotron Advantages	16
Minirhizotron Limitations.....	16
Root Core Method.....	17
Sampling Equipment.....	17
Sampling Strategy	19
Processing Soil Core Samples	20
Advantages of the Root Core Method	20
Limitations of the Root Core Method.....	21

Applications in Corn	21
Root Analysis Conclusion.....	22
SUMMARY	23
REFERENCES	24
Chapter 2 - Impact of phosphorus placement on corn rooting dynamics under long-term strip- tillage	40
ABSTRACT.....	40
INTRODUCTION	41
MATERIALS AND METHODS.....	43
Site Description.....	43
Root Observations.....	44
Root Cores	46
Soil Test Phosphorus Sampling	46
Statistical Analysis.....	47
RESULTS	48
Manhattan, Kansas Weather	48
Soil Test Phosphorus by Depth.....	48
Root Biomass	49
Root Imaging	49
Total Root Length.....	50
Root Length by Depth.....	50
Mean Root Diameter.....	52
Root Diameter by Depth	52
Grain Yield.....	53
DISCUSSION.....	53
CONCLUSIONS	57
REFERENCES	59
TABLES	64
FIGURES.....	65
APPENDIX A. Supplemental Information	76
APPENDIX B. SAS Code	83

List of Figures

Figure 2.1 Weather summary throughout growing season..	65
Figure 2.2 Soil test phosphorus (Mehlich-III P) by depth..	66
Figure 2.3 Square root transformed root biomass (g) by depth.	67
Figure 2.4 Total root length 2015	68
Figure 2.5 Total root length 2016.	69
Figure 2.6 Square root transformed root length by depth 2015.....	70
Figure 2.7 Square root transformed root length by depth 2016.....	71
Figure 2.8 Mean root diameter 2015.....	72
Figure 2.9 Mean root diameter 2016.....	73
Figure 2.10 Root diameter by depth 2015.	74
Figure 2.11 Root diameter by depth 2016.	75

List of Tables

Table 2.1 Manhattan, KS weather data	64
--	----

Acknowledgements

There are many people who deserve recognition for their support in the completion of this project. First, to my major professor Dr. Colby Moorberg. Thank you for giving me the opportunity to work with you and earn this degree. I have learned much from you, and without your guidance and support this research would not have been possible. Thank you also to my committee members, Drs. Kraig Roozeboom and Dorivar Ruiz Diaz, who provided invaluable advice and direction throughout this process.

Thank you to Dana Mayer, Cristie Edwards, and the staff of the Agronomy North Farm for all of their assistance. I would also like to thank my undergraduate workers, Keren Duerksen and Ben Hamill, who dedicated many hours to the completion of this project. Thank you also to the many other faculty members, the Agronomy office staff, and fellow graduate students that answered questions and pointed me in the right direction, your support does not go unnoticed.

Thank you to my parents and my siblings for your constant love and reassurance throughout my entire life. Especially to my brother Nate, your phone calls and advice throughout this project were so appreciated.

Most of all, I would like to thank my husband Landon, who has sacrificed much so I can complete my degree. I love you and would not have been able to do this without your encouragement over the last two years.

Above all else, everything I have been given is a gift from God. “So neither the one who plants nor the one who waters is anything, but only God, who makes things grow.” 1 Corinthians 3:7.

Chapter 1 - Literature Review

INTRODUCTION

Understanding soil-root interactions is often a complicated area of study, as our current knowledge of this “hidden half” of nature lags behind what we understand of the above ground counterparts (Eshel and Beeckman, 2013). While there is much to learn, significant progress has been made in regard to how varying agricultural management practices impact root system development, morphology, and nutrient uptake efficiency. In this chapter, methods of root analysis and impacts of phosphorus fertilization and tillage management on corn rooting dynamics will be reviewed.

PHOSPHORUS

Plant Phosphorus

The macronutrient, phosphorus (P) plays a critical role in plant growth and development. Second to nitrogen (N), P is the most important nutrient due to its vital role in many fundamental plant processes, including root development, stalk/stem strength, and energy storage and transfer (Vance et al., 2003).

Soil Phosphorus

Although a soil may have high concentrations of total P, only a small fraction of P is available for plant uptake. Phosphorus is stable and immobile in comparison to other mobile nutrients such as N. Approximately 80% of P is unavailable for plants at any given moment (Holford, 1997). The availability of P is primarily governed by mineral equilibria (dissolution-precipitation), interaction of dissolved P and soil solid surfaces (sorption-desorption), and biologically-mediated conversions between organic and inorganic P forms (mineralization-immobilization), with small amounts (0.1-10 μmol) present in the soil solution (Hinsinger,

2001). Total P concentrations are usually greatest in the top 15 cm, but can vary (50 to 3,000 mg P kg⁻¹ soil) as a function of parent material, soil type, vegetation, and management practices (Sims and Pierzynski, 2005).

The two major pools of soil P are organic P and inorganic P. Organic P represents approximately 30 to 65% of the total soil P, and consists predominately of inositol phosphates, phospholipids, and nucleic acids within soil humus or plant and microbial residues (Turner et al., 2002; E. K. Bünemann, 2011). Organic P can also be classified as dissolved organic P (DOP) based on size and solubility (E. K. Bünemann, 2011). Animal manures are an excellent source of organic P but must undergo mineralization via P-solubilizing microorganisms before it is useful to plants (Gyaneshwar et al., 2002; E. K. Bünemann, 2011). Soil microbes utilize a variety of enzymes to mineralize organic P, including phosphatases, phytases, and phosphonatasases (Sharma et al., 2013). Mineralization processes are highly influenced by soil moisture, temperature, chemical properties, and pH. Microbial populations have the ability to mobilize or immobilize P depending on Carbon (C) to P ratios (C:P ratios) and the amount of organic matter present (Havlin, 2005). Net immobilization occurs when substrate C:P ratios are larger than 300, while net mineralization occurs with C:P ratios below 200 (Paul, 2014). Plants can take up small amounts of DOP, however primarily take up P as the orthophosphate form (Menzies, 2009).

The primary source of fertilizer P is the mineral apatite. The weathering of primary P minerals found in the sand fractions of the soil is much too slow to meet crop demand because of low solubility and low rate of dissolution (Menzies, 2009). Soluble or adsorbed soil P predominates as orthophosphates, specifically H₂PO₄⁻ in acidic soils and HPO₄²⁻ in alkaline soils. These anions adsorb to negatively charged colloids through divalent or trivalent cation bridges, which primarily include calcium (Ca²⁺), ferric iron (Fe³⁺), or aluminum (Al³⁺) (Bünemann,

2011). The amount of P adsorbed on these soil constituents depends on many factors, but most importantly soil pH, and in the case of iron, redox status. Calcium phosphates adsorbed to clay minerals are the main mineral sources of phosphate in moderately weathered soils with neutral to alkaline pH, while Fe and Al phosphates adsorbed to clay minerals dominate in weathered soils with more acidic conditions (Sims and Pierzynski, 2005). Adsorption mechanisms are driven by inner-sphere and outer-sphere complexations. Inner-sphere complexes are a chemical reaction which result in strong, highly specific complexes in which nutrients are not readily available to plants. Outer-sphere complexes are a result of localized charge imbalances, are not specific, and contain ions available for plant uptake. Phosphate adsorption onto oxides is an inner-sphere complex that is dominated by ligand exchange. Bridging complexes are formed in which OH_2 and OH^- are displaced by a single phosphate anion, resulting in a stable complex (Barber, 2002). Phosphate adsorbed on the surface of a soil mineral may also become trapped if any oxide coating is precipitated on the mineral, referred to as a phosphorus occlusion (Wandruszka, 2006). The strength of the inner-sphere complexes or occlusions can lead to phosphorus retention or fixation. This explains why concentrations of plant-available P may be insufficient for crop growth, despite high concentrations of total soil P. In some cases it is a reversible reaction, as P can be released by desorption reactions creating labile P in solution (Shen et al., 2011).

Phosphorus Uptake

Plants acquire nutrients through three methods, mass flow, diffusion, and interception (Barber, 1966). Mass flow involves the delivery of nutrients to the root as those nutrients move with water towards the root during water uptake (Barber, 1966). Nutrients such as potassium (K) or N are acquired through mass flow. Interception occurs when a growing root comes in direct contact with nutrients (Barber, 1966). In diffusion, nutrients move through the soil solution from

areas of high concentration to areas of low concentration – specifically, near the root where nutrient uptake would decrease nutrient concentration (Barber, 1966). Plants acquire P predominately as orthophosphates (Richardson et al., 2009). A small fraction of P uptake is through mass flow and root interception – approximately 5% and 2.5% of the required P, respectively (Lambers et al., 1998). Due to the immobility of P, the majority of P acquisition by a plant occurs through diffusion (Turner and Gilliam, 1976). However, efficiency of plant P uptake is rather low. Marschner (1995) calculated the distance of movement of H_2PO_4^- in solution to be about 0.13 mm per day. Conditions that favor greater diffusion rates include high soil solution temperature, high volumetric water content, low solution buffering capacity, and low tortuosity.

The ability of a corn crop to acquire phosphorus from the soil is largely affected by root length, number of root hairs, and plant age (Jungk and Barber, 1974). As roots explore the soil volume, uptake generally occurs at the root tip where the P is transported into the plant by movement across root cell membranes. As P is taken up by the plant, the concentration in the soil zone at the root-soil interface decreases, resulting in a concentration gradient that facilitates diffusion (Barber et al., 1963). Mackey and Barber (1985) observed that increased soil P concentration resulted in greater root growth and rates of diffusion, thus increasing plant uptake.

Plant Adaptations to Limited Phosphorus

Because of the prevalence of P-limiting conditions in many agronomic systems, plants have undergone adaptations for enhanced P acquisition. These adaptations are aimed at conservation of use and enhanced acquisition or uptake (Vance, 2001), which include morphological, biochemical, and physiological responses (Yuan and Liu, 2008).

Vance et al. (2003) describes root architecture as the complexity of root system spatial configuration in response to soil conditions. It is well documented that root architecture follows P distribution throughout the soil profile (Drew and Saker, 1978; Mollier and Pellerin, 1999; Vance et al., 2003; Costa et al., 2009; Richardson et al., 2009; Costa et al., 2010). Root architecture refers to root morphology, topology, and distribution patterns (Lynch, 1995). This is important as the plant investigates more areas of the soil profile to acquire P via diffusion. Drew and Saker (1978) observed localized modifications to root growth, namely in lateral roots or cluster roots, in areas of the soil with high P concentrations. This growth was seen to largely compensate for the other parts of the root system that were in P-deficient soil. Williamson et al. (2001) observed that soil P availability dramatically affects root architecture. In soils low in available phosphate, lateral root growth was favored over primary root growth in regard to root density and length. This was mediated by reduced cell elongation in the primary roots. One more adaptation to P-deficiency is the increase of density and length of root hairs, which make up 77% of the root surface in P deficient environments (Gahoonia and Nielsen, 1998). These changes in root architecture vary between species and between genotypes within a species.

Enhanced P uptake can be mediated by physiological responses via chemical changes in the rhizosphere. The secretion of enzymes by enhanced expression of acid phosphatase and ribonuclease genes help to release phosphate from inorganic and organic P sources in the rhizosphere and lower the pH in alkaline soils (Yuan and Liu, 2008). Some plants also can secrete organic acids, which help facilitate the breakdown of more insoluble complexes (Vance et al., 2003; Hammond and White, 2008). Roots are able to sense and make adjustments to changes in P availability through changes in the transcription of genes, degradation of specific transcripts and proteins, and changes in plant growth regulators (Lynch, 1995; Hammond and

White, 2011). Other physiological root responses to low phosphorus soils include stimulating root growth via the allocation of more carbon to roots, enhancing the expression of P transporters, increasing P use efficiency, and forming thinner roots (Zhang et al., 2012).

Phosphorus Deficiency

As many as 5.7 billion ha worldwide lack sufficient available P, resulting in decreased crop yield (Batjes, 1997). Under P-limited conditions, symptoms of deficiency are typically observed in older plant tissues, due to the ability of plants to relocate available P for utilization in new growth (Duff et al., 1991). Deficiencies in corn are usually evident on young corn plants as delayed growth, dark green to red or purple leaves, thin stems, or underdeveloped root systems (Grant et al., 2011). Excluding nitrogen, P is the most deficient nutrient in the soils of Kansas and the Great Plains that can have major impacts on grain yield (Whitney and Lamond, 2005).

Phosphorus Fertilization

Phosphorus fertilization is an important issue worldwide, as studies suggest that the Earth's non-renewable P reserves could be depleted by as early as 2050 (Vance et al., 2003; Yuan and Liu 2008). Unfertilized agricultural soils cannot release enough P to keep up with plant growth demand (Schachtman et al., 1998), and as stated previously, rates of plant uptake of P are rather low. Consequently, applied P fertilizer is a major concern for producers striving to achieve maximum economic yield. For corn grown in the Great Plains the critical soil test level for P is 20 ppm (Leikam et al., 2003), with a yield response to P fertilization over 50% of the time when STL is below 20 ppm (Dodd et al., 2005).

Traditional sources of P fertilizer include ground rock phosphate and organic materials such as manures. These sources are still the primary sources of P in developing countries today. In modern production agriculture most commercial P fertilizers are formed from rock phosphate

treated with sulfuric acid to form water soluble phosphoric acid (Whitney, 1988). The fertilizer is dissolved in water and expressed as a percentage of P_2O_5 by weight of the dissolved sample. Some fertilizer is less soluble, and is expressed as citrate soluble. This is the fraction of the fertilizer that is dissolved in a 1 N ammonium citrate solution, and again is expressed as a percentage of P_2O_5 by weight of the sample. The amount of phosphorus in the fertilizer available to plants guaranteed on a fertilizer label, is the sum of the water-soluble and citrate-soluble phosphate (Whitney, 1988).

The most common forms of phosphate fertilizers are triple superphosphate (0-46-0), monoammonium phosphate (11-52-0), diammonium phosphate (18-46-0), and ammonium polyphosphate (10-34-0). Fertilizers created with phosphoric acid that is 55% P_2O_5 are called orthophosphates and are used to make dry triple superphosphate and dry ammonium phosphate fertilizers. The concentration of the acid used can increase, forming polyphosphoric acid. This concentrated acid is reacted with ammonia to form liquid 10-34-0 (Penas and Sander, 1982) and must undergo a hydrolysis reaction to convert from a polyphosphate to an orthophosphate that the plants can utilize (Stewart, 2002). Liquid sources of phosphorus fertilizer have no real benefit over dry fertilizer because adequate soil moisture is present under normal conditions to dissolve dry fertilizers. However, liquid fertilizer does have some advantages in that a homogeneous blend of more than one nutrient can uniformly be applied to a field or used in fertigation or in hydroponic studies (Whitney, 1988).

Phosphorus Fertilizer Application

Choosing a fertilizer application method is an important management decision for producers, from both efficiency and environmental standpoints. Due to P being relatively immobile in the soil profile, application distance is important in order to maximize P uptake

efficiency (Eghball and Sander, 1989). It is also known that P movement in water runoff causes eutrophication, which promotes algal growth in bodies of water, an increasing environmental concern. The excess of mineral nutrients results in the over production of autotrophs leading to high bacterial populations and high respiration rates, often resulting in fresh water fish kills and major shifts in species composition (Correll, 1998). There are many factors that control this runoff, including erosion potential, surface soil P concentration, P fertilization rates, and timing of fertilizer and manure applications (Sharpley et al., 1993).

The two most common application methods are broadcast or banded fertilization. Broadcast application uniformly distributes fertilizer over the entire soil surface. Banded fertilizer applications concentrate nutrients below, above, or on the side of the seedling.

Broadcast P application is often the simplest method and is suited for higher rates of fertilizer (Borkert and Barber, 1985a). In conventional systems, broadcasting before plowing produces uniform P distribution within the soil profile. This maximizes fertilizer contact with soil constituents over a larger volume of soil, thus increasing potential for P fixation (Barber, 1980). Many producers are converting to conservation tillage or no-till systems, which minimize or eliminate the incorporation of broadcast P fertilizers, resulting in concentrated P at or near the soil surface (Lal et al., 1990; Morrison and Chichester, 1994). Because of strong P adsorption to soil particles, broadcast application increases risk of runoff pollution of P-laden sediment to nearby rivers and streams. In a review of P loss, Hart et al. (2004) reported that between 0.7 and 42% of fertilizer P was lost via runoff.

Because P movement within the soil profile is limited (Hinsinger, 2001), band application of P can provide many advantages over broadcast application at low soil test levels (STL), especially in no-till or strip-till systems (Hairston et al., 1990). Chaudhary and Prihar (1974)

found that banded P in wheat increased uptake by 42% in uncompacted soils, and by as much as 98% in compacted soils. Deep banding results in zones of elevated concentrations of P in the soil at a location easily accessed by the root systems of young plants (Randall et al., 2001). By fertilizing only a small volume of soil, soil-to-fertilizer contact is reduced, resulting in decreased P fixation and increased plant uptake (Matar and Brown, 1989). Eghball and Sander (1989) examined the effect of distance and distribution of P fertilization on corn plant P. They observed that P moves outward from the point of injection in a sphere shape. In early stages of growth, fertilizer placement in close proximity to the plant resulted in greater levels of plant tissue P. This has been attributed to early and longer contact of roots with fertilizer (Eghball and Sander, 1989). At maturity, little to no effect of P application distance was observed, as a greater proportion of plant P was from soil P acquired late in the growing season, once the root systems had grown enough to explore a larger volume of soil further from the plant. Bly and Woodard (1997) did a similar study on soybeans and concluded that the distance of the P band from the row was more important than the P concentration in the band. They saw the greatest P uptake and grain yield when the band was less than 9 cm from the row.

Even in systems with high nutrient levels, certain conditions can limit nutrient availability, which can be alleviated with the use of starter fertilizer (Ketcheson, 1968). With early planting and low soil temperatures, root growth and nutrient uptake is minimal (Mackay and Barber, 1985; Havlin, 2005). Starter fertilizer can help plants to overcome these limitations. In reduced tillage systems, research suggests that starter application with deep banding P generally results in a more even vertical distribution of soil P (Martin, 2009).

LONG-TERM STRIP-TILL MANAGEMENT

The Tillage Effect

Conservation tillage systems are widely used in Kansas (Wade et al., 2015). No-till and reduced tillage systems have a profound effect on soil water relationships as well as other soil properties including soil health, aggregation, and erosion potential. By utilizing crop residues to protect the soil surface, no-till systems can dramatically reduce sediment losses. No-till systems also reduce energy, labor, and machinery inputs for the producer. One of the most important reported impacts of tillage on a cropping system is the influence on root development and function (Mosaddeghi et al., 2009). Chassot et al., (2001) reported that lower temperatures of topsoil in no-till was the main cause of reduction in root growth of maize seedlings when compared to a conventionally tilled system. For that reason, strip-till systems are a popular alternative as they give the producer ground coverage of a no-till system between rows while also providing a warmer, drier seedbed in the rows for earlier planting dates and better seedling root growth (Licht and Al-Kaisi, 2005). Similar yields have been seen under conventional and strip-till systems (Randall et al., 2001), as well as increased plant populations with strip-till when compared to no-till (Dudenhoeffer, 2012). Strip-till implements disturb the soil to a depth of 17-20 centimeters and create a 10-15 centimeter wide by 2-5 centimeter high mound of soil into which the seed can be directly planted (Randall et al., 2001), while simultaneously providing an opportunity to knife fertilizer below the soil surface.

Findings suggest that strip-till can be equally as effective as no-till in conserving soil moisture, while increasing soil temperature in the top 5 cm (Licht and Al-Kaisi, 2005). With its influence on soil moisture levels, reduced tillage systems also affect P uptake as moisture levels impact the diffusion of P from the soil to the root (Olsen et al., 1961). Moisture level has less

effect at high soil P levels because diffusion rate corresponds with the amount of P in the soil solution (Mahtab et al., 1971).

Effect of Tillage on Soil Nutrients

Yields are frequently influenced by factors such as climate, nutrient management, cropping system, or soil properties (Pittelkow et al., 2015) and have been documented to both increase (Norwood, 1999; Grandy et al., 2006) and decrease (Kumar et al. 2012; Vetsch et al. 2007; Vetsch and Randall 2002; Pittelkow et al. 2015) with long-term no-till management. A common drawback of long-term no-till or conservation tillage is significant stratification of immobile nutrients such as P (Deubel et al., 2011). No-till systems often limit the producer to broadcast fertilizer applications with little opportunity for incorporation, causing high concentrations of P at the soil surface, and stratification by depth. Nutrient stratification at the soil surface decreases P levels at deeper depths in the soil profile where higher moisture contents are present, potentially decreasing P uptake and reducing yields (Mackay and Barber, 1985). However, Boomsma et al. (2007) noted that when no-till conserves acceptable moisture near the surface, high soil P near the surface is not necessarily unfavorable because of water availability for P uptake. The strip-till system allows for banding of the nutrients in concentrated zones typically 15 cm below the surface where moisture is present and the roots can readily access it, potentially decreasing P fixation by limiting soil contact. Deep band application is also one strategy used to mitigate P pollution as it increases soil P below the crop row while decreasing soil P at the surface where soil particles are at higher risk of being eroded (Fernández and Schaefer, 2012; Fernández and White, 2012). With overall better soil conditions and a concentrated zone of nutrients, strip-till deep band (STDB) often results in a competitive advantage for nutrient uptake and crop production relative to no-till systems (Fernández and

White 2012; Farmaha et al., 2012). In a study done on soybeans, STDB had 23% greater shoot P accumulation and uptake rate per unit of root surface area compared to no-till. While the strip-till system may be a good alternative to no-till or conventional tillage, successive fertilizer applications without incorporation can still lead to uneven distribution of soil nutrients, thus impacting root growth.

Effect of Tillage on Root Growth

Larger root growth is frequently observed in no-till systems, which is often attributed to greater and deeper water accumulation throughout the soil profile (Lampurlanés et al., 2001; Sheng et al., 2012). No-till systems have been reported to increase shoot and root biomass, root surface area, diameter, volume, and root length density (Sheng et al., 2012). No-till also has been reported to increase mechanical impedance, especially at the soil surface, in some cases limiting root distribution and downward progression (Mosaddeghi et al., 2009). However, this is often a temporary impact, as long-term no-till operations have reported lower bulk densities due to higher organic matter and improved soil physical properties (Soane, 1990).

Root growth patterns often are plant-specific responses to nutrient deficiencies in the local soil environment (Richardson et al., 2009). It is well documented that root growth follows P distribution (Borkert and Barber, 1985b; Costa et al., 2009, 2010). In a study conducted on soybeans, Farmaha et al. (2012) concluded that no-till broadcast P application produced and maintained greater root length when compared to STDB P application. Irrespective of root system size however, STDB had 23% greater P accumulation and greater nutrient uptake per unit of root surface area indicating that this system provides overall better soil environments for P uptake and crop production. Greater yields also have been reported with STBD compared to no-till treatments (Fernández and White, 2012).

Our understanding of rooting dynamics and how they change under different management practices is an integral part of understanding the cropping system as a whole. The following section will discuss methods of root analysis used in root research.

METHODS OF ROOT ANALYSIS

Minirhizotron Method

The minirhizotron method is an adaptation of one of the earliest methods used to study roots – the transparent wall method, commonly known as the rhizotron (Böhm 1979). Rhizotrons typically consist of an underground cellar or walkway with walls or windows that come into direct contact with the natural soil profile (Taylor et al. 1990). Large rhizotrons however, are expensive and therefore limit the number of replications that can be performed, which gave way to the development of the minirhizotron (MR) system (Rewald and Ephrath, 2013).

The MR system allows for the *in situ* study of rooting dynamics over time in a non-destructive way. The system includes a transparent observation tube inserted into the soil vertically, horizontally, or at an angle through the soil. Bates (1937) first described a system of inserting glass tubes into the soil and viewing the roots using a mirror mounted on a rod that was lowered into the tube. Waddington (1971) is considered the first to use the technique as we know it today in a greenhouse study observing the growth of wheat (*Triticum aestivum* L.) roots using a fiber-optic probe (Taylor et al., 1990). Since the 1970's, the mirror mounted on the rod has been replaced with fiber optics (Sanders and Brown, 1978), endoscopes (Maertens and Clauzel, 1982), periscopes (Richards, 1984), video cameras (Upchurch and Ritchie, 1983; Cunningham et al., 1989), and other image scanning devices (Meier and Leuschner 2008). Today the most widely used methods utilize a digital video camera or scanner-based MR (Rewald and Ephrath,

2013) with an indexing handle to ensure the same soil location for every subsequent imaging session (Ferguson and Smucker, 1989; Johnson and Meyer, 1998).

Observations on root numbers (Crocker et al., 2003), root length densities (Liao et al., 2015), root lifespan (Johnson et al., 2001), morphology (Withington et al., 2003), as well as biological and pathological (Allen, 2007) studies have all been performed using the MR technique. Because this method is non-destructive, all measurements and observations can be made over time to track temporal changes.

Tube Design and Installation

Traditionally the MR tubes were made out of glass, but have since been made out of acrylic, lexan, and polybuterate (Brown and Upchurch, 1987). Withington et al. (2003) concluded that the transparent material used often has little effect on root production but can influence root survival in some species. Rigid plastic tubes are usually preferred because of their durability and relatively cheap cost (Rewald and Ephrath, 2013). The diameter of the tube varies depending on the type of camera used and the equipment available to make the access hole.

Installation of the observation tubes is a critical step of the MR technique. Installation should occur before planting or just after planting when root and shoot biomass is low. Holes to insert the observation tube are generally made using an auger or soil corer often facilitated by mechanical drilling devices (Brown and Upchurch, 1987). Ideally, tubes should be in complete and uniform contact with the soil matrix, affecting root growth only as much as other large objects such as stones (Rewald and Ephrath, 2013). However, achieving this is extremely difficult. A tight fit that prevents gaps and tube rotation can cause soil compaction during tube insertion that could hinder root growth to the tube (Johnson et al., 2001). On the other hand, large gaps could facilitate preferential root paths that can artificially increase root growth (Van

Noordwijk et al., 1985). Minirhizotron observation tubes are commonly inserted at an angle, but also have been installed horizontally and vertically. Early research suggested that roots preferentially followed tubes that were installed vertically (Brown and Upchurch, 1987). Angled tubes, commonly positioned at a 45⁰ or 30⁰ angle from the vertical, reduce this preferential pathway (Bragg et al., 1983) and can reach underneath multiple plants in a row crop system.

After observation tube installation, enough time must be allowed for the disturbed soil to settle against the minirhizotron tube. The time allotted before the first measurements varies depending on the species being studied. In disturbed soils such as an agricultural system, researchers have waited from as little as a week to a year before taking the first measurements (Johnson et al., 2001). This is based on both the short period of time allowed for an annual crop combined with the need for tillage in the row (as with strip-till), and on the assumption that disturbed agricultural soils can resettle following disturbances faster than an undisturbed native soil or perennial agricultural soils. In soils with established root systems, such as prairie or forest ecosystems, a longer wait period of over a year is suggested (Burke and Raynal, 1994).

Image Acquisition

Many authors have custom made their own MR camera using webcams or microscopes (Faget et al., 2010; Amato et al., 2012). The most commonly used commercial systems have been produced by Bartz Technology Corporation (Carpinteria, CA, USA).

Image acquisition with an optical scanner has been a preferred method as improvements in lighting sources and scanner technology have given way to greater image contrast over larger areas of the soil profile (Costa et al., 2000). Many authors have used traditional desktop scanners to capture images of excavated roots (Kaspar and Ewing, 1997). Recently, CID Bio-Science (Camas, WA, USA) has developed a high resolution linear scanner (CI-600 In-situ Root Imager)

that can be inserted into the MR tube, generating an image capturing almost the entire 360° circumference of the MR tube (Kobiela et al. 2016). The CI-600 generates bigger pictures (approximately 20 x 22 cm), which allows for data acquisition of larger parts of the branching root system in comparison to other MR image capturing techniques (Rewald and Ephrath, 2013).

Minirhizotron Advantages

Arguably the biggest advantage of the MR system is its ability to give researchers a way to study roots *in situ* in a non-destructive way (Ephrath et al. 1999). Unlike many methods, the MR technique allows for frequent and repeated observations of the same root system throughout the growing period. Johnson et al. (2001) argues that the greatest strength of the MR is the ability to monitor specific roots of interest from birth to death without significantly impacting fine root processes. Not only does the MR limit soil disturbance, but it also allows for more observation numbers. The MR also is versatile and can be used in a variety of ecological conditions (Kobiela et al. 2016). Aside from initial investments in the imaging device, the technique is cost effective relative to operational costs of other methods such as soil coring (Brown and Upchurch, 1987). After image acquisition, software such as WinRHIZO (Regent Instruments Inc., Ottawa, ON Canada), RootSnap!™ (CID Bio-Science, Camas, WA, USA), and many others are well adapted for image analysis.

Minirhizotron Limitations

Although the MR technique is by far the least destructive method of *in situ* root studies, there is still minor soil disturbance that must be taken into account. Proper tube installation is critical to ensure good soil/tube contact and to minimize preferential root growth paths along the viewing surface of the tube (Brown and Upchurch, 1987; Johnson et al., 2001). Because excavating the soil can stimulate the release of N, it is critical that there is sufficient time for the

soil dynamics to return to that of undisturbed areas (Joslin and Wolfe, 1999). Some soil textures can also cause issues with visibility on the tube surface. For example, a wet clayey soil could smear the viewing surface if not installed properly (Johnson et al. 2001).

The biggest limiting factor of MR technique however, is the substantial amount of labor involved in analyzing the sample images. The frequency of image collection depends on the species and root parameter being studied. Johnson et al. (2001) developed a simulation model to evaluate the importance of sampling interval on root turnover and found that the proportion of roots missed increased with the length of the sampling interval. Reducing the number of images analyzed per tube also comes at the cost of increasing variation in the data especially in perennial woody systems (Johnson et al., 2001). This is less of a concern in annual systems, as root mortality is generally low until the end of the season (Rees et al., 2005).

Root Core Method

The most commonly used method for obtaining soil samples is the root-soil auger method, also referred to as the root core method (Bengough et al., 2000). Root distribution can be quantified by taking soil cores from the soil profile and carefully washing the roots from the surrounding soil for further analysis. This method can give further information to the researcher about root mass and length of living and dead roots, root number, root health, as well as many other parameters per unit volume of soil (Schroth and Kolbe, 1994). The method is also often used to validate other methods of root analysis (Mackie-Dawson and Atkinson, 1991).

Sampling Equipment

Using a hand driven corer is the simplest method for taking soil samples from the field. There are many types of hand augers, however the most cited model was developed by Schuurman and Goedewaagen (1971). A hand-held auger has a cylindrical tube of a given length

and diameter that is commonly made of stainless steel or Plexiglas (Bengough et al., 2000). Above the cylinder is a shaft fixed at 100 cm. Extendable bi-partite models are also currently available to facilitate greater sampling depths (Böhm, 1979). The cylinder sampling tube typically is serrated or beveled at the edge to cut through the soil and roots and to minimize soil disruption. To extract the sample, the auger is pressed into the ground while being turned until the desired depth is reached. To force the core out of the tube, early studies describe a system with an auger consisting of two halves of the cylinder held together by a metal ring that come apart to expose the intact core (Oliveira et al., 2000). More commonly, augers are made with a disc at the bottom and a rod or spring-activated system that acts as a plunger. Currently, root augers like these are commercially available from Eijkelkamp (Royal Eijkelkamp, Lathum, The Netherlands).

The diameter of the core is an important consideration. If the core diameter is too small, the cutting edges can cause too much resistance between the core and the soil (Böhm, 1979). In addition, when using a small core diameter, the number of necessary replications increases to an inconvenient and laboriously high number. The most common diameter that researchers work with is approximately 7 cm (Böhm, 1979).

When working in difficult soils, a few adaptations may be necessary. When sampling in clayey or hard soils, Schuurman and Goedewaagen (1971) suggested dipping the auger into a pail of water before every sample. A lubricant, such as cooking spray or WD-40, is commonly used to prevent the soil from sticking to the probe or auger. Research has confirmed no significant effect on macro and micronutrients of the sample when such products are used (Midwest Laboratories, personal communication, 2017). With a modified T-handle, a hammer may also be utilized to force the auger into the ground. Mechanized techniques have greatly

reduced the time and labor it takes to retrieve cores, however they may not be suited for every situation as the vehicle carrying the sampler may cause damage to the surrounding crop and compact the soil.

Sampling Strategy

Designing a sampling scheme in terms of sample size, replication, and position in the field depends on the system being studied as well as the heterogeneity of the root distribution. Heterogeneity can occur because of certain soil properties (i.e. rootability), the plant, or the soil/plant interaction (i.e. branching patterns due to localized nutrient supplies) (Van Noordwijk et al., 1985). In grasslands or similar ecosystems, a completely random design is suggested (Oliveira et al., 2000). For row crops, samples must be taken within and between rows (Van Noordwijk et al., 1985). Spatial variability requires a larger number of replicates to obtain representative data. The number of subsamples often is similar to what is done for aboveground measurements, and can vary anywhere from three to ten measurements per experimental unit (Oliveira et al., 2000). Buczko et al. (2009) evaluated how many samples are necessary for representative estimates of root length density (RLD) and root morphology of corn. By calculating ratios of RLD in the plant row to RLD midway between rows, they yielded reasonable estimates with an average of eight cores, but suggested at least ten cores be taken when sampling in a more random manner. The appropriate time of the year to sample depends on the species and root parameters being studied. Mengel and Barber (1974) looked at the distribution of corn roots in intervals between planting and harvest and concluded that length and fresh weight increased rapidly for 80 days following planting, remained constant for 14 days, and then decreased rapidly when the plants were in the reproductive stage.

Processing Soil Core Samples

Once the soil cores are taken from the field, the roots are carefully washed from the surrounding soil over a sieve or by hand for further analysis (Metcalf et al. 2007; Prathapar et al. 1989). Elutriation or automatic root sieve-washing systems also have been used (Qin et al., 2005; Chotte et al., 2008; Benjamin and Nielsen, 2004). Researchers must be cognizant of the potential for underestimating the amount of root material due to missing roots during these processes (Metcalf et al., 2007). Using sieves with finer mesh diameters assures that excessive amounts of root material isn't lost passing through the sieve. However, finer mesh diameters also leave a greater amount of soil and other material to be picked through by hand.

After separating the roots, quantifying the root parameters can be done in a number of ways. Early methods include a system in which roots are laid out on a flat grid surface and a count is made of the number of intersections between the roots and random straight lines (Newman, 1966). Since then, digital image analysis has led to automated techniques to measure root parameters such as length, diameter, and surface area (Coelho and Or, 1999; Metcalf et al., 2007). After digital analysis, the roots can be dried and weighed to obtain dry mass per soil volume.

Advantages of the Root Core Method

The root core method is much less destructive than pit excavations or similar techniques. Core sampling can be done reasonably quickly, especially with the use of hydraulic powered or other mechanized equipment. The amount of time can be significantly reduced with the core break method.

Augers can be adapted for use on a variety of soil textures and plant species. A major advantage of the hand auger is that it can be used on small research plots when damage to the surrounding area needs to be minimized.

Limitations of the Root Core Method

The relatively small diameter size of the cores can increase the number of replications needed to obtain a representative picture of root distribution. Also the type of soil may not be ideal for the auger method. Soils that are stony, dry, or hard make the auger method difficult. Problems also can occur depending on the species being studied and the nature of their root system (i.e. fibrous tree roots). Heavy equipment when utilizing a mechanized technique may be risky because it can compact the cores, compact the soil, and damage the above ground plant material as well (Oliveira et al., 2000). The auger method does not give information about small-scale variability within a plot (Schroth and Kolbe, 1994). The biggest limitation however, is not in the time it takes to acquire the root cores from the field, but instead in the amount of time spent in the lab processing the data (Persson, 1990). Persson (1990) estimated that sorting a single core sample may take as much as 4 to 8 hours.

Applications in Corn

Corn roots have the ability to adapt to varying environmental conditions, yet there is a basic pattern of root distribution, which can be modified but is not fundamentally changed (Liedgens and Richner, 2001). Liedgens and Richner (2001) used the MR technique to study the spatial distribution of the corn root system and concluded that root density increased to a max at a depth of 25 cm, while decreasing at greater depths. Density of roots decreased with increasing distance from the plant row, however soil depth was found to have a greater influence. Majdi et al. (1992) and Liao et al. (2015) compared the MR to the monolith method (a mounted profile of

the entire root system taken from a soil pit). Liao et al. (2015) concluded that for both methods the growth rate of RLD decreased as soil depth increased. They did find a few discrepancies between the two methods, as the MR technique underestimated RLD at milk and maturity stages but overestimated RLD during tasseling (Liao et al., 2015). Overall, Liao et al. (2015) deemed the MR technique a reliable method to nondestructively study corn root growth.

Work has also been done to compare the MR technique to the root core method. Wiesler and Horst (1994) looked at differences in corn cultivars and found an underestimation of root density in the topsoil with the MR and a linear decline of root density with depth below 30 cm whereas RLD in the soil cores decreased exponentially with depth. Jose et al. (2001) cited a slight, but not significant, underestimation of corn root biomass in the top 30 cm of soil by the MR when compared to core sampling. They found similar distributions of predicted root biomass and found that root area measurements from the MR and fine root biomass from soil cores exhibited a significant linear relationship (2001).

Root Analysis Conclusion

Both the MR method and root core methods have distinct advantages as well as unique limitations. The root core method is well established in the literature. Whether using hand-held augers or utilizing mechanized techniques, the root core method can be a reliable source of root information. The MR allows for long-term root studies in a way that previous root analysis methods could not. The technique is versatile and can be adapted to answer diverse research questions. Because of the limited information on rooting dynamics as impacted by management systems, there is a great need for more research that utilizes minirhizotron and root core methods, particularly for new management practices such as strip-till, STDB, and the use of starter fertilizer in strip-till.

SUMMARY

It is clear that soil P plays a significant role in how cropping systems are managed. Phosphorus is essential to many important plant processes such as energy storage and transfer. Management decisions in agricultural systems impact root system development, morphology, and nutrient uptake efficiency. Phosphorus has a unique relationship with soil constituents, often becoming unavailable for plant utilization, making P fertilization decisions an important part of nutrient management. Plants adapt to P deficiencies in various ways, including morphological changes as root architecture follows P in the soil profile. This becomes increasingly important as nutrient stratification associated with conservational tillage practices have many producers worried about meeting the nutrient needs of their crop. Broadcast and banded application methods create differing soil nutrient profiles that can impact rooting dynamics. Research has long demonstrated the crop response to low soil test P levels. However, research involving root response to both low and high P levels is still needed. Various root analysis techniques such as the minirhizotron and root core methods can help to elucidate this impact of P application methods on rooting dynamics in corn.

REFERENCES

- Allen, M.F. 2007. Mycorrhizal fungi: highways for water and nutrients in arid soils. *Vadose Zone J.* 6(2): 291–297.
- Amato, M., F. Lupo, G. Bitella, R. Bochicchio, M. Abdel Aziz, and G. Celano. 2012. A high quality low-cost digital microscope minirhizotron system. *Comput. Electron. Agric.* 80: 50–53.
- Barber, S.A. 1980. Twenty-five years of phosphate and potassium fertilization of a crop rotation. *Fertil. Res.* 1(1): 29–36.
- Barber, T.M. 2002. Phosphate adsorption by mixed and reduced iron phases in static and dynamic systems. Thesis, Stanford Univ.
- Barber, S.A., J.M. Walker, and E.H. Vasey. 1963. Mechanisms for movement of plant nutrients from soil and fertilizer to plant root. *J. Agric. Food Chem.* 11(3): 204–207.
- Batjes, N.H. 1997. A world dataset of derived soil properties by FAO–UNESCO soil unit for global modelling. *Soil Use Manag.* 13(1): 9–16.
- Bengough, A.G., A. Castrignano, L. Pagès, and M. van Noordwijk. 2000. Sampling Strategies, Scaling, and Statistics. p. 147–173. *In* Smit, D.A.L., Bengough, D.A.G., Engels, P.D.C., Noordwijk, D.M. van, Pellerin, D.S., Geijn, D.S.C. van de (eds.), *Root Methods*. Springer. Berlin Heidelberg.
- Benjamin, J.G., and D.C. Nielsen. 2004. A method to separate plant roots from soil and analyze root surface area. *Plant Soil* 267(1–2): 225–234.

- Bly, A., and H.J. Woodard. 1997. Soybean growth and yield response to residual fertilizer phosphorus bands. *J. Plant Nutr.* 20(11): 1527–1538.
- Böhm, W. 1979. *Methods of studying root systems*. Springer Berlin Heidelberg, New York.
- Boomsma, C.R., M. Canepa, and T.J. Vyn. 2007. Factors affecting the relative benefit of deep-banding versus broadcast application of phosphorus and potassium for corn and soybean. *North Cent. Ext.-Ind. Soil Fertil. Conf.* 23: 55–63.
- Borkert, C.M., and S.A. Barber. 1985a. Predicting the most efficient phosphorus placement for soybeans. *Soil Sci. Soc. Am. J.* 49(4): 901–904.
- Borkert, C.M., and S.A. Barber. 1985b. Soybean shoot and root growth and phosphorus concentration as affected by phosphorus placement. *Soil Sci. Soc. Am. J.* 49(1): 152–155.
- Bragg, P.L., G. Govi, and R.Q. Cannell. 1983. A comparison of methods, including angled and vertical minirhizotrons, for studying root growth and distribution in a spring oat crop. *Plant Soil* 73(3): 435–440.
- Brown, D.A., and D.R. Upchurch. 1987. Minirhizotrons: a summary of methods and instruments in current use. In: Taylor HM (ed) *Minirhizotron observation tubes: methods and applications for measuring rhizosphere dynamics*. ASA special publication number 50. American Society of Agronomy, Madison, WI, pp 15-30
- Buczko, U., R. Kuchenbuch, and H. Gerke. 2009. Evaluation of a core sampling scheme to characterize root length density of maize. *Plant Soil* 316(1/2): 205–215.

- Burke, M.K., and D.J. Raynal. 1994. Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. *Plant Soil* 162(1): 135–146.
- Chassot, A., P. Stamp, and W. Richner. 2001. Root distribution and morphology of maize seedlings as affected by tillage and fertilizer placement. *Plant Soil* 231(1): 123–135.
- Chaudhary, M.R., and S.S. Prihar. 1974. Comparison of banded and broadcast fertilizer applications in relation to compaction and irrigation in maize and wheat. *Agron. J.* 66(4): 560–564.
- Chotte J.L., Laurent J.Y. and Rossi J.P. 1995. A modified hydropneumo-elutriation apparatus for quantitative root separation from large soil core samples. *Comm. Soil Sci. Plant Anal.* 26, 2703–2709.
- Coelho, E.F., and D. Or. 1999. Root distribution and water uptake patterns of corn under surface and subsurface drip irrigation. *Plant Soil* 206(2): 123–136.
- Correll, D.L. 1998. The role of phosphorus in the eutrophication of receiving waters: a review. *J. Environ. Qual.* 27(2): 261–266.
- Costa, C., L.M. Dwyer, R.I. Hamilton, C. Hamel, L. Nantais, and D.L. Smith. 2000. A sampling method for measurement of large root systems with scanner-based image analysis. *Agron. J.* 92(4): 621.
- Costa, S.E.V.G. de A., E.D. de Souza, I. Anghinoni, J.P.C. Flores, E.G. Cao, and M.J. Holzschuh. 2009. Phosphorus and root distribution and corn growth related to long-term tillage systems and fertilizer placement. *R. Bras. Ci. Solo*, 33:1237-1247, 2009.

- Costa, S.E.V.G.A., E.D. Souza, I. Anghinoni, J.P.C. Flores, F.C.B. Vieira, A.P. Martins, and E.V.O. Ferreira. 2010. Patterns in phosphorus and corn root distribution and yield in long-term tillage systems with fertilizer application. *Soil Tillage Res.* 109(1): 41–49.
- Crocker, T.L., R.L. Hendrick, R.W. Ruess, K.S. Pregitzer, A.J. Burton, M.F. Allen, J. Shan, and L.A. Morris. 2003. Substituting root numbers for length: improving the use of minirhizotrons to study fine root dynamics. *Appl. Soil Ecol.* 23(2): 127–135.
- Cunningham, M., M.B. Adams, R.J. Luxmoore, W.M. Post, and D.L. DeAngelis. 1989. Quick estimates of root length, using a video image analyzer. *Can. J. For. Res.* 19(3): 335–340.
- Deubel, A., B. Hofmann, and D. Orzessek. 2011. Long-term effects of tillage on stratification and plant availability of phosphate and potassium in a loess chernozem. *Soil Tillage Res.* 117: 85–92.
- Dodd, J.R., and A.P. Mallarino. 2005. Soil-test phosphorus and crop grain yield responses to long-term phosphorus fertilization for corn-soybean rotations. *Soil Sci. Soc. Am. J.* 69(4): 1118–1128.
- Drew, M.C., and L.R. Saker. 1978. Nutrient Supply and the Growth of the Seminal Root System in Barley III. Compensatory increases in growth of lateral roots, and in rates of phosphate uptake, in response to a localized supply of phosphate. *J. Exp. Bot.* 29(2): 435–451.
- Dudenhoeffer, C.J. 2012. Enhanced efficiency phosphorus application for corn. Thesis, Univ. of Missouri.

- Duff, S.M., W.C. Plaxton, and D.D. Lefebvre. 1991. Phosphate-starvation response in plant cells: de novo synthesis and degradation of acid phosphatases. *Proc. Natl. Acad. Sci.* 88(21): 9538–9542.
- E. K. Bünemann, A.O. 2011. Phosphorus in action – Biological processes in soil phosphorus cycling. *Soil Biology* 26: 483.
- Eghball, B., and D.H. Sander. 1989. Distance and distribution effects of phosphorus fertilizer on corn. *Soil Sci. Soc. Am. J.* 53(1): 282–287.
- Ephrath, J.E., M. Silberbush, and P.R. Berliner. 1999. Calibration of minirhizotron readings against root length density data obtained from soil cores. *Plant Soil* 209(2): 201–208.
- Eshel, A., and T. Beeckman. 2013. *Plant Roots: The Hidden Half*. 4th Edition. CRC Press, Boca Raton, FL.
- Faget, M., M. Liedgens, P. Stamp, P. Flütsch, and J.M. Herrera. 2010. A minirhizotron imaging system to identify roots expressing the green fluorescent protein. *Comput. Electron. Agric.* 74(1): 163–167.
- Farmaha, B.S., F.G. Fernández, and E.D. Nafziger. 2012. Distribution of soybean roots, soil water, phosphorus and potassium concentrations with broadcast and subsurface-band fertilization. *Soil Sci. Soc. Am. J.* 76(3): 1079–1089.
- Ferguson, J.C., and A.J.M. Smucker. 1989. Modifications of the minirhizotron video camera system for measuring spatial and temporal root dynamics. *Soil Sci. Soc. Am. J.* 53(5): 1601–1605.

- Fernández, F.G., and D. Schaefer. 2012. Assessment of soil phosphorus and potassium following real time kinematic-guided broadcast and deep-band placement in strip-till and no-till. *Soil Sci. Soc. Am. J.* 76(3): 1090–1099.
- Fernández, F.G., and C. White. 2012. No-till and strip-till corn production with broadcast and subsurface-band phosphorus and potassium. *Agron. J.* 104(4): 996–1005.
- Gahoonia, T.S., and N.E. Nielsen. 1998. Direct evidence on participation of root hairs in phosphorus (^{32}P) uptake from soil. *Plant Soil* 198(2): 147–152.
- Grandy, A.S., T.D. Loecke, S. Parr, and G.P. Robertson. 2006. Long-term trends in nitrous oxide emissions, soil nitrogen, and crop yields of till and no-till cropping systems. *J. Environ. Qual.* 35(4): 1487–1495.
- Grant, C.A., D.N. Flaten, D.J. Tomasiewicz, and S.C. Sheppard. 2011. The importance of early season phosphorus nutrition. *Can. J. Plant Sci.* 81:211–224.
- Gyaneshwar, P., G.N. Kumar, L.J. Parekh, and P.S. Poole. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant Soil* 245(1): 83–93.
- Hairston, J.E., W.F. Jones, P.K. McConnaughey, L.K. Marshall, and K.B. Gill. 1990. Tillage and fertilizer management effects on soybean growth and yield on three Mississippi soils. *J. Prod. Agric.* 3(3): 317–323.
- Hammond, J.P., and P.J. White. 2008. Sucrose transport in the phloem: integrating root responses to phosphorus starvation. *J. Exp. Bot.* 59(1): 93–109.

- Hammond, J.P., and P.J. White. 2011. Sugar signaling in root responses to low phosphorus availability. *Plant Physiol.* 156(3): 1033–1040.
- Hart, M.R., B.F. Quin, and M.L. Nguyen. 2004. Phosphorus runoff from agricultural land and direct fertilizer effects: a review. *J. Environ. Qual.* 33(6): 1954–1972.
- Havlin, J. 2005. *Soil fertility and fertilizers: an introduction to nutrient management.* 7th edition.. Pearson Prentice Hall, Upper Saddle River, NJ.
- Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237(2): 173–195.
- Holford, I.C.R. 1997. Soil phosphorus: Its measurement, and its uptake by plants. *Aust. J. Soil Res.* 35:227–240. doi:10.1071/S96047
- Johnson, M.G., and P.F. Meyer. 1998. Mechanical advancing handle that simplifies minirhizotron camera registration and image collection. *J. Environ. Qual.* 27(3): 710–714.
- Johnson, M.G., D.T. Tingey, D.L. Phillips, and M.J. Storm. 2001. Advancing fine root research with minirhizotrons. *Environ. Exp. Bot.* 45(3): 263–289.
- Jose, S., A.R. Gillespie, J.R. Seifert, and P.E. Pope. 2001. Comparison of minirhizotron and soil core methods for quantifying root biomass in a temperate alley cropping system. *Agrofor. Syst.* 52(2): 161–168.
- Joslin, J.D., and M.H. Wolfe. 1999. Disturbances during minirhizotron installation can affect root observation data. *Soil Sci. Soc. Am. J.* 63(1): 218–221.

- Jungk, A., and S.A. Barber. 1974. Phosphate uptake rate of corn roots as related to the proportion of the roots exposed to phosphate. *Agron. J.* 66(4): 554–557.
- Kaspar, T.C., and R.P. Ewing. 1997. ROOTEDGE: Software for measuring root length from desktop scanner images. *Agron. J.* 89(6): 932–940.
- Ketcheson, J.W. 1968. Effect of controlled air and soil temperature and starter fertilizer on growth and nutrient composition of corn (*Zea mays* L.)1. *Soil Sci. Soc. Am. J.* 32(4).
- Kobiela, B., M. Biondini, and K. Sedivec. 2016. Comparing root and shoot responses to nutrient additions and mowing in a restored semi-arid grassland. *Plant Ecol.*: 217:303–314.
- Kumar, S., A. Kadono, R. Lal, and W. Dick. 2012. Long-term no-till impacts on organic carbon and properties of two contrasting soils and corn yields in Ohio. *Soil Sci. Soc. Am. J.* 76(5): 1798–1809.
- Lal, R., T.J. Logan, and N.R. Fausey. 1990. Long-term tillage effects on a mollic ochraqualf in North-west Ohio. III. Soil nutrient profile. *Soil Tillage Res.* 15(4): 371–382.
- Lampurlanés, J., P. Angás, and C. Cantero-Martínez. 2001. Root growth, soil water content and yield of barley under different tillage systems on two soils in semiarid conditions. *Field Crops Res.* 69(1): 27–40.
- Leikam, D.F., R.E. Lamond, and D.B. Mengel. 2003. Soil test interpretations and fertilizer recommendations. Kansas State University Agricultural Experiment Station. Department of Agronomy. MF-2568.

- Liao, R., Y. Bai, H. Liang, S. An, S. Ren, Y. Cao, Z. Le, J. Lu, and J. Liu. 2015. Root growth of maize as studied with minirhizotrons and monolith methods. *Arch. Agron. Soil Sci.* 61(10): 1343–1356.
- Licht, M.A., and M. Al-Kaisi. 2005. Strip-tillage effect on seedbed soil temperature and other soil physical properties. *Soil Tillage Res.* 80(1–2): 233–249.
- Liedgens, M., and W. Richner. 2001. Minirhizotron observations of the spatial distribution of the maize root system. *Agron. J.* 93(5): 1097–1104.
- Lynch, J. 1995. Root architecture and plant productivity. *Plant Physiol.* 109(1): 7–13.
- Mackay, A.D., and S.A. Barber. 1985. Soil moisture effects on root growth and phosphorus uptake by corn. *Agron. J.* 77(4): 519–523.
- Mackie-Dawson, L.A., and D. Atkinson. 1991. Methodology for the study of roots in field experiments and the interpretation of results. *Plant Root Growth*: 25–47.
- Maertens, C. and Y. Clauzel. 1982. First observations on the use of endoscopy in in situ root studies of cultivated plants (*Sorghum vulgare* and *Lolium multiflorum*). *Agronomie* 2:677-680.
- Mahtab, S.K., C.L. Godfrey, and A.R. Swoboda. 1971. Phosphorus diffusion in soils. I. Effect of applied P, clay content, and water content. *Soil Sci. Soc. Am. J.* (35): 393–397.
- Majdi, H., A.J.M. Smucker, and H. Persson. A comparison between minirhizotron and monolith sampling methods for measuring root growth of maize (*Zea mays* L.). *Plant Soil* 147(1): 127–134.

- Marschner, H. 1995. Mineral nutrition of higher plants. 2nd ed. Academic Press, London.
- Martin, K.L. 2009. Effect of phosphorus placement in reduced tillage crop production. Thesis, Kansas State Univ.
- Matar, A.E., and S.C. Brown. 1989. Effect of rate and method of phosphate placement on productivity of durum wheat in a Mediterranean climate. *Fertil. Res.* 20(2): 83–88.
- Mengel, D.B., and S.A. Barber. 1974. Development and distribution of the corn root system under field conditions. *Agron. J.* 66(3): 341–344.
- Menzies, N. 2009. The science of phosphorus nutrition: forms in the soil, plant uptake, and plant response. Available at < <https://grdc.com.au/Research-and-Development/GRDC-Update-Papers/2009/02/THE-SCIENCE-OF-PHOSPHORUS-NUTRITION-FORMS-IN-THE-SOIL-PLANT-UPTAKE-AND-PLANT-RESPONSE>> (verified 28 April 2017).
- Metcalf, D.B., M. Williams, L.E.O.C. Aragão, A.C.L. Da Costa, S.S. De Almeida, A.P. Braga, P.H.L. Gonçalves, J. De Athaydes, S. Junior, Y. Malhi, and P. Meir. 2007. A method for extracting plant roots from soil which facilitates rapid sample processing without compromising measurement accuracy. *New Phytol.* 174(3): 697–703.
- Midwest Laboratories. 2017. Soil probe lubrication: to lubricate or not? Personal communication: memorandum. Midwest Laboratories, Omaha, Nebraska..
- Mollier, A., and S. Pellerin. 1999. Maize root system growth and development as influenced by phosphorus deficiency. *J. Exp. Bot.* 50(333): 487–497.

- Morrison, J.E., and F.W. Chichester. 1994. Tillage system effects on soil and plant nutrient distributions on vertisols. *J. Prod. Agric.* 7(3): 364–373.
- Mosaddeghi, M.R., A.A. Mahboubi, and A. Safadoust. 2009. Short-term effects of tillage and manure on some soil physical properties and maize root growth in a sandy loam soil in western Iran. *Soil Tillage Res.* 104(1): 173–179.
- Newman, E.I. 1966. A method of estimating the total length of root in a sample. *J. Appl. Ecol.* 3(1): 139–145.
- Norwood, C.A. 1999. Water use and yield of dryland row crops as affected by tillage. *Agron. J.* 91(1): 108–115.
- Oliveira, M. do R.G., M. van Noordwijk, S.R. Gaze, G. Brouwer, S. Bona, G. Mosca, and K. Hairiah. 2000. Auger sampling, ingrowth cores and pinboard methods. p. 175–210. *In* Smit, D.A.L., Bengough, D.A.G., Engels, P.D.C., Noordwijk, D.M. van, Pellerin, D.S., Geijn, D.S.C. van de (eds.), *Root Methods*. Springer Berlin Heidelberg.
- Olsen, S.R., F.S. Watanabe, and R.E. Danielson. 1961. Phosphorus absorption by corn roots as affected by moisture and phosphorus concentration. *Soil Sci. Soc. Am. J.* 25(4): 289–294.
- Paul, E.A. 2014. *Soil Microbiology, Ecology and Biochemistry*. Academic Press, San Diego, CA.
- Penas, E.J., and D.H. Sander. 1982. G82-601 Using phosphorus fertilizers effectively. *Ext. Bull.* G601. Soil Resource Management. Univ. Nebraska-Lincoln Ext., Univ. of Nebraska-Lincoln, Lincoln, NE.

- Persson, H. 1990. Methods of studying root dynamics in relation to nutrient cycling. *Nutrient Cycling in Terrestrial Ecosystems: Field Methods Application, and Interpretation*. Ed. A F Harrison, P Ineson and O W Heal: 198–217. London/Elsevier Applied Science, New York.
- Pittelkow, C.M., B.A. Linquist, M.E. Lundy, X. Liang, K.J. van Groenigen, J. Lee, N. van Gestel, J. Six, R.T. Venterea, and C. van Kessel. 2015. When does no-till yield more? A global meta-analysis. *Field Crops Res.* 183: 156–168.
- Prathapar, S., W. Meyer, and F. Cook. 1989. Effect of cultivation on the relationship between root length density and unsaturated hydraulic conductivity in a moderately swelling clay soil. *Soil Res.* 27(4): 645–650.
- Qin, R., P. Stamp, and W. Richner. 2005. Impact of tillage and banded starter fertilizer on maize root growth in the top 25 centimeters of the soil. *Agron. J.* 97(3): 674–683.
- Randall, G.W., J.A. Vetsch, and T.S. Murrell. 2001. Corn response to phosphorus placement under various tillage practices. *Better Crops* 85(3): 12–15.
- Rees, R.M., I.J. Bingham, J.A. Baddeley, and C.A. Watson. 2005. The role of plants and land management in sequestering soil carbon in temperate arable and grassland ecosystems. *Geoderma* 128(1–2): 130–154.
- Rewald, B., and J.E. Ephrath. 2013. Minirhizotron techniques. Chapter 42. In: Eshel, A. & Beeckman, T. (Eds.) *Plant roots: The hidden half*. 4th edition. Barber, S.A. 1966. The role of root interception, mass-flow and diffusion in regulating the uptake of ions by plants from soil.

- Richards, J.H. 1984. Root growth response to defoliation in two Agropyron bunchgrasses: field observations with an improved root periscope. *Oecologia* 64(1): 21–25.
- Richardson, A.E., J.-M. Barea, A.M. McNeill, and C. Prigent-Combaret. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* 321(1–2): 305–339.
- Sanders, J.L., and D.A. Brown. 1978. A new fiber optic technique for measuring root growth of soybeans under field conditions. *Agron. J.* 70(6): 1073–1076.
- Schachtman, D.P., R.J. Reid, and S.M. Ayling. 1998. Phosphorus uptake by plants: from soil to cell. *Plant Physiol.* 116(2): 447–453.
- Schroth, G., and D. Kolbe. 1994. A method of processing soil core samples for root studies by subsampling. *Biol. Fertil. Soils* 18(1): 60–62.
- Schuurman, J.J., and M.A.J. Goedewaagen. 1971. Methods for the examination of root systems and roots. *Cent. Agric. Pub Doc.*
- Sharma, S.B., R.Z. Sayyed, M.H. Trivedi, and T.A. Gobi. 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* 2: 587.
- Sharpley, A.N., T.C. Daniel, and D.R. Edwards. 1993. Phosphorus movement in the landscape. *J. Prod. Agric.* 6(4): 492–500.

- Shawn M. Kaeppler, J.P.L. 2005. Zhu J M, Kaeppler S M, Lynch J P. Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays*). *Funct Plant Biol*, 2005. *Funct. Plant Biol.* 32(8).
- Shen, J., L. Yuan, J. Zhang, H. Li, Z. Bai, X. Chen, W. Zhang, and F. Zhang. 2011. Phosphorus Dynamics: From Soil to Plant. *Plant Physiol.* 156(3): 997–1005.
- Sheng, M., R. Lalande, C. Hamel, N. Ziadi, and Y. Shi. 2012. Growth of corn roots and associated arbuscular mycorrhizae are affected by long-term tillage and phosphorus fertilization. *Agron. J.* 104(6): 1672–1678.
- Sims, J.T., and G.M. Pierzynski. 2005. Chemistry of Phosphorus in Soils. *In* Tabatabai, M.A. and D.L. Sparks (eds.) *Chemical Processes in Soils*, pp. 151–192. Soil Science Society of America, Inc. Madison, WI, USA.
- Soane, B.D. 1990. The role of organic matter in soil compactibility: A review of some practical aspects. *Soil Tillage Res.* 16(1): 179–201.
- Stewart, W.M. 2002. Commercial phosphorus fertilizer: know your sources. *News and Views.* Potash and Phosphate Institute, February 2002.
- Taylor, H.M., D.R. Upchurch, and B.L. McMichael. 1990. Applications and limitations of rhizotrons and minirhizotrons for root studies. *Plant Soil* 129(1): 29–35.
- Turner, F.T., and J.W. Gilliam. 1976. Increased P diffusion as an explanation of increased p availability in flooded rice soils. *Plant Soil* 45(2): 365–377.

- Turner, B.L., I.D. McKelvie, and P.M. Haygarth. 2002. Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biol. Biochem.* 34(1): 27–35.
- Upchurch, D.R., and J.T. Ritchie. 1983. Root observations using a video recording system in mini-rhizotrons. *Agron. J.* 75(6): 1009–1015.
- Van Noordwijk, M., A. de Jager, and J. Floris. 1985. A new dimension to observations in minirhizotrons: a stereoscopic view on root photographs. *Plant Soil* 86: 447–453.
- Vance, C.P. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. *Plant nutrition in a world of declining renewable resources.* *Plant Physiol.* 127(2): 390–397.
- Vance, C.P., C. Uhde-Stone, and D.L. Allan. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157(3): 423–447.
- Vetsch, J.A., and G.W. Randall. 2002. Corn production as affected by tillage system and starter fertilizer. *Agron. J.* 94(3): 532–540.
- Vetsch, J.A., G.W. Randall, and J.A. Lamb. 2007. Corn and soybean production as affected by tillage systems. *Agron. J.* 99(4): 952–959.
- Waddington, J. 1971. Observation of plant roots in situ. *Can. J. Bot.* 49(10): 1850–1852.
- Wade, T., R. Claassen, and S. Wallander. 2015. Conservation-Practice Adoption Rates Vary Widely by Crop and Region. EIB-147. U.S. Department of Agriculture, Economic Research Service., December 2015.

- Wandruszka, R. von. 2006. Phosphorus retention in calcareous soils and the effect of organic matter on its mobility. *Geochem. Trans.* 7: 6.
- Whitney, D.A. 1988. Phosphorus Facts: Soil, Plant, and Fertilizer. Ext Bull C-665 Kansas State University Cooperative Extension Service, Manhattan, Kansas, USA.
- Whitney, D., and R. Lamond. 2015. Nutrient Planning Reference Guide: Managing Soil and Applied Nutrients. Kansas State Research Ext., Manhattan, KS.
- Wiesler, F., and W.J. Horst. 1994. Root growth of maize cultivars under field conditions as studied by the core and minirhizotron method and relationships to shoot growth. *Z. Für Pflanzenernähr. Bodenkd.* 157(5): 351–358.
- Williamson, L.C., S.P.C.P. Ribrioux, A.H. Fitter, and H.M.O. Leyser. 2001. Phosphate availability regulates root system architecture in *Arabidopsis*. *Plant Physiol.* 126(2): 875–882.
- Withington, J.M., A.D. Elkin, B. Bułaj, J. Olesiński, K.N. Tracy, T.J. Bouma, J. Oleksyn, L.J. Anderson, J. Modrzyński, P.B. Reich, and D.M. Eissenstat. 2003. The impact of material used for minirhizotron tubes for root research. *New Phytol.* 160(3): 533–544.
- Yuan, H., and D. Liu. 2008. Signaling components involved in plant responses to phosphate starvation. *J. Integr. Plant Biol.* 50(7): 849–859.
- Zhang, Y., P. Yu, Y.-F. Peng, X.-X. Li, F.-J. Chen, and C.-J. Li. 2012. Fine root patterning and balanced inorganic phosphorus distribution in the soil indicate distinctive adaptation of maize plants to phosphorus deficiency. *Pedosphere* 22(6): 870–877.

Chapter 2 - Impact of phosphorus placement on corn rooting dynamics under long-term strip-tillage

ABSTRACT

Soil profile phosphorus (P) distribution is known to influence rooting dynamics. However, it's unknown if P placement in long-term no-till management influences root development in high P-testing soils. The research objective was to compare impacts of P placement on corn (*Zea mays*, L.) root development. Replicated field trials were conducted in Manhattan, KS on a long-term, strip-tilled, corn-soybean-wheat rotation. Five P treatments were applied to the corn rotation for 11 years and included a control (0 kg), 22 kg starter and 67 kg broadcast (BC+ST), 90 kg broadcast (BC), 22 kg starter and 67 kg deep band (DB+ST), and 90 kg deep band (DB) of P₂O₅ per hectare. This study was conducted in 2015 and 2016 – years 10 and 11 of the long-term study, respectively. All treatments tested above 20 ppm P, with highest concentrations at 10-15 cm for DB, and 0-5 cm for all other treatments. Root length treatment differences were observed primarily in the upper 35 cm during vegetative growth. In 2015, both total root length (TRL) and root length by depth were significantly less for starter P treatments. Though no TRL differences were observed in 2016, BC+ST exhibited reduced root length compared to BC across multiple depths. The BC and DB treatments had similar root growth in 2015. In 2016, greater TRL and root length across multiple depths were observed for BC compared to DB. Although grain yield was not affected, this study showed that P placement in high testing soils impacts root development, particularly during vegetative growth.

INTRODUCTION

Phosphorus (P) management in no-till or reduced tillage systems can have significant agronomic and environmental impacts. Phosphorus bound to sediment in surface water runoff contributes to eutrophication in freshwater systems (Correll, 1998). Phosphorus is also one of the most limiting nutrients in Kansas agriculture, and plays a critical role in root development, stalk and stem strength, and energy storage and transfer (Vance et al., 2003). This is due, in part, to the relative immobility of P caused by the formation of stable bonds with soil constituents (Sims and Pierzynski, 2005). This results in more than 80% of P becoming unavailable for plant uptake after fertilization (2005).

Minimal tillage systems are becoming increasingly popular in Kansas (Wade et al., 2015). However, no-till systems often limit the producer to broadcast fertilizer applications with little opportunity for incorporation, resulting in high P concentrations at the soil surface and low concentrations below. When such stratification occurs, dry surface conditions can potentially limit P diffusion resulting in decreased plant uptake and subsequent yield loss (Mackay and Barber, 1985). In strip-till systems P fertilizer is banded in concentrated zones typically 15 cm below the surface where moisture is present and the roots can readily access it. In addition, P fixation is limited due to the decreased volume of soil that's fertilized. Deep band application can also mitigate P pollution by decreasing P concentration at the surface, thus limiting delivery of sediment-bound P to streams through water erosion (Fernández and Schaefer, 2012; Fernández and White, 2012). Martin (2009) reported that when P was broadcast in reduced tillage, shallow soil depths continued to have high soil test P, while deep band application increased soil P up to the 15 cm depth. The use of starter P fertilizer (5 cm below and to the side of the seed) with deep banding of P resulted in more even vertical nutrient stratification within the row. With overall

better soil conditions and a concentrated zone of nutrients, strip-till deep band (STDB) often poses a competitive advantage for nutrient uptake and crop production relative to no-till broadcast systems (Fernández and White 2012; Farmaha et al., 2012).

Phosphorus placement has been studied extensively as a way to increase crop yields both in low and high testing soils. In low P soils, it is well known that there is a consistent yield response to P fertilization regardless of placement (Bordoli and Mallarino, 1998; Barker, 1998). In high testing soils, crop response to P fertilization is not uncommon, especially when applied as a starter in minimal tillage systems with earlier planting dates and cooler soil temperatures (Griffith, 1992). Some speculate that yield differences among hybrids may be the result of differing rooting dynamics (Gordon et al., 1997).

Root response to P placement is well documented (Vance et al., 2003; Costa et al., 2010). Phosphorus availability is seen as one of the biggest drivers of root architecture (Williamson et al., 2001) with plant responses to P deficiencies resulting in morphological, biochemical, and physiological changes to better survive in low P conditions (Yuan and Liu, 2008), including localized modifications for enhanced root growth in nutrient enriched zones of the soil profile (Drew and Saker, 1978). Costa (2009) reported that root distribution of corn followed P distribution for no-till and strip-till management. The improvement of plant establishment with zones of high P concentration from banding or starter applications has resulted in increased use of these practices (Beegle et al., 2014). However high P and increased root growth in a small fraction of the soil is not always favorable, as this concentrated root distribution can result in localized reductions in water and thus P diffusion to the roots (Davies and Zhang, 1991). Root distribution throughout the entire soil profile is important to meet nutrient and water needs, especially in later developmental stages.

Research on corn root response to P placement under long-term reduced tillage management is limited. There are also very few studies that monitor corn root growth over time. The objective of this study was to examine the impact of broadcast, deep band, and starter P application methods on corn rooting dynamics throughout the growing season under strip-till management. The hypothesis was that long-term P application would impact P distribution in the soil profile and that root distribution would follow P distribution with increased root growth either at the surface or at 15 cm below the surface with broadcast and deep band P application, respectively. These findings should help in identifying changes in rooting dynamics as a result of stratified nutrients due to long-term reduced tillage.

MATERIALS AND METHODS

Site Description

The research was conducted in Manhattan, Kansas, USA. The replicated field site is non-irrigated and has been managed as a long-term reduced tillage corn-soybean-wheat rotation under strip-till for 11 years, thus P stratification was expected to exist. Data collection for this study was conducted during years 10 and 11. The primary soil is Smolan silt loam (fine, smectitic, mesic Pachic Argiustolls). Weather data from an on-site weather station are reported in

Figure 2.1 and Table 2.1. The corn variety used for both years was Pioneer® P1105AM™. Fertilizer placement treatments were designed to compare broadcast and deep-band application both with and without starter application. The five different P treatments and application rates included a control (0 kg), 22 kg starter and 67 kg broadcast (BC+ST), 90 kg broadcast (BC), 22 kg starter and 67 kg deep band (DB+ST), and 90 kg deep band (DB) of P₂O₅ per hectare applied to the corn rotation. The starter treatments also received 45 kg of broadcast P

applied to the soybean rotation. The fertilizer rate was chosen at the initiation of the long-term study to ensure adequate P for corn production even at high yields, without masking a placement effect. Dry triple super phosphate (0-46-0) was used as the broadcast fertilizer source and was applied just prior to planting. Ammonium polyphosphate solution (10-34-0) was used for the starter and deep band treatments. It was injected in the row using the strip-till implement approximately 15 cm below the surface for deep band treatments, and 5 cm below and 5 cm to the side of the seed for the starter band. All treatments were strip-tilled regardless of fertilizer rate and application method to eliminate any tillage effect on the results. Nitrogen application rates were balanced for all treatments to prevent an N effect, and applied when the field was strip-tilled and again side dressed at the v6 growth stage for a total of 200 kg N/ha applied.

The field site was strip-tilled in the early spring before planting and planted on May 11th and May 6th for the 2015 and 2016 research years, respectively with 76 cm row spacing and a seed population of 84,000 per hectare.

Root Observations

Minirhizotron (MR) observation tubes were installed in the field within three to four days of plant emergence. Each treatment plot (3.0 x 24.4 m) was four rows wide, and had two MR tubes installed at both the north and south ends of the middle two rows, at least one meter from the plot border. The center 7.6 m was harvested for grain yield, with the MR tubes installed between the harvested area and the end of the row. A motorized, handheld auger and wooden jig were used to bore holes approximately 7.5 cm in diameter and 90 cm long at a 30° angle from the vertical in the plant row. The auger holes were enlarged to 9 cm in diameter and deepened as necessary with a bucket auger. A one meter acrylic MR observation tube with a waterproof plug at the bottom was inserted into each auger hole. Insulated caps were used to close the exposed

tubes, regulate the in-tube temperature, and keep the imaging surface clean and dry. In 2015, friction between the soil and tube was relied upon to hold the tube in place. However, rises in the water table following rain events occasionally raised some tubes out of the auger holes in the first two weeks before the soil had completely settled after installation. To avoid this in 2016, tubes were anchored in place with wooden stakes and plastic ties.

Root images were collected weekly using a CI-600 In-Situ Root Imager (CID-Bioscience, Camas, WA, USA) throughout the growing season. This portable MR utilizes a linear scanner-based system to collect near-360° scans (21.59 x 19.56 cm), and is powered and controlled using a tablet computer. Images with 23.6 dot mm⁻¹ (600 DPI resolution) were collected at four depth positions in each tube via an indexing handle. Images were analyzed using RootSnap! (version 1.3.2.23, CID-Bioscience, Camas, WA, USA).

Significant data processing was required to utilize the raw RootSnap! data files. In order to quantify the root data by depth, each image was split into 5 cm depth increments according to pixel number. Four images at four different depths were taken per MR observation tube for a given imaging session. The physical size of each image was given in both pixels (2273 x 2550) and in centimeters (19.24 x 21.59), allowing for the calculation of pixels/cm scale. Each root was given a physical X and Y pixel location on the image. Using the Y pixel location, roots were binned by depth in the soil profile. Because a portion of each MR tube was above the soil surface, bins were adjusted for depth based on the number of pixels from the top of the image to the “true” soil surface. In addition to the soil surface adjustment, images two, three, and four had the total pixel height of the images above them added to it to simulate a single, seamless scan instead of four individual images. Because the RootSnap! software does not treat the four images as one, any single root that grew from one image into another was counted as two individual

roots, essentially doubling any root data at an image break. To avoid the overestimation, depth intervals containing the image breaks were removed from data analysis.

Root Cores

In the first year, a manual hand-driven auger (Equipment for Soil Research B.V. Eijkelkamp, Lathum, The Netherlands) was utilized to take volumetric soil cores during grain fill. The sampler consists of a cylindrical sampling tube 15 cm long and 7.6 cm wide with a serrated edge. The auger had a spring-activated platform to push the soil core from the sampling tube and an indexing handle to adjust for sampling depth. Samples were taken in conjunction with each MR observation tube, with one sample drawn directly in the plant row and another drawn 38 cm from the plant row. The cores were taken to a depth of 75 cm and sub-divided into nine depth intervals: 0-5 cm, 5-10 cm, 10-15 cm, 15-20 cm, 20-25 cm, 25-30 cm, 30-45 cm, 45-60 cm, and 60-75 cm. The samples were stored at 5°C until processing.

The soil was carefully hand-washed from the roots using running tap water over a sieve. Once all the soil and extraneous debris was removed, the roots were rinsed once more with deionized water. The roots were then oven dried at 60°C for at least 48 hr and weighed to determine dry biomass weight.

Soil Test Phosphorus Sampling

In 2015 soil fertility samples were collected from the soil profile using a push probe following corn harvest. Samples were collected to a depth of 60 cm at two locations – in the plant row and between rows (38 cm from the plant row). The soil cores were subdivided into the same depth intervals described previously for root cores. Mixed composite samples were collected for each depth at both locations using ten subsamples per plot. Samples were submitted

to the Kansas State Soil Testing Laboratory (Manhattan, Kansas, USA) for determination of extractable P using the Mehlich-III method (Mehlich, 1984).

Statistical Analysis

The study used a randomized complete block design. Depth was nested within each MR observation tube. Each tube was considered a subsample of the plot, with two tubes per plot and three replicates per treatment. A PROC MIXED analysis with a 95% confidence interval was performed in SAS version 9.4 software (The SAS Institute, Cary, NC 2010) to analyze treatment differences for soil test P by depth, root biomass by depth, total root length by depth, total root length (TRL), mean root diameter by depth. For parameters analyzed by depth, depth was treated as a repeated measure, and each depth interval is reported based on the shallowest depth (e.g. the 5-10 cm depth interval is reported as 5 cm). The block was treated as a random variable, Square root transformations were used for root length by depth and biomass by depth. Soil test P was analyzed separately for in row and between row samples.

Parameters were analyzed over time by splitting the entire growing season into four corn growth periods and combining all imaging sessions within a given period. The corn growth periods were defined as VE to V10, V10 to VT, VT to R3, and R3 to R5. The error bars on all graphs represent the 95% confidence interval. Graphs by depth have error bars only on the control treatments to depict variability within the data. The p-values listed for each depth in the figures represent the depth slice of the treatment-depth interaction term, indicating whether there was a significant treatment difference at each depth.

RESULTS

Manhattan, Kansas Weather

Weather data are reported in Figure 2.1 and Table 2.1. Maximum temperatures throughout the 2015 and 2016 growing season were similar. Rainfall in 2015 was similar to the U.S. Climate Normals, with the exception of above-normal rainfall in the month of May (274.3 mm). The biggest discrepancy between years came in June of 2016, when there was only 32.3 mm of total precipitation, the majority coming in a single rain event, compared to 137.4 mm in June of 2015. However, after a dry June, precipitation in July, August, and September of 2016 was above average.

Soil Test Phosphorus by Depth

Concentrations of soil test P (STP) by depth for samples collected in the plant row and between plant rows are shown in Figure 2.2. Highest STP was observed at the surface for all treatments except DB, which had the highest concentration at the 10 cm depth. This pattern was observed for both in row and between row measurements. Significant treatment differences were observed at 0, 5, and 10 cm depths for both sampling locations, with no significant treatment differences below 10 cm.

For soil samples collected in the plant row the BC, BC+ST, and DB+ST treatments had surface STP concentrations of 154, 125, and 168 ppm, respectively, which were significantly greater ($p < 0.0001$) than both the control (40 ppm) and DB (51 ppm). At the 5 cm depth, BC, DB, and DB+ST had significantly greater STP compared to the control ($p = 0.0395, 0.0003, \text{ and } 0.0015$, respectively). The DB treatment increased to a peak STP concentration of 107 ppm at the 10 cm depth, which was significantly greater than the control, BC, and DB+ST ($p < 0.0001$ for each).

Soil test samples from between the plant rows had STP distributions and concentrations similar to those of the samples from within the row. At the surface, the BC, BC+ST, and DB+ST each had greater STP than the control at concentrations of 182, 120, and 144 ppm, respectively. At the 5 cm depth, all treatments were significantly greater than the control. The DB treatment had lower STP than DB+ST at the 5 cm depth ($p=0.0046$), but higher STP at the 10 cm depth ($p=0.0227$). Although the peak STP concentration for the DB treatment occurred at 10 for both in row and between row locations, the peak concentrations between rows (68 ppm) was lower than in the row (107 ppm).

Root Biomass

Phosphorus fertilizer placement only affected root biomass in the top 5 cm of the soil profile. Root biomass by depth for samples collected in the row and between rows at tasseling are reported in Figure 2.3. From samples collected in the row, the addition of starter fertilizer facilitated greater in row root biomass near the surface. The surface root biomass for the BC+ST and DB+ST treatments were 1.15 and $1.0 \text{ g}^{1/2} \text{ cm}^{-3/2}$ (1.33 and 1.0 g cm^{-3}), respectively; which was significantly ($p=0.01$) larger than BC and DB, which had $0.70 \text{ g}^{1/2} \text{ cm}^{-3/2}$ (0.49 g cm^{-3}) and $0.62 \text{ g}^{1/2} \text{ cm}^{-3/2}$ (0.38 g cm^{-3}), respectively. There were no treatment differences for root biomass collected between the plant rows.

Root Imaging

Root senescence was observed in the R3 to R5 growth period, during which corn allocates all available resources to grain fill (Mengel and Barber, 1974). Root data from that growth period are not germane to the objectives of this study, as maximum root growth would have already been achieved. Thus, only results for the first three growth periods will be discussed.

Total Root Length

Total root length treatment differences generally occurred during the vegetative growth periods and were no longer significant by the reproductive stages. Total root length in 2015 is shown in Figure 2.4. The addition of starter fertilizer resulted in less TRL with broadcast P application (9.37 m) relative to the BC treatment (20.9 m, $p=0.0084$) during the VE to V10 period. While there wasn't a difference between the BC and DB treatments at any point in 2015, there was a significant difference between the BC+ST and the DB+ST treatment ($p=0.0468$) during the VE to V10 period. By the V10 to VT growth period, the starter treatments exhibited less total root length for both deep band and broadcast treatments. The TRL was 53.4 m in the DB+ST treatment compared to 86.0 m for DB ($p=0.0233$). The TRL was 49.0 m in the BC+ST treatment, compared to 81.5 m for BC ($p=0.0235$). Although the mean TRL for both starter treatments were less than for the treatments without starter, the difference was only significant for BC+ST by the VT to R3 period.

In contrast to 2015, there was no starter fertilizer effect on TRL in 2016 (Figure 2.5). There was a significant difference in TRL between the BC treatment (21.5 m) and the DB treatment (13.2 m, $p=0.0425$) during the VE to V10 period, which was not observed in 2015. Both BC and BC+ST (18.9 m) had significantly greater total root growth than the control (10.3 m; $p=0.0080$ and $p=0.0421$, respectively) during the VE to V10 period. No other significant treatment differences in TRL were observed in 2016.

Root Length by Depth

Root length by depth showed similar patterns in both years (Figure 2.6 and Figure 2.7). A decline in root growth was observed at the 10 cm depth interval. Root growth then increased

with depth and was relatively uniform across depths up to 60 and 80 cm before declining at greater depth.

All treatments except BC+ST had greater root length than the control at one or more depth intervals between 0-45 cm at VE to V10. A starter fertilizer treatment effect was observed in root length by depth for the broadcast treatments, with the BC treatment having greater root length than BC+ST at VE to V10 at 0, 25, 30, and 35 cm ($p=0.0221$, $p=0.0328$, $p=0.0396$, and $p=0.0088$, respectively). However, these differences were not observed thereafter. While there was a difference in TRL between BC+ST and BC in the V10 to VT growth period, there were no significant differences in root length for any given depth. The DB+ST and DB treatments had similar root growth at VE to V10, however during the V10 to VT growth period, significantly greater root length was observed for DB compared to DB+ST for the 10, 15, and 35 cm depth intervals ($p=0.0251$, $p=0.0423$, and $p=0.0230$, respectively). By the third reproductive period, DB still had greater root length than DB+ST at the 35 cm depth interval ($p=0.0430$). There were no treatment differences between DB and BC across all depths and growth periods.

Similar to 2015, most treatment differences in root length by depth in 2016 were observed during the VE to V10 period. There was extensive root growth in the V10 to VT growth period at 60-80 cm in depth for all treatments. Both broadcast treatments had significantly greater root length compared to the control for four or more depth increments at VE to V10. The BC treatment had greater root length than BC+ST at the surface and 15 cm depths ($p=0.0431$ and $p=0.0279$, respectively). This pattern was observed throughout the V10 to VT growth period and VT to R3 growth period, with reduced root length in BC+ST compared to BC at depths up to the 15 cm depth increment. Greater root growth was observed for DB+ST compared to DB at the 70 and 85 cm depth intervals during VE to V10. No other treatment

differences were observed for DB+ST and DB across all other depths and growth periods. There were treatment differences between DB and BC in 2016, compared to none in 2015. The BC treatment had greater root growth than DB for more than one depth interval in both vegetative growth periods. However, by the VT to R3 growth period, similar root length between DB and BC was observed.

Mean Root Diameter

Mean root diameters for each treatment for 2015 and 2016 are shown in Figure 2.8 and Figure 2.9, respectively. The greatest mean diameters occurred during VE to V10. The mean diameter averaged across all treatments was 0.55 and 0.53 mm in 2015 and 2016, respectively. By the VT to R3 growth period, the mean diameter averaged across all treatments dropped to 0.38 and 0.39 mm in 2015 and 2016, respectively. During the V10 to VT period in 2015 the DB+ST treatment had significantly smaller root diameter (0.42 mm) than the control (0.52 mm, $p=0.0497$). No other treatment differences in 2015 were observed. In 2016, the DB treatment had significantly smaller mean root diameters than the control, BC, and DB+ST ($p=0.0076$, $p=0.0128$, and $p=0.0479$, respectively) at VE to V10, and significantly smaller mean root diameter than BC ($p=0.0398$) in the V10 to VT period.

Root Diameter by Depth

Root diameter by depth is shown in Figure 2.10 and Figure 2.11 for 2015 and 2016, respectively. **Error! Reference source not found.** Root diameter by depth was highly variable during VE to V10 of 2015. In the V10 to VT growth period all treatments had significantly smaller diameter than the control at the 45, 60, and 85 cm depth intervals, but those differences were no longer significant by the VT to R3 growth period. No treatment differences were observed below the 10 cm depth in the VT to R3 growth period of 2015. In the VE to V10

growth period of 2016, differences in root diameter were noted deeper in the soil profile. The BC treatment exhibited larger root diameter than DB at the 55 cm ($p=0.0356$) and 80 cm ($p=0.0177$) depth, and BC+ST and the 75 cm depth ($p=0.0151$). There were no significant treatment differences in the V10 to VT growth period of 2016. In both years, the broadcast treatments had significantly smaller root diameter with the addition of starter fertilizer.

Grain Yield

Average yields in 2015 and 2016 were 12.4 and 9.8 Mg/ha (se ± 0.3886), respectively. There were no significant treatment differences in grain yield in either year.

DISCUSSION

The precipitation during the growing season varied greatly from year to year (Figure 2.1 and Table 2.1). The biggest discrepancy came in June of 2016 where there was only 32.3 mm of total precipitation, the majority coming in a single rain event, compared to 137.4 mm in June of 2015. Maximum root length by depth was a good indicator of soil moisture conditions. In 2015 the largest root length in the V10 to VT growth period was observed at the surface and the 30-45cm depth increment. While in 2016 under dry conditions, the maximum root length for the same growth period was observed much deeper in the soil profile (60-80 cm) where moisture was present.

The soils of the research site tested well above the critical level of P of 20 ppm (Leikam et al., 2003), though high concentrations were restricted to P hotspots, i.e. localized areas of high P concentrations. The P hot spot development and high STP can be attributed to both long-term strip-till management and successive fertilizer applications (Deubel et al., 2011). As expected, the concentration of STP was associated with application method, with BC applications resulting

in high STP at the surface and low STP below, and DB applications resulting in high STP in the row and 10 cm in depth. Both STP distributions can be explained by low P mobility (Hinsinger, 2001). The effect of starter fertilization on STP distribution was most notable in the DB+ST treatment, which had a more even vertical distribution of STP relative to the DB treatment, consistent with distributions observed by Martin (2009). Higher STP at the surface in BC and at 15 cm in DB did not result in greater root growth at these positions in the soil profile. This may be due to the fact that STP was not limiting for any treatment

The majority of root biomass was found in the upper 20 cm of the soil, which is consistent with work done by Fan et al. (2016). Starter fertilizer facilitated greater root biomass in the top 5 cm. This root response could be attributed to greater root density in the localized zone of nutrients available to the seedling (Drew and Saker, 1978). Qin et al. (2005) reported similar findings, with increased root length density in the top 5 cm of soil under banded starter fertilizer, which could explain the increase in root biomass. However, in 2015 using the MR method during the same growing period, we saw no differences in root length between treatments with and without starter at the surface, and smaller root diameter in BC+ST compared to BC. This is most likely the result of an underestimation of root length by the MR method in the most superficial soil layers (Liao et al., 2015).

Root length by depth showed similar patterns in both years. The decline in root growth observed at the 10 cm depth interval could be due in part to changes in soil bulk density or other soil physical properties at this depth.

Differences in TRL and root length by depth were mainly observed in the vegetative growth periods. As the plant reaches maturity, the majority of plant P is acquired from soil P late

in the growing season once the roots have explored a large area of the soil volume, with little to no affect due to P placement (Eghball and Sander, 1989).

In low testing soils, it is well known that the plant invests more resources to the expansion of the root system (Vance et al., 2003; Grossman and Rice, 2012). The reduction in TRL with the starter fertilizer could be related to P placement and availability even with high STP. The starter fertilizer places a concentrated zone of nutrients 5 cm below and to the side of the seed that the young root system can readily access. Even though STP was not a limiting factor for all treatments, soil exploration was increased in the early growth periods in the absence of starter fertilizer. This is likely due to the importance of P availability in the early corn growth stages (Grant et al., 2011). The impact of the starter fertilizer is further evidenced by the distribution of root length by depth. Under BC application, the addition of a starter resulted in decreased root length for certain depths up to 35 cm in the first growth period, with no differences with and without the starter observed by the second growth period. With DB application however, the starter had no effect on root length in the VE to V10 growth period but did produce smaller root length in depths up to 35 cm during the V10 to VT growth period. Root system size does not always limit the plant's ability to meet nutrient requirements, as there is a trade-off between the amount of soil volume explored and efficiency. While larger root systems can explore a greater volume of the soil profile, efficiency is minimized (Berntson, 1994). Krannitz et al. (1991) found that as the size of the root system increased, phosphorus uptake per unit root length declined. Larger root systems also create competition within the system, as two nearby roots can restrict the nutrient supply to the other by generating a depletion zone (Nye and Tinker, 1977). Although differences in root system size were noted, grain yield was not compromised.

The effect of starter fertilizer on TRL was not seen in 2016, as the starter treatments had similar root length to treatments without starter. The differences in the two growing seasons can be attributed largely to rainfall distribution. June of 2016 was very dry, which would impact root growth during the vegetative growth periods. Dry soil conditions can greatly reduce diffusion rates of P in the soil solution and negatively affect the ability of the plant to meet uptake requirements (Misra and Tyler, 1999). Absence of TRL differences between treatments could be the result of increased soil exploration for water acquisition. While TRL was not affected, some depths showed reduced root length for BC+ST compared to BC, similar to the 2015 season. In 2016, DB+ST actually had larger root length than DB for two depth locations in the VE to V10 growth period deep in the soil profile (70 and 85 cm). However, this difference is most likely not a nutrient response, but instead due to differences in soil moisture or soil physical properties.

There were no significant differences in BC and DB root length in 2015 for any depth. In 2016 however, BC had larger TRL in the VE to V10 growth period and larger root length for one or more depths in both vegetative growth periods, consistent with work done on soybeans by Farmaha et al., (2012) showing greater root growth under BC fertilization compared to DB. In the same study, despite having the smaller root system, DB application had greater nutrient uptake and P accumulation. This could help to explain why no yield difference was observed between the BC and DB treatments despite reduced root length in the vegetative periods under DB application.

Root diameter was largest in the VE to V10 growth period and decreased as the season progressed. In cereal crops, seminal roots develop first followed by the nodal root system that dominates later in the growing season. As branching order increases, root diameter decreases which is why a reduction in root diameter was observed over time (Wu et al., 2016).

The RootSnap! software had many limitations that complicated data analysis. The software is not adjusted to analyze by depth for any increments smaller than the physical size of the image, which is why significant data processing occurred in order to bin all the data in 5 cm increments. The use of four different images for a given tube also created issues when trying to simulate a continuous scan. If a single root lengthened across two imaging windows, the software would essentially treat this as two separate roots. This necessitated the removal of certain depths where an image break would occur to avoid any overestimations in root length. A way to alleviate this would be to composite the four separate images into one image of the entire length of the MR tube before analysis, which would allow for easier and more accurate analysis by depth. It would also be beneficial if the software could account for the true soil surface instead of assuming that the top of the first image is at the soil surface, as those adjustments needed to be made manually.

CONCLUSIONS

The objective of this study was to examine the impact of broadcast, deep band, and starter P application methods on corn rooting dynamics throughout the growing season under strip-till management.

The concentration of STP was consistent with application method, with BC applications resulting in high STP at the surface and low STP below; and DB applications resulting in high STP in the row and 10 cm in depth.

Mean root diameter was largest in the VE to V10 growth period and smallest in the VT to R3 period, which was consistent with the development of larger diameter primary roots early on, and development of smaller diameter secondary and tertiary roots later in the growing season. For root length, the majority of treatment differences occurred during the vegetative growth

period. The addition of starter fertilizer did impact root development based on root length observations. For the BC+ST and DB+ST treatments in 2015 and the BC+ST treatment in 2016, the use of starter fertilizer resulted in reduced root growth for depths predominately in the top 35 cm of the soil profile. This resulted in reduced TRL for one or both vegetative growth periods in 2015. Though root growth was reduced with starter fertilizer additions, grain yield was not compromised. No differences in root length between BC and DB treatments were observed in 2015. However, in 2016 the BC treatment exhibited greater root growth than DB for one or more depths in both vegetative growth periods, and greater TRL in the VE to V10 growth period. Differences in rainfall amount and distribution between 2015 and 2016 likely contributed to some differences between the two years, indicating that the influences of P placement under long-term strip-till practices is dependent on rainfall and soil moisture conditions.

The findings from this study identified changes in rooting dynamics as a result of stratified nutrients due to long-term reduced tillage. While no treatment differences in grain yield were observed, this study showed that P placement in high testing soils does impact root growth – particularly during the early vegetative growth periods. This may have implications for crop establishment during early vegetative periods, during which uniform crop development is crucial for realizing maximum yields. Further, it is possible that yield differences due to P placement under long-term strip-tillage management may occur under more extreme soil moisture conditions, in low P-testing soils, or other growing conditions resulting in considerable plant stress. Thus more research examining corn rooting dynamics throughout the growing season is needed.

REFERENCES

- Arguez, A., I. Durre, S. Applequist, M. Squires, R. Vose, X. Yin, and R. Bilotta. 2010. NOAA's U.S. Climate Normals (1981-2010). Daily. NOAA National Centers for Environmental Information.
- Barker, D. 1998. Phosphorus and potassium placement for corn and soybean managed with two conservation tillage systems. Theses diss., Iowa State Univ.
- Beegle, D.B., G.W. Roth, and D.D. Lingenfelter. 2014. Starter Fertilizer. Agron. Facts 51. Penn State Ext.
- Berntson, G.M. 1994. Modelling root architecture: are there tradeoffs between efficiency and potential of resource acquisition? *New Phytol.* 127(3): 483–493.
- Bordoli, J.M., and A.P. Mallarino. 1998. Deep and shallow banding of phosphorus and potassium as alternatives to broadcast fertilization for no-till corn. *Agron. J.* 90(1): 27–33.
- Correll, D.L. 1998. The role of phosphorus in the eutrophication of receiving waters: a review. *J. Environ. Qual.* 27(2): 261–266.
- Costa, S.E.V.G. de A., E.D. de Souza, I. Anghinoni, J.P.C. Flores, E.G. Cao, and M.J. Holzschuh. 2009. Phosphorus and root distribution and corn growth related to long-term tillage systems and fertilizer placement. *R. Bras. Ci. Solo*, 33:1237-1247, 2009.

- Costa, S.E.V.G.A., E.D. Souza, I. Anghinoni, J.P.C. Flores, F.C.B. Vieira, A.P. Martins, and E.V.O. Ferreira. 2010. Patterns in phosphorus and corn root distribution and yield in long-term tillage systems with fertilizer application. *Soil Tillage Res.* 109(1): 41–49.
- Davies, W.J., and J. Zhang. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42:55-76.
- Deubel, A., B. Hofmann, and D. Orzessek. 2011. Long-term effects of tillage on stratification and plant availability of phosphate and potassium in a loess chernozem. *Soil Tillage Res.* 117: 85–92.
- Drew, M.C., and L.R. Saker. 1978. Nutrient supply and the growth of the seminal root system in barley III. Compensatory increases in growth of lateral roots, and in rates of phosphate uptake, in response to a localized supply of phosphate. *J. Exp. Bot.* 29(2): 435–451.
- Eghball, B., and D.H. Sander. 1989. Distance and distribution effects of phosphorus fertilizer on corn. *Soil Sci. Soc. Am. J.* 53(1): 282–287.
- Fan, J., B. McConkey, H. Wang, and H. Janzen. 2016. Root distribution by depth for temperate agricultural crops. *Field Crops Res.* 189: 68–74.
- Farmaha, B.S., F.G. Fernández, and E.D. Nafziger. 2012. Distribution of soybean roots, soil water, phosphorus and potassium concentrations with broadcast and subsurface-band fertilization. *Soil Sci. Soc. Am. J.* 76(3): 1079–1089.

- Fernández, F.G., and D. Schaefer. 2012. Assessment of soil phosphorus and potassium following real time kinematic-guided broadcast and deep-band placement in strip-till and no-till. *Soil Sci. Soc. Am. J.* 76(3): 1090–1099.
- Fernández, F.G., and C. White. 2012. No-Till and Strip-Till Corn Production with Broadcast and Subsurface-Band Phosphorus and Potassium. *Agron. J.* 104(4): 996–1005.
- Gordon, W.B., D.L. Fjell, and D.A. Whitney. 1997. Corn hybrid response to starter fertilizer in a no-tillage, dryland environment. *J. Prod. Agric.* 10(3): 401–404.
- Grant, C.A., D.N. Flaten, D.J. Tomasiewicz, and S.C. Sheppard. 2011. The importance of early season phosphorus nutrition. *Can. J. Plant Sci.* 81:211–224.
- Grossman, J.D., and K.J. Rice. 2012. Evolution of root plasticity responses to variation in soil nutrient distribution and concentration. *Evol. Appl.* 5(8): 850.
- Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant Soil* 237(2): 173–195.
- Kansas State University Mesonet. 2017. Historical Weather. Available at < <http://mesonet.k-state.edu/weather/historical/>> (verified 28 April 2017).
- Krannitz, P.G., L.W. Aarssen, and D.D. Lefebvre. 1991. Correction for non-linear relationships between root size and short term Pi uptake in genotype comparisons. *Plant Soil* 133(2): 157–167.
- Leikam, D.F., R.E. Lamond, and D.B. Mengel. 2003. Soil test interpretations and fertilizer recommendations. Kansas state University Agricultural Experiment Station. Department

of Agronomy. MF-2568.

Liao, R., Y. Bai, H. Liang, S. An, S. Ren, Y. Cao, Z. Le, J. Lu, and J. Liu. 2015. Root growth of maize as studied with minirhizotrons and monolith methods. *Arch. Agron. Soil Sci.* 61(10): 1343–1356.

Mackay, A.D., and S.A. Barber. 1985. Soil moisture effects on root growth and phosphorus uptake by corn. *Agron. J.* 77(4): 519–523.

Martin, K.L. 2009. Effect of phosphorus placement in reduced tillage crop production. Thesis, Kansas State Univ.

Mehlich, A. 1984. Mehlich 3 soil extractant: A modification of Mehlich 2 extractant. *Commun. Soil Sci. Plant Anal.* 15: 1409–1416.

Mengel, D.B., and S.A. Barber. 1974. Development and distribution of the corn root system under field conditions. *Agron. J.* 66(3): 341–344.

Misra, A., and G. Tyler. 1999. Influence of soil moisture on soil solution chemistry and concentrations of minerals in the calicoles phleum phleoides and veronica spicata grown on a limestone soil. *Ann. Bot.* 84: 401–410.

Nye, P.H., and Philip B. Tinker. 1977. Solute movement in the soil-root system. Oxford: Blackwell.

Qin, R., P. Stamp, and W. Richner. 2005. Impact of tillage and banded starter fertilizer on maize root growth in the top 25 centimeters of the soil. *Agron. J.* 97(3): 674–683.

- Sims, J.T., and G.M. Pierzynski. 2005. Chemistry of Phosphorus in Soils. *In* Tabatabai, M.A. and D.L. Sparks (eds.) *Chemical Processes in Soils*, pp. 151–192. Soil Science Society of America, Inc. Madison, WI, USA.
- Vance, C.P., C. Uhde-Stone, and D.L. Allan. 2003. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytol.* 157(3): 423–447.
- Wade, T., R. Claassen, and S. Wallander. 2015. Conservation-Practice Adoption Rates Vary Widely by Crop and Region. USDA.
- Williamson, L.C., S.P.C.P. Ribrioux, A.H. Fitter, and H.M.O. Leyser. 2001. Phosphate availability regulates root system architecture in arabidopsis. *Plant Physiol.* 126(2): 875–882.
- Wu, Q., L. Pages, and J. Wu. 2016. Relationships between root diameter, root length and root branching along lateral roots in adult, field-grown maize. *Ann. Bot.* 117: 379–390.
- Yuan, H., and D. Liu. 2008. Signaling components involved in plant responses to phosphate starvation. *J. Integr. Plant Biol.* 50(7): 849–859.

TABLES

Table 2.1 Manhattan, KS weather data. Monthly average maximum temperature and total precipitation for Manhattan, KS for the U.S. Climate Normals for 1981-2010 (Arguez et al., 2010) and for 2015 and 2016 (Kansas State University Mesonet, 2017)

Month	Average Maximum Temperature (°C)	Average Precipitation (mm)
-----Normals-----		
May	24.8	129.3
June	30.1	144.8
July	33.1	111.8
August	32.3	104.1
September	27.7	86.4
-----2015-----		
May	22.9	274.3
June	30.9	137.4
July	31.9	139.2
August	30.7	96.0
September	29.7	82.3
-----2016-----		
May	23.8	150.9
June	33.2	32.3
July	32.3	176.8
August	30.4	149.6
September	28.6	157.0

FIGURES

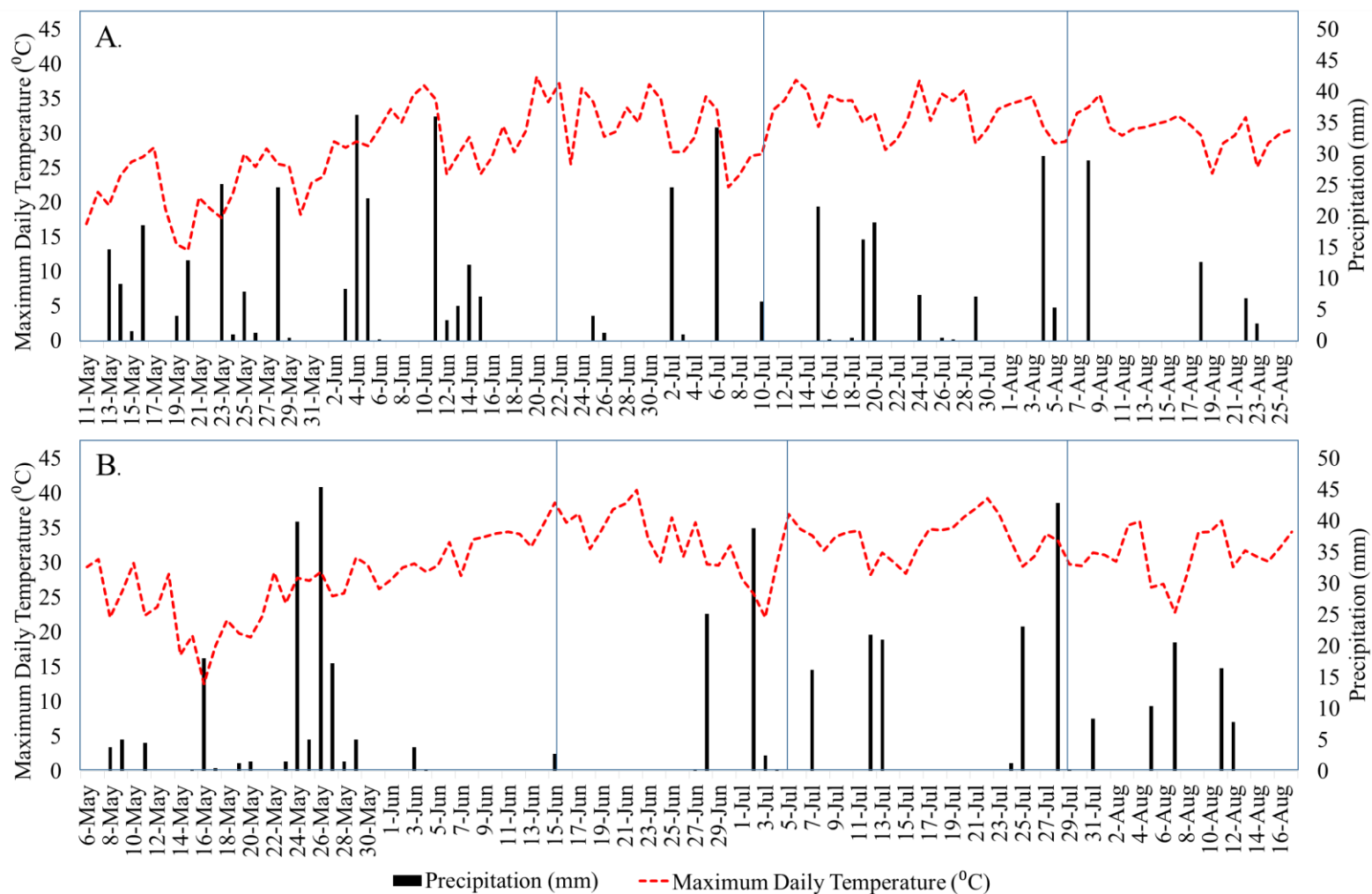


Figure 2.1 Daily precipitation and maximum temperature for the 2015 (A) and 2016 (B) growing seasons at Manhattan, KS. Vertical lines denote corn growth periods VE to V10, V10 to VT, VT to R3, and R3 to R5 (from left to right).

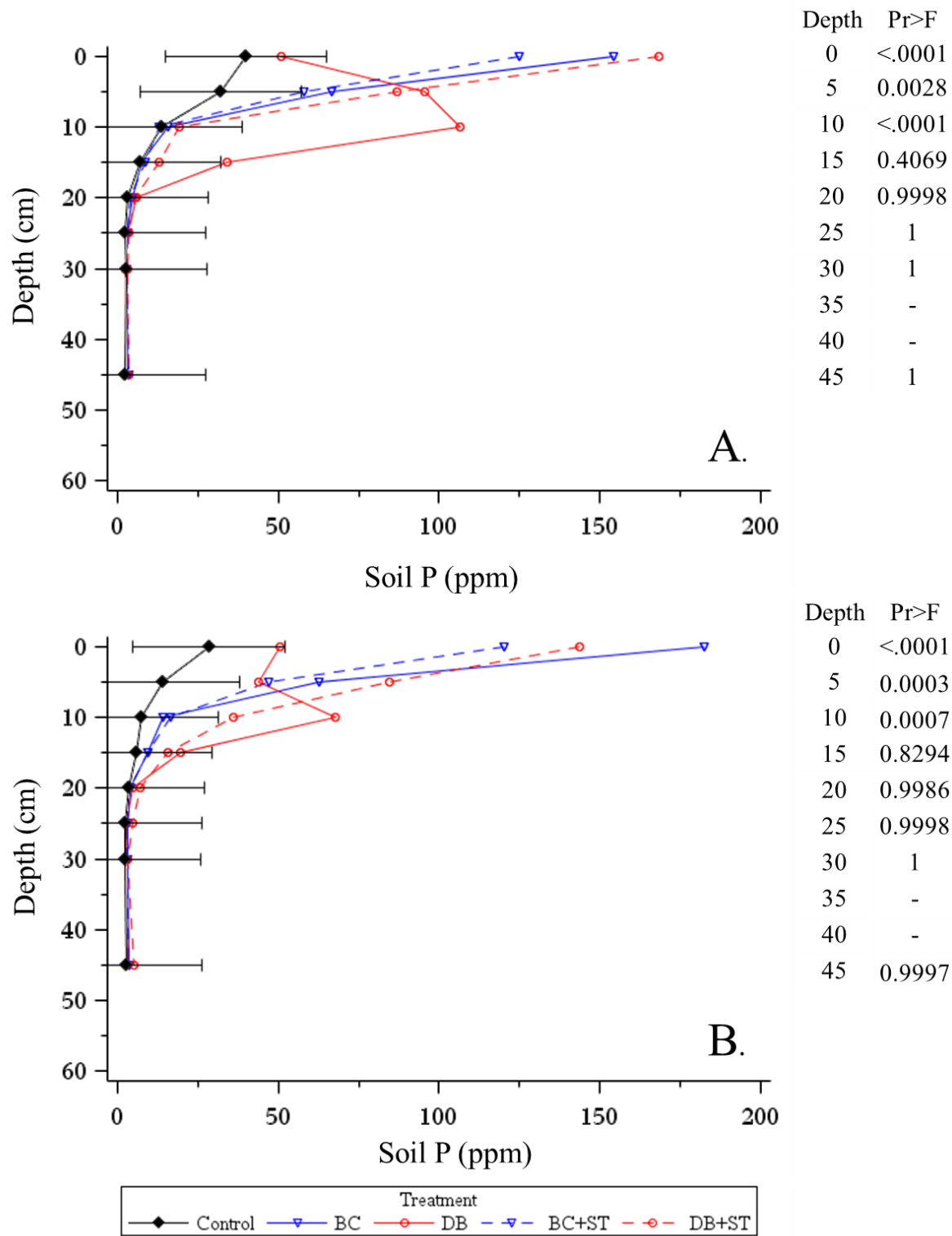


Figure 2.2 Soil test phosphorus (Mehlich-III P) by depth. Samples were taken after corn in a corn-soybean-wheat rotation in 2015 in Manhattan, KS to determine soil phosphorus concentrations in the plant row (A) and in between plant rows (B). P-values less than 0.05 denote significant treatment differences at the given depth.

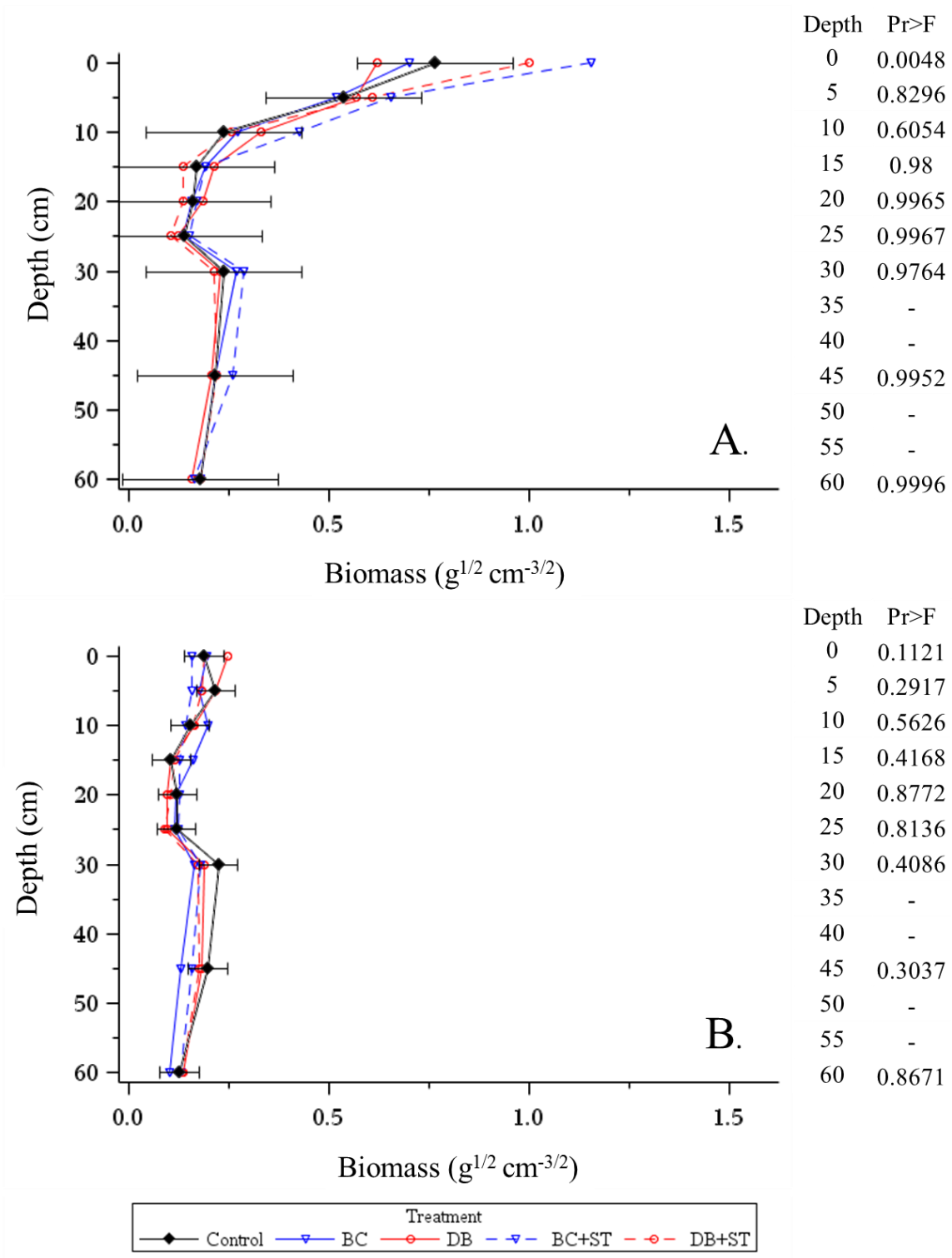


Figure 2.3 Square root transformed root biomass (g) by depth for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS. Samples were taken in 2015 in during corn grain fill to determine dry weight root biomass in the plant row (A) and in between plant rows (B). P-values less than 0.05 denote significant treatment differences at the given depth.

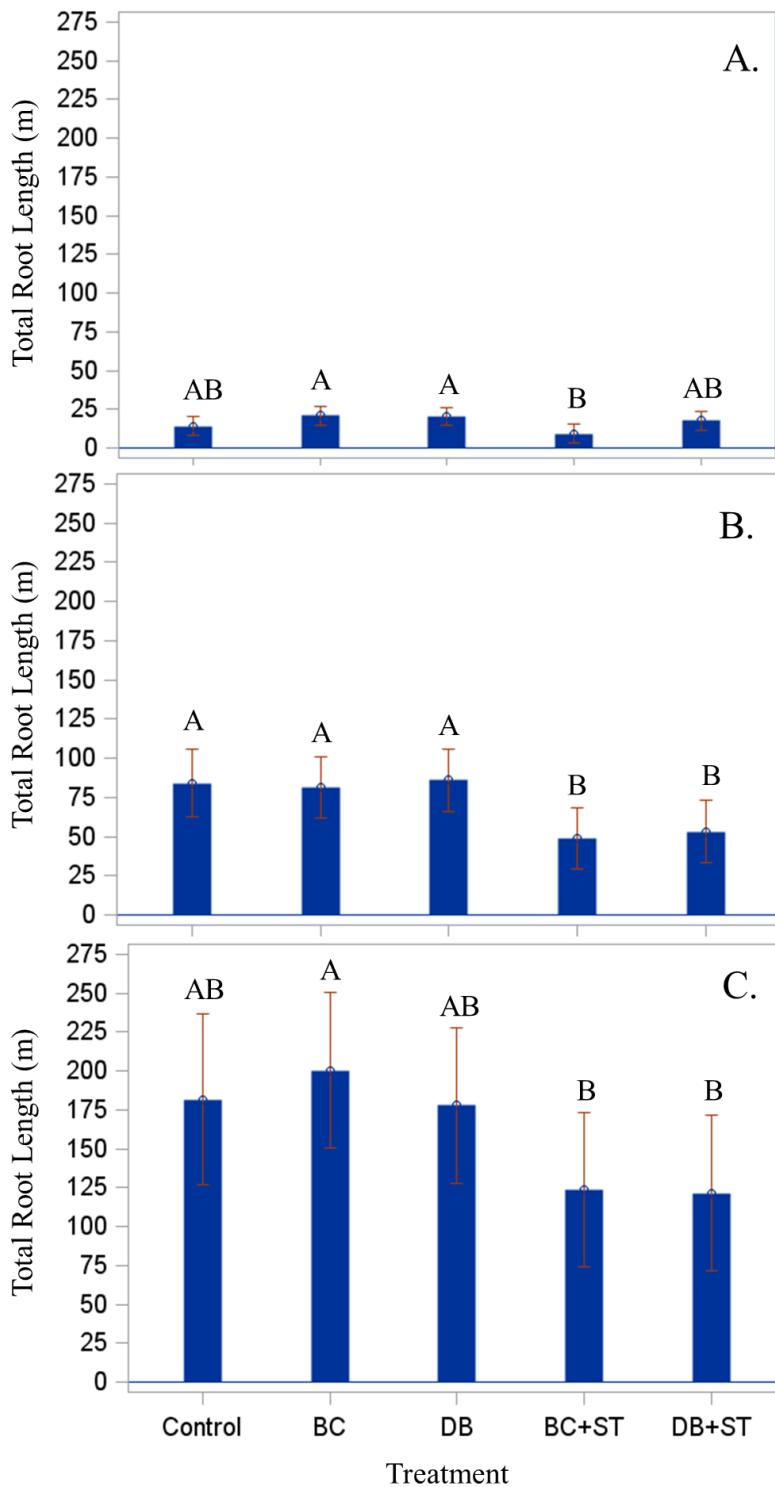


Figure 2.4 Total root length of corn for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2015 in the VE to V10 (A), V10 to VT (B), and VT to R3 (C) corn growth stages. Treatments with the same letter are not significantly different (alpha=0.05).

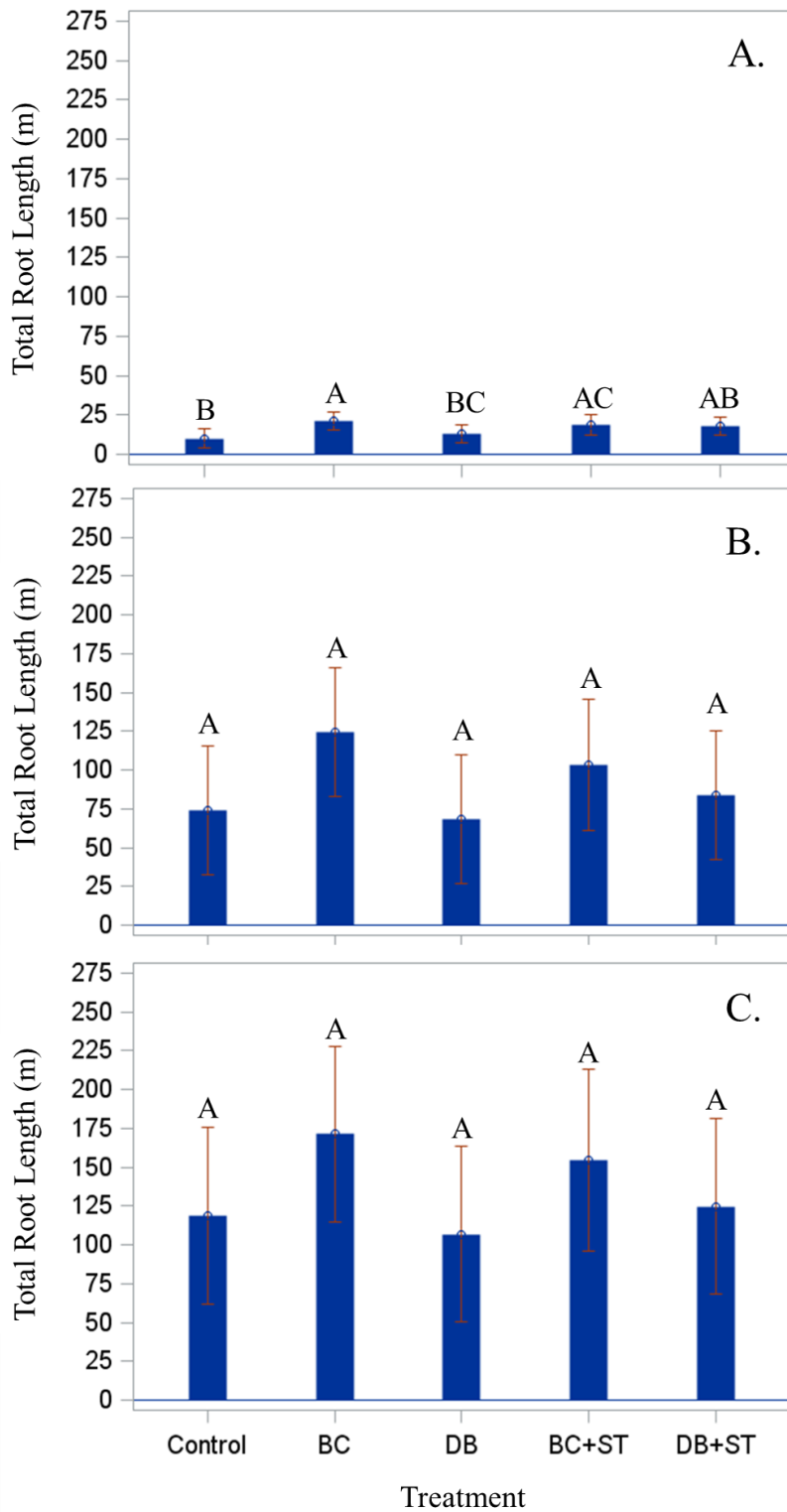


Figure 2.5 Total root length of corn for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2016 in the VE to V10 (A), V10 to VT (B), and VT to R3 (C) corn growth stages.. Treatments with the same letter are not significantly different (alpha=0.05).

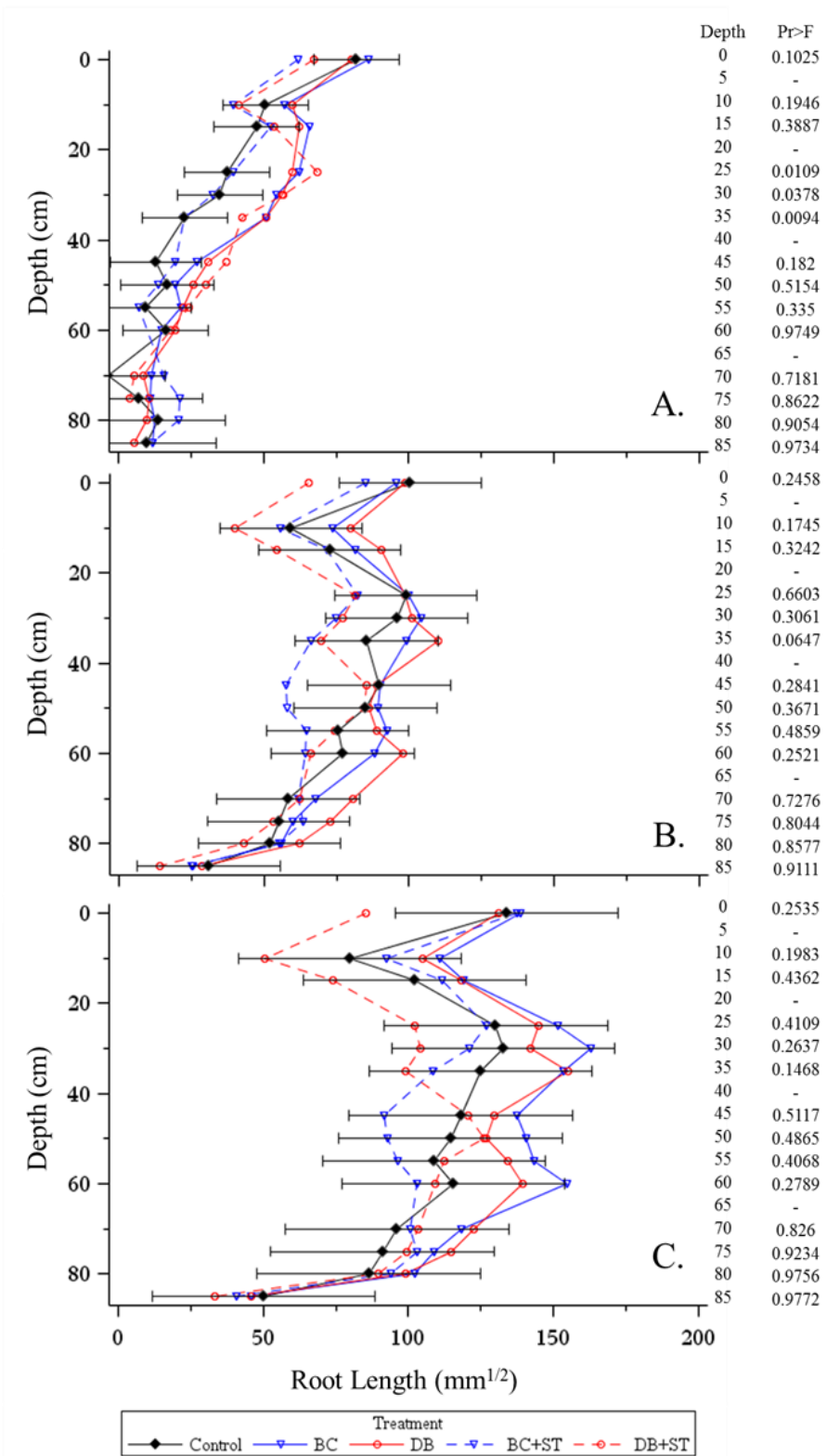


Figure 2.6 Square root transformed root length by depth of corn for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2015 in the VE to V10 (A), V10 to VT (B), and VT to R3 (C) corn growth stages.. P-values less than 0.05 denote significant treatment differences at the given depth.

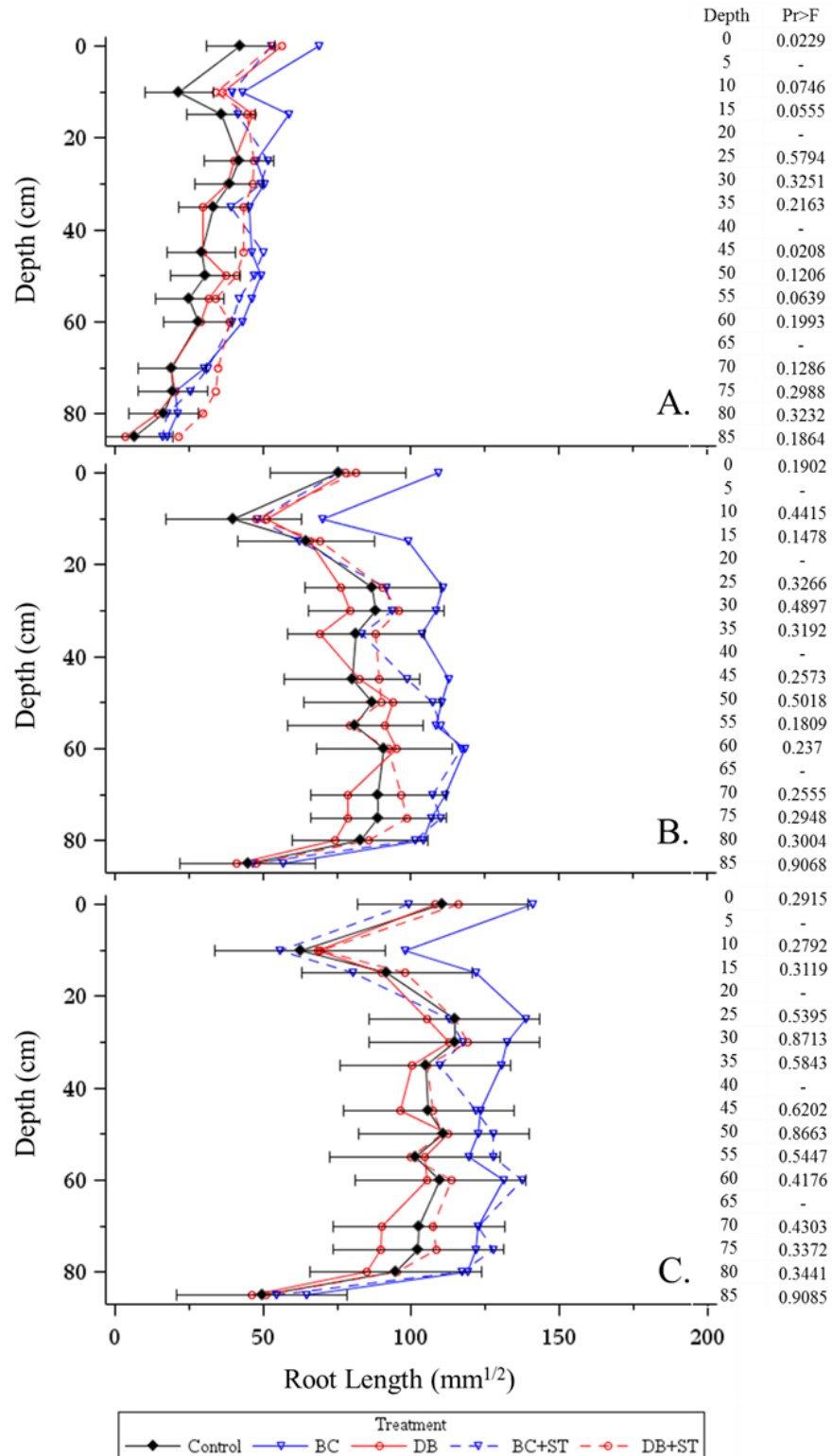


Figure 2.7 Square root transformed root length by depth of corn for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2016 in the VE to V10 (A), V10 to VT (B), and VT to R3 (C) corn growth stages.. P-values less than 0.05 denote significant treatment differences at the given depth.

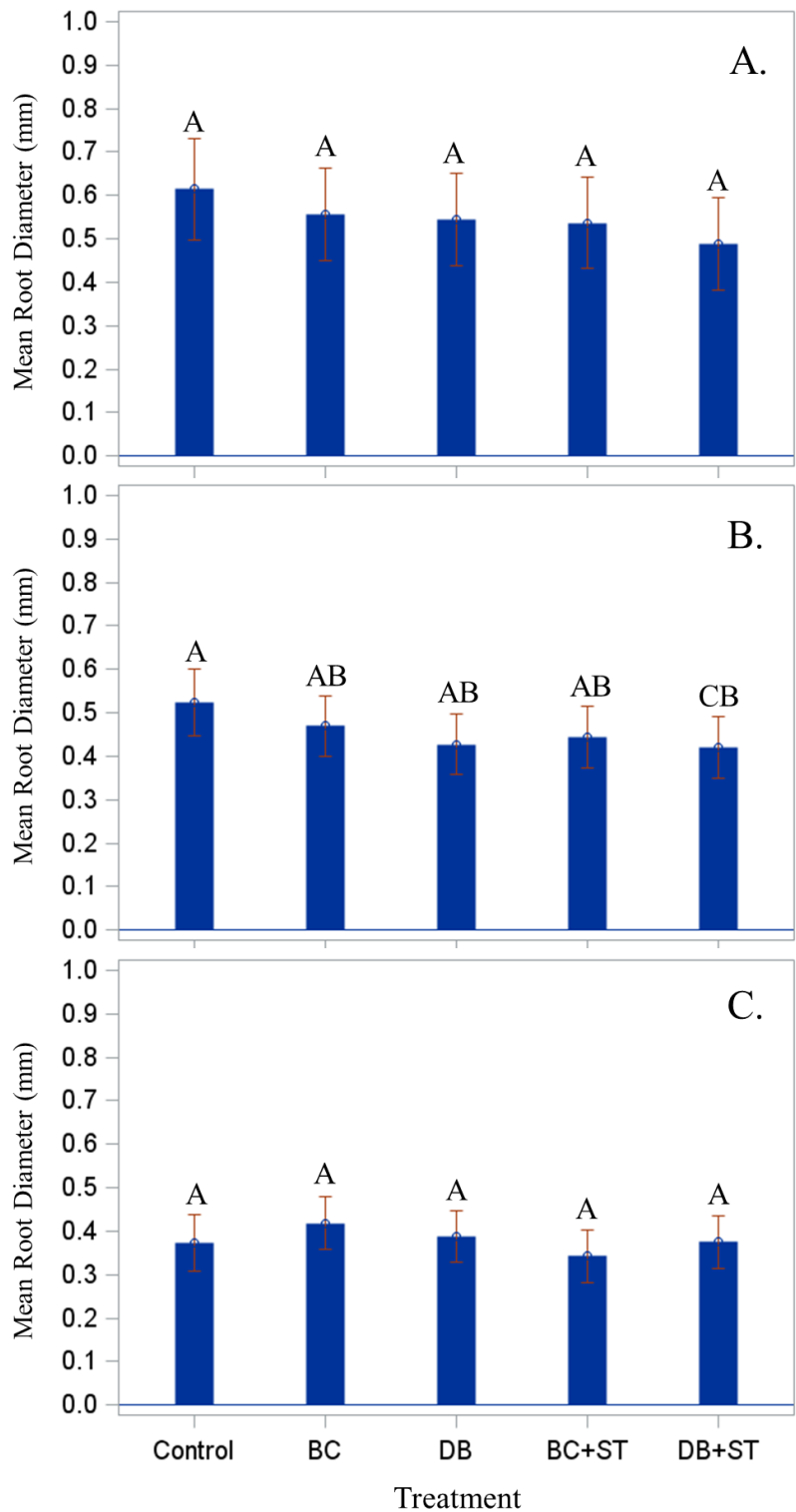


Figure 2.8 Mean root diameter of corn for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2015 in the VE to V10 (A), V10 to VT (B), and VT to R3 (C) corn growth stages. Treatments with the same letter are not significantly different ($\alpha=0.05$).

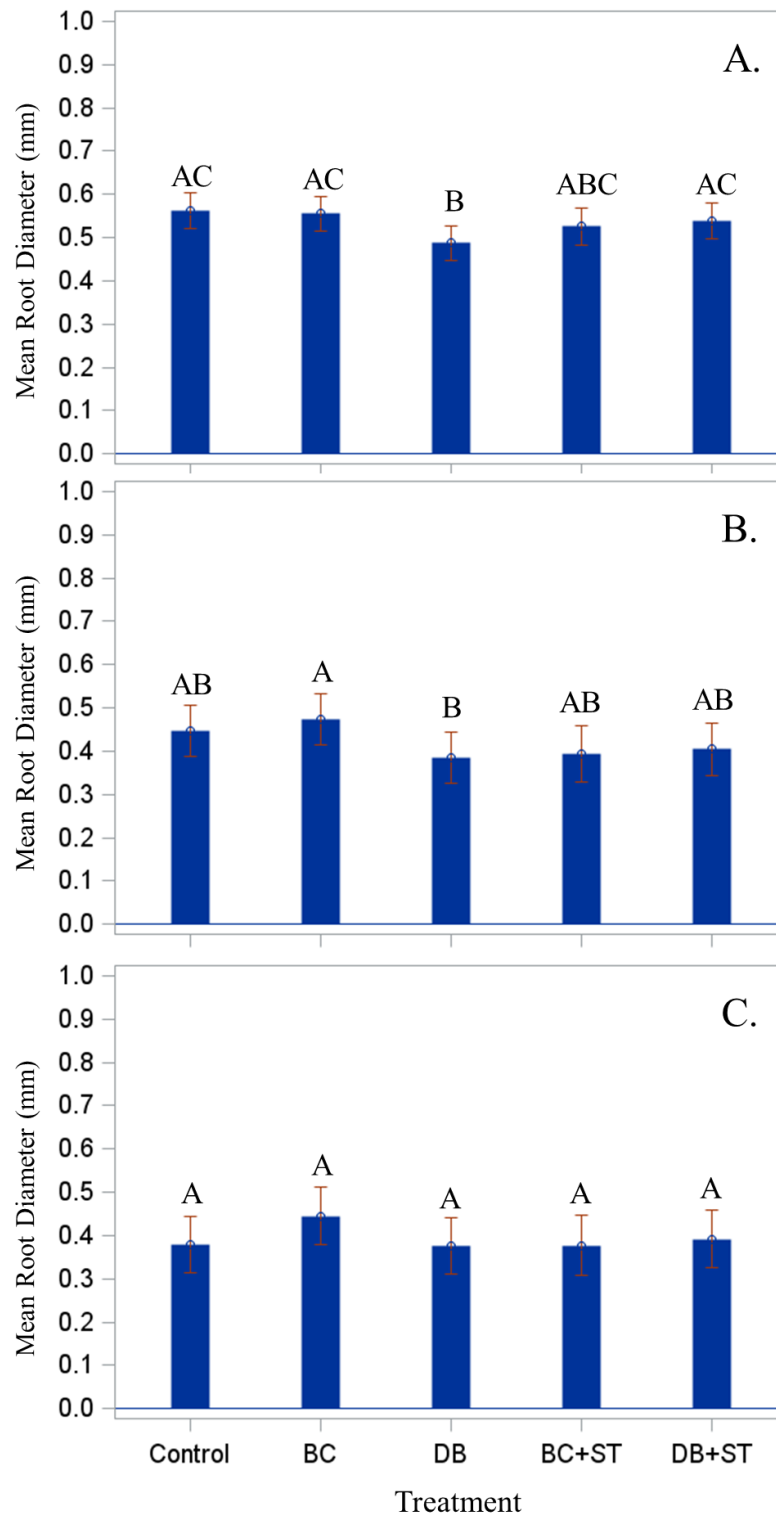


Figure 2.9 Mean root diameter of corn for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2016 in the VE to V10 (A), V10 to VT (B), and VT to R3 (C) corn growth stages. Treatments with the same letter are not significantly different (alpha=0.05).

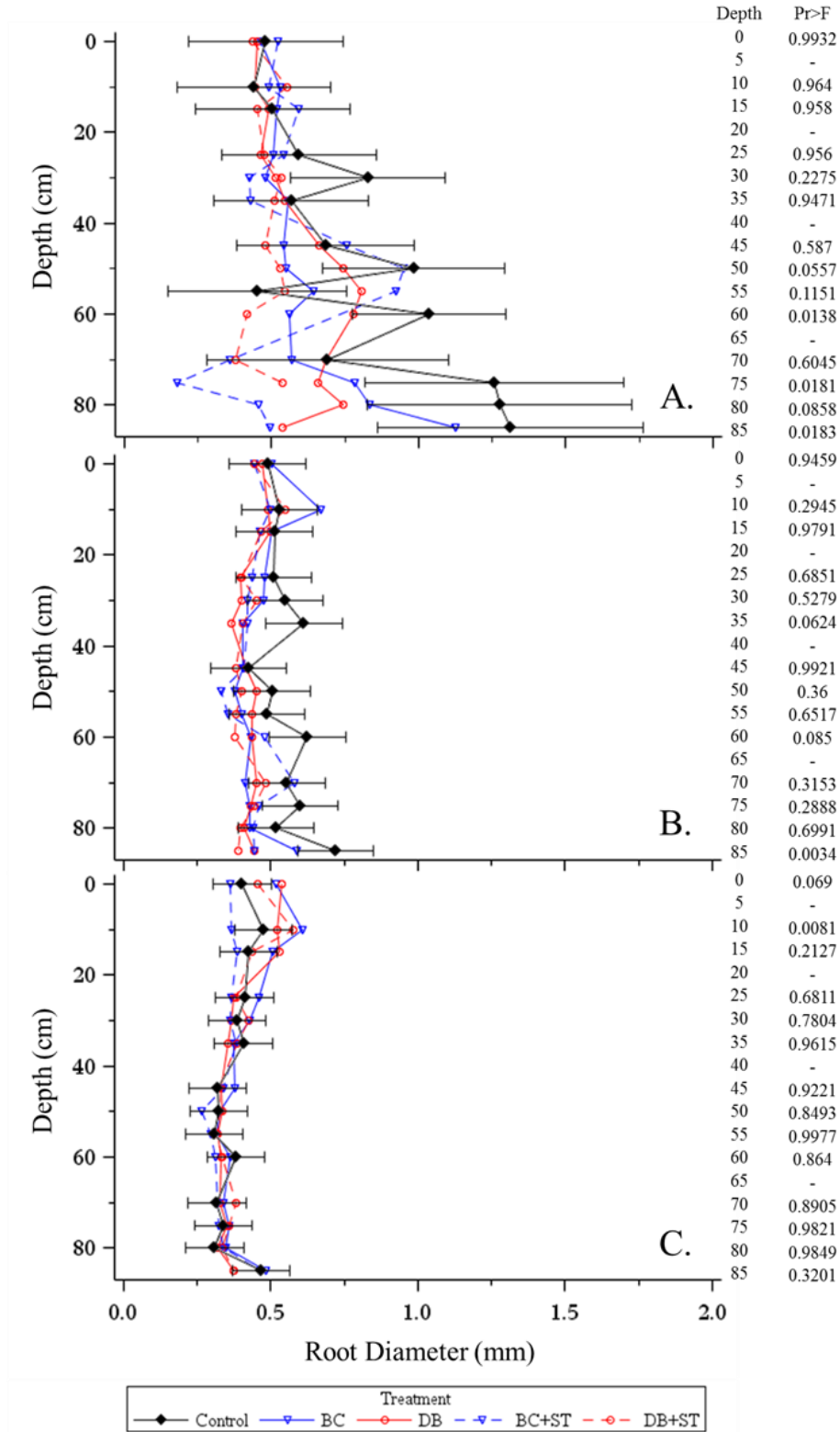


Figure 2.10 Root diameter by depth of corn for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2015 in the VE to V10 (A), V10 to VT (B), and VT to R3 (C) corn growth stages.

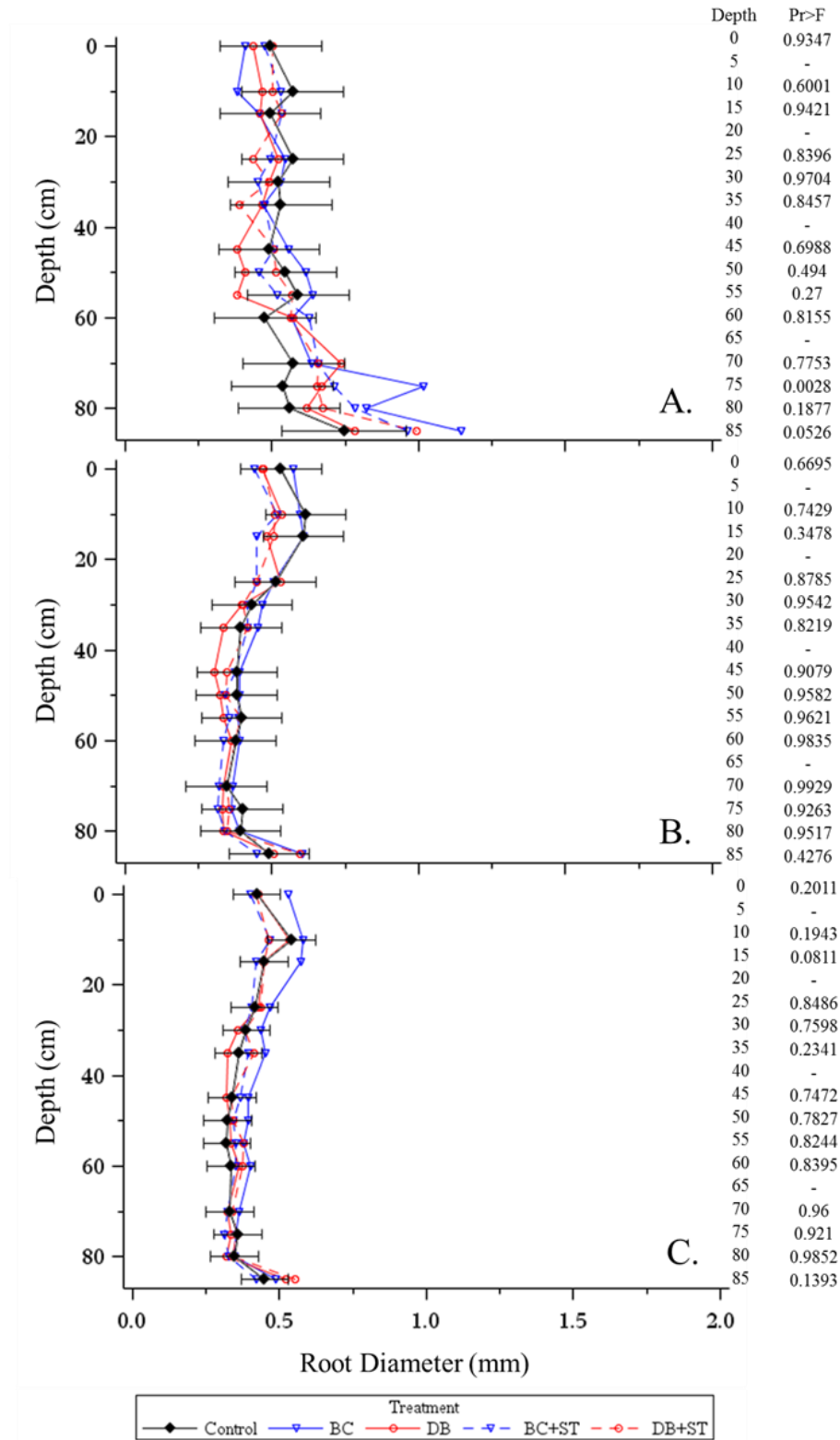


Figure 2.11 Root diameter by depth of corn for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2016 A in the VE to V10 (A), V10 to VT (B), and VT to R3 (C) corn growth stages.

APPENDIX A. Supplemental Information

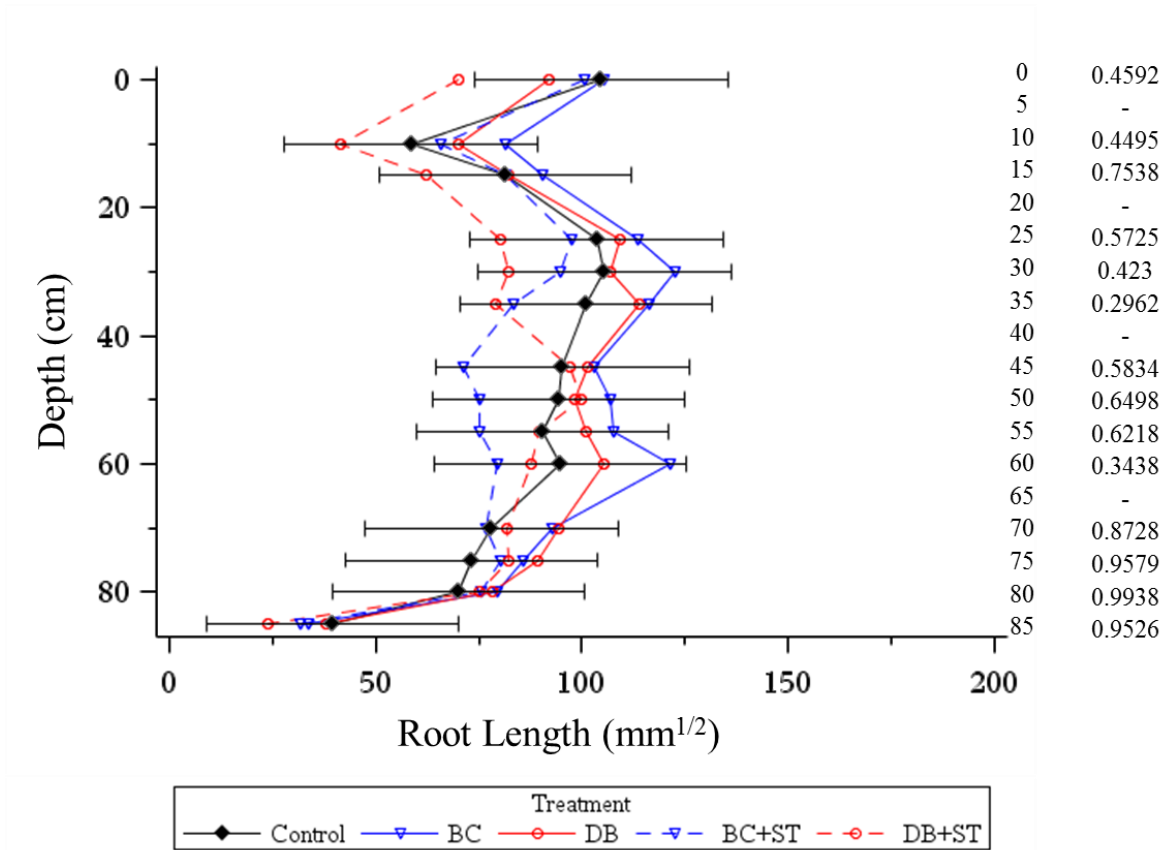


Figure A.1 Square root transformed root length by depth during corn growth stages R3 to R5 for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2015.

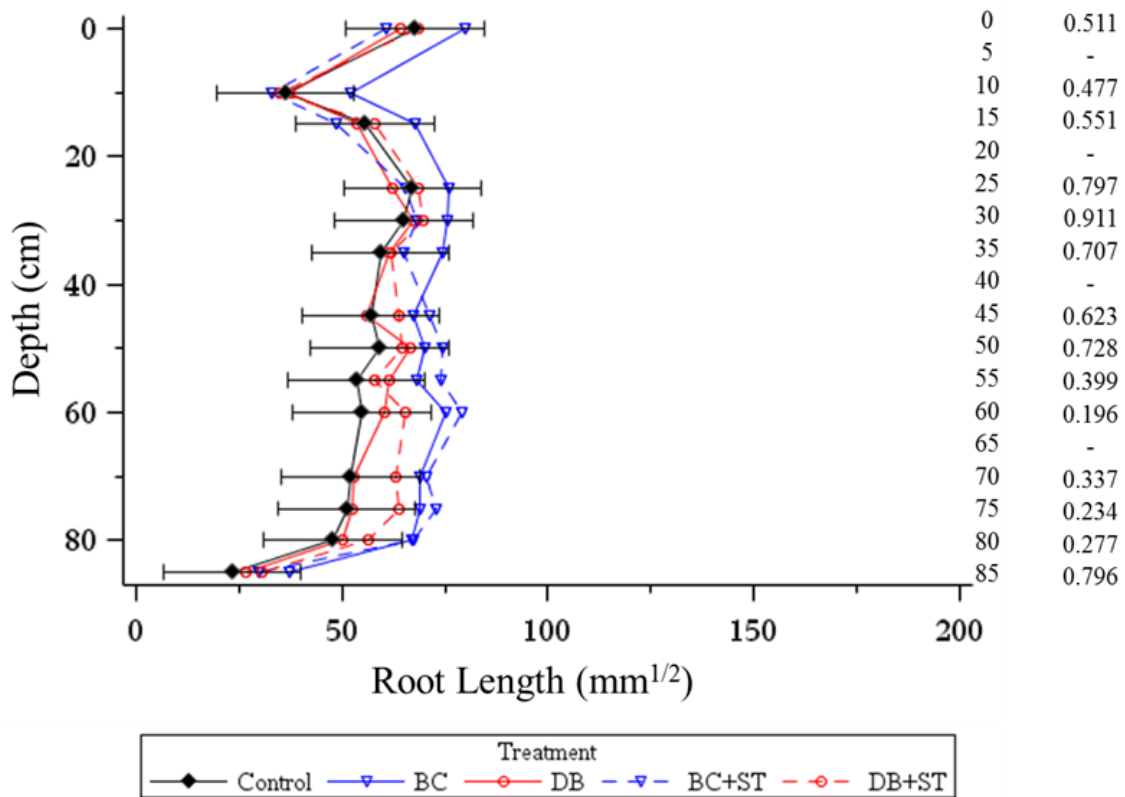


Figure A.2 Square root transformed root length by depth during corn growth stages R3 to R5 for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2016.

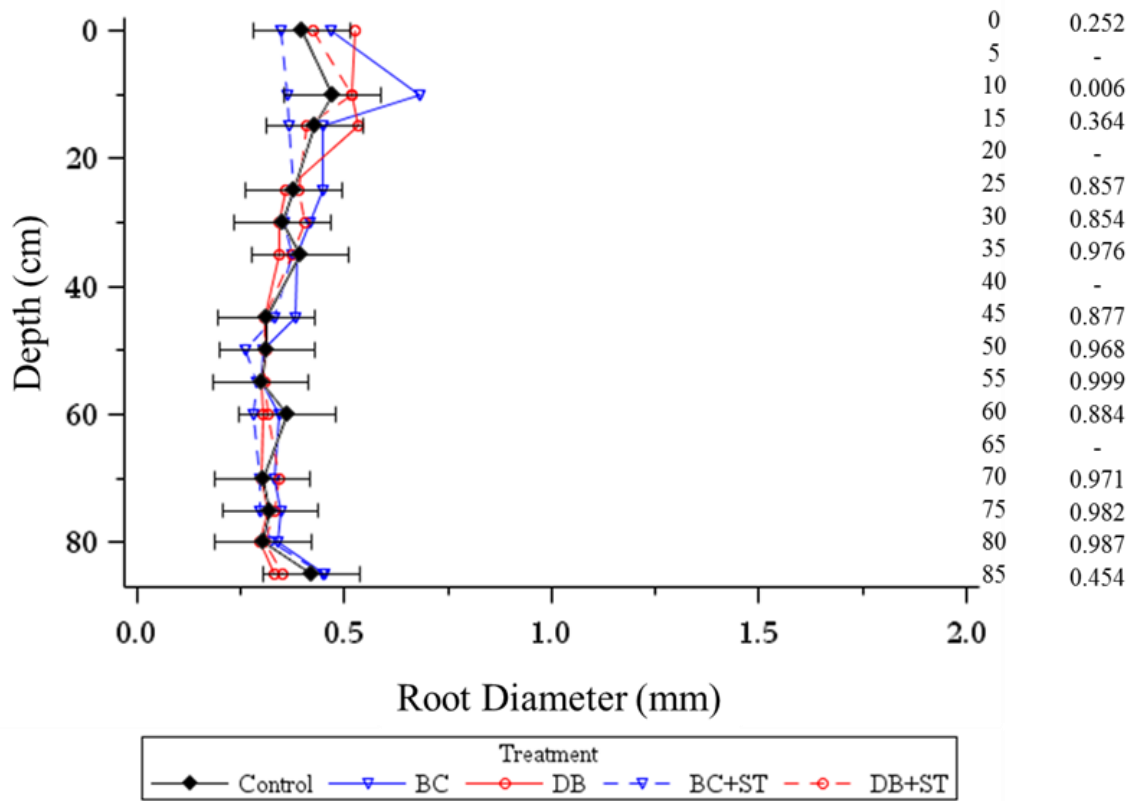


Figure A.3 Root diameter by depth during corn growth stages R3 to R5 for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2015.

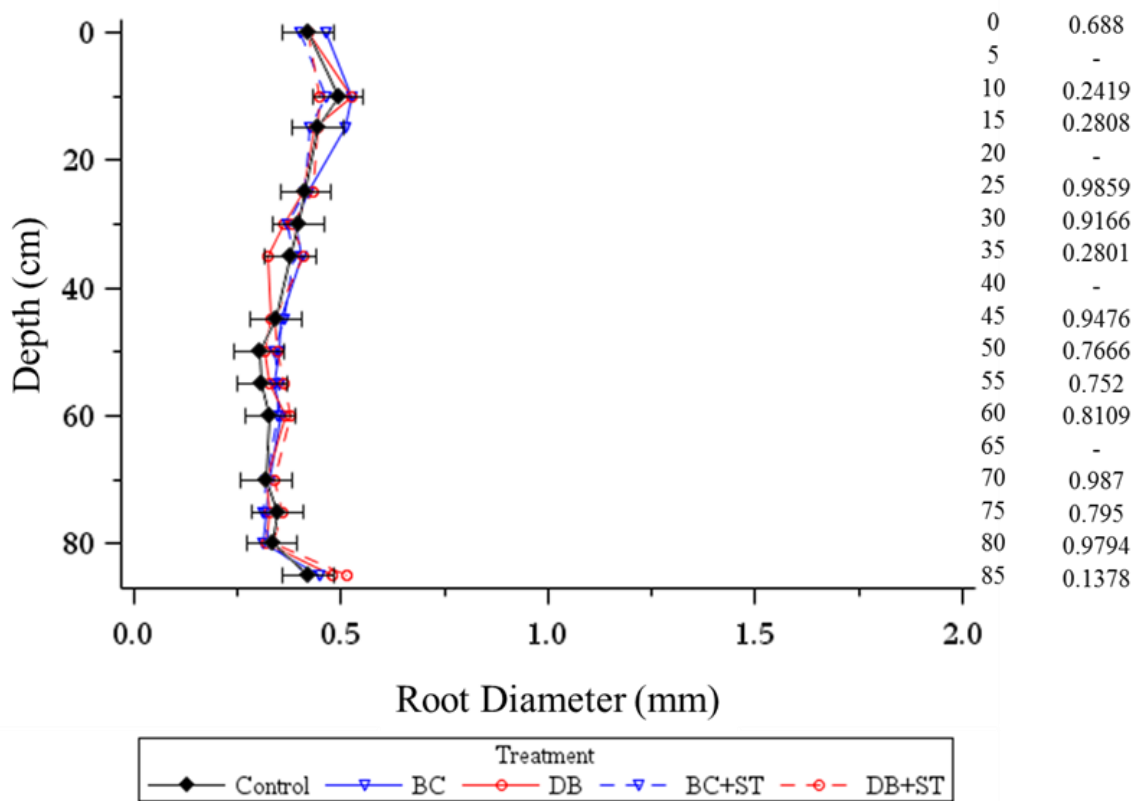


Figure A.4 Root diameter by depth during corn growth stages R3 to R5 for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2016.

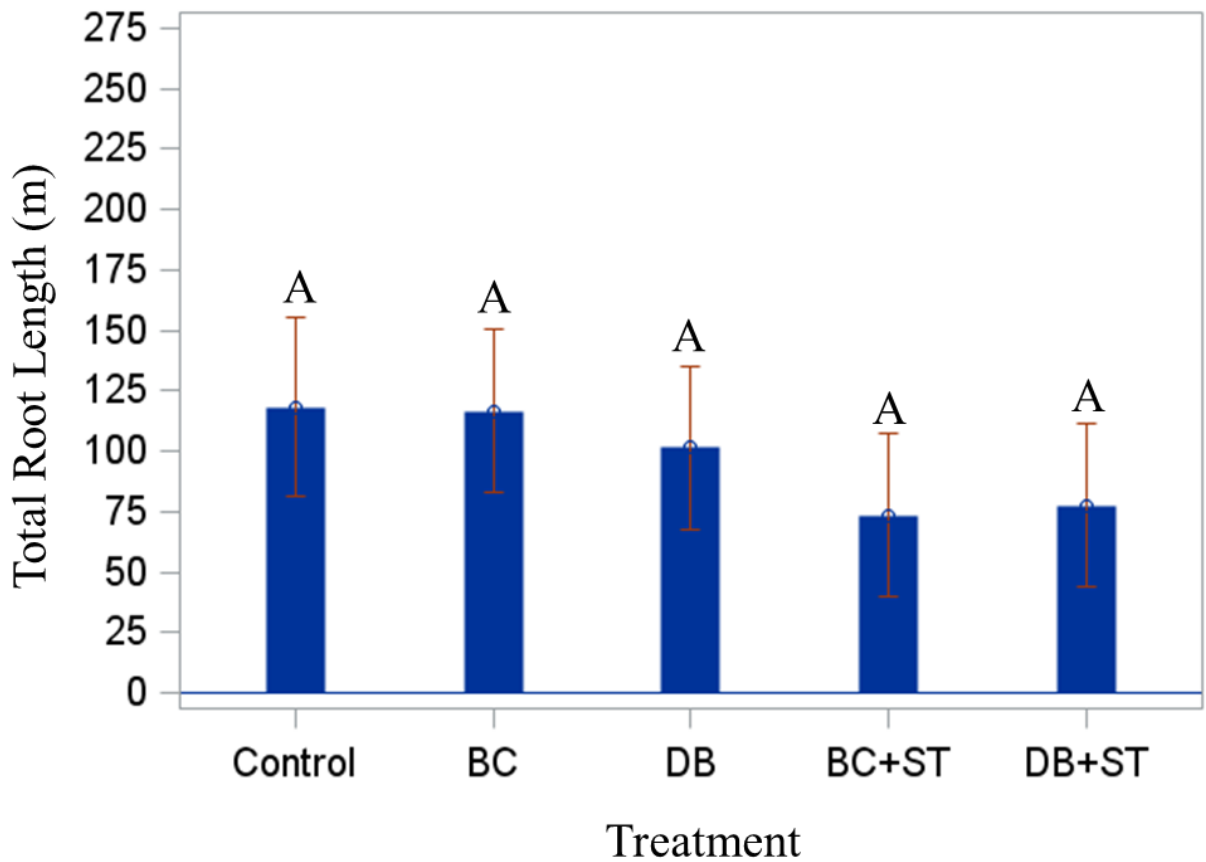


Figure A.5 Total root length during corn growth stages R3 to R5 for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2015. Treatments with the same letter are not significantly different (alpha=0.05).

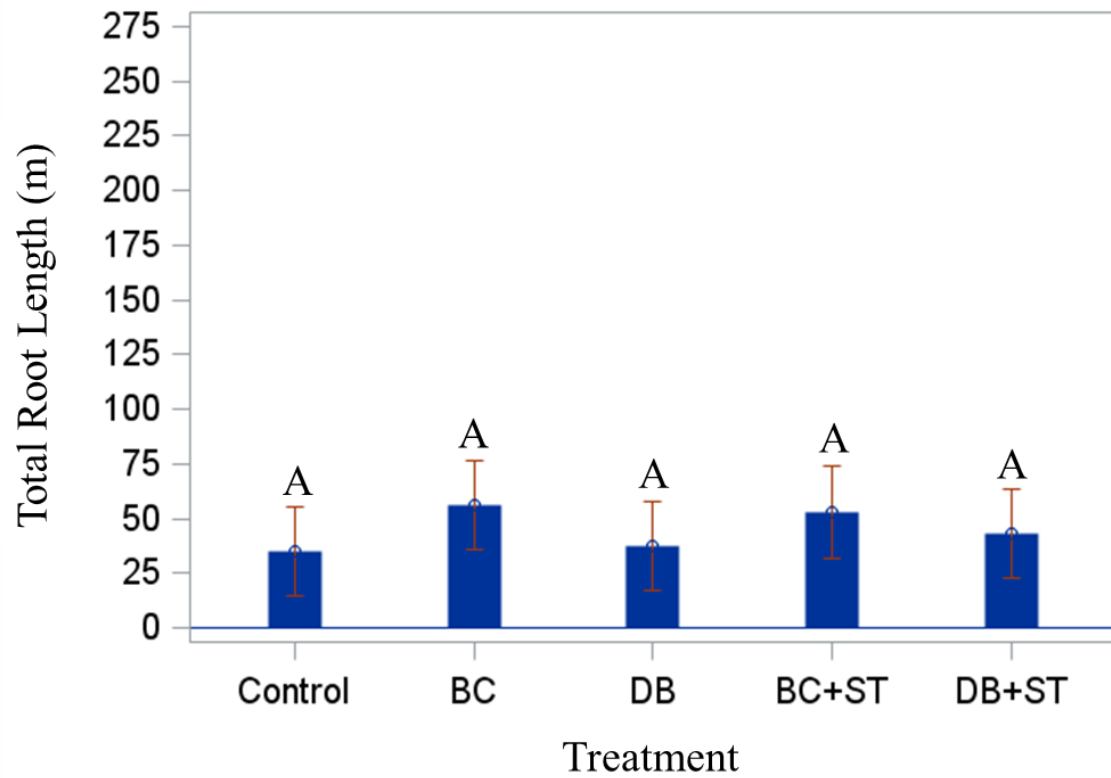


Figure A.6 Total root length during corn growth stages R3 to R5 for five phosphorus fertilizer placement methods in a strip-tilled corn-soybean-wheat rotation at Manhattan, KS in 2016. Treatments with the same letter are not significantly different (alpha=0.05).

Table A.1 Corn grain yield from Manhattan, KS.

Treatment	Yield ^{†‡} (Mg/ha)	
	-----2015-----	-----2016-----
Control	11.4	9.0
BC	12.2	9.7
DB	12.7	10.0
BC+ST	12.1	10.0
DB+ST	13.4	10.5

† No significant treatment differences were observed for both 2015 and 2016.

‡ All values had SE=0.8690.

APPENDIX B. SAS Code

Importing Data

```
proc sort data= Marcie.NFcorn2015rtsummerge;  
by tubenum winnum sessionnum rootid;  
run;  
data Marcie.NFcorn2015final;  
merge      marcie.nfcorn2015ptsummerge  
           marcie.nfcorn2015rtsummerge;  
by tubenum winnum sessionnum rootid;  
run;
```

```
proc sort data= Marcie.NFcorn2016rtsummerge;  
by tubenum winnum sessionnum rootid;  
run;  
data Marcie.NFcorn2016final;  
merge      marcie.nfcorn2016ptsummerge  
           marcie.nfcorn2016rtsummerge;  
by tubenum winnum sessionnum rootid;  
run;
```

```
proc sort data= Marcie.NFcorn2015final;  
by tubenum winnum sessionnum rootid;  
run;  
proc sort data= Marcie.NFcorn2016final;  
by tubenum winnum sessionnum rootid;  
run;  
data Marcie.NFcorn20152016;  
merge      marcie.nfcorn2015final  
           marcie.nfcorn2016final;  
by tubenum winnum sessionnum rootid;  
run;
```

Summarizing Data

```
PROC MEANS NOPRINT DATA=marcie.Nfcorn20152016;
  CLASS Year Block Trt TubeNum SessionNum DepthNum RootID;
  VAR RtLength AveDiam Area Volume;
  OUTPUT OUT =      marcie.rootsummaryF
              MEAN(RtLength AveDiam Area Volume) =
MeanRtLength MeanAveDiam MeanArea MeanVolume;
PROC PRINT DATA = marcie.rootsummaryF;
  Title 'Root Summary F';
RUN;

DATA marcie.rootsummaryG;
  SET marcie.rootsummaryf;
  IF nmiss(of _numeric_) + cmiss(of _character_) > 0 then DELETE;
run;

PROC SORT DATA=marcie.rootsummaryg;
  BY Year Block Trt TubeNum SessionNum DepthNum;

PROC MEANS NOPRINT DATA=marcie.rootsummaryg;
  BY Year Block Trt TubeNum SessionNum DepthNum;
  VAR MeanRtLength MeanAveDiam MeanArea MeanVolume;
  OUTPUT OUT =      marcie.rootsummaryH
              MEAN(MeanRtLength MeanAveDiam MeanArea
MeanVolume) = MeanRtLength2 MeanAveDiam2 MeanArea2 MeanVolume2
              MAX(MeanRtLength MeanAveDiam MeanArea
MeanVolume) = MaxRtLength MaxAveDiam MaxArea MaxVolume
              MIN(MeanRtLength MeanAveDiam MeanArea
MeanVolume) = MinRtLength MinAveDiam MinArea MinVolume
              MEDIAN(MeanRtLength MeanAveDiam MeanArea
MeanVolume) = MedRtLength MedAveDiam MedArea MedVolume
              N(MeanRtLength) = RootCount
              STDDEV(MeanRtLength MeanAveDiam MeanArea
MeanVolume) = StdDevRtLength StdDevAveDiam StdDevArea StdDevVolume
              SUM(MeanRtLength MeanArea MeanVolume) =
SumRtLength SumArea SumVolume;
PROC PRINT DATA = marcie.rootsummaryH;
  Title 'Root Summary H';
RUN;

PROC CONTENTS data=marcie.rootsummaryH;
run;

DATA marcie.rootsummaryh;
  SET marcie.rootsummaryh;
  LABEL      Block = 'Block'
            DepthNum = 'Depth'
            MaxArea = 'Maximum Area'
            MaxAveDiam = 'Maximum Diameter'
            MaxRtLength = 'Maximum Length'
            MaxVolume = 'Maximum Volume'
            MeanArea2 = 'Mean Area'
            MeanAveDiam2 = 'Mean Diameter'
```

```

MeanRtLength2 = 'Mean Length'
MeanVolume2 = 'Mean Volume'
MedArea = 'Median Area'
MedAveDiam = 'Median Diameter'
MedRtLength = 'Median Length'
MedVolume = 'Median Volume'
MinArea = 'Minimum Area'
MinAveDiam = 'Minimum Diameter'
MinRtLength = 'Minimum Length'
MinVolume = 'Minimum Volume'
RootCount = 'Root Count'
SessionNum = 'Session'
StdDevArea = 'Std Dev Area'
StdDevAveDiam = 'Std Dev Diameter'
StdDevRtLength = 'Std Dev Length'
StdDevVolume = 'Std Dev Volume'
SumArea = Area 'Sum'
SumRtLength = 'Length Sum'
SumVolume = 'Volume Sum'
Trt = 'Treatment'
TubeNum = 'Tube'
Year = 'Year'
_FREQ_ = '_FREQ_'
_TYPE_ = '_TYPE_';

```

run;

PROC CONTENTS data=marcie.rootsummaryh;

run;

DATA Marcie.rootsummary2015;

SET Marcie.rootsummaryH;

IF year= '2016' THEN delete;

run;

DATA Marcie.rootsummary2016;

SET Marcie.rootsummaryH;

IF year= '2015' THEN delete;

run;

DATA Marcie.rootsummary2015;

SET Marcie.rootsummary2015;

IF SessionNum='01' THEN GSTAGE='Veg1';

IF SessionNum='02' THEN GSTAGE='Veg1';

IF SessionNum='03' THEN GSTAGE='Veg1';

IF SessionNum='04' THEN GSTAGE='Veg1';

IF SessionNum='05' THEN GSTAGE='Veg1';

IF SessionNum='06' THEN GSTAGE='Veg1';

IF SessionNum='07' THEN GSTAGE='Veg2';

IF SessionNum='08' THEN GSTAGE='Veg2';

IF SessionNum='09' THEN GSTAGE='Veg2';

IF SessionNum='10' THEN GSTAGE='Veg2';

IF SessionNum='11' THEN GSTAGE='Rep1';

IF SessionNum='12' THEN GSTAGE='Rep1';

IF SessionNum='13' THEN GSTAGE='Rep1';

IF SessionNum='14' THEN GSTAGE='Rep1';

IF SessionNum='15' THEN GSTAGE='Rep1';

IF SessionNum='16' THEN GSTAGE='Rep2';

```
IF SessionNum='17' THEN GSTAGE='Rep2';
IF SessionNum='18' THEN GSTAGE='Rep2';
RUN;
```

```
DATA Marcie.rootsummary2016;
SET Marcie.rootsummary2016;
IF SessionNum='01' THEN GSTAGE='Veg1';
IF SessionNum='02' THEN GSTAGE='Veg1';
IF SessionNum='03' THEN GSTAGE='Veg1';
IF SessionNum='04' THEN GSTAGE='Veg2';
IF SessionNum='05' THEN GSTAGE='Veg2';
IF SessionNum='06' THEN GSTAGE='Veg2';
IF SessionNum='07' THEN GSTAGE='Rep1';
IF SessionNum='08' THEN GSTAGE='Rep1';
IF SessionNum='09' THEN GSTAGE='Rep1';
IF SessionNum='10' THEN GSTAGE='Rep2';
IF SessionNum='11' THEN GSTAGE='Rep2';
IF SessionNum='12' THEN GSTAGE='Rep2';
RUN;
```

```
DATA Marcie.rootsummary2015;
SET Marcie.rootsummary2015;
IF TubeNum= '01' THEN Plot= '201';
IF TubeNum= '06' THEN Plot= '201';
IF TubeNum= '02' THEN Plot= '202';
IF TubeNum= '07' THEN Plot= '202';
IF TubeNum= '03' THEN Plot= '206';
IF TubeNum= '08' THEN Plot= '206';
IF TubeNum= '04' THEN Plot= '208';
IF TubeNum= '09' THEN Plot= '208';
IF TubeNum= '05' THEN Plot= '211';
IF TubeNum= '10' THEN Plot= '211';
IF TubeNum= '11' THEN Plot= '325';
IF TubeNum= '16' THEN Plot= '325';
IF TubeNum= '12' THEN Plot= '328';
IF TubeNum= '17' THEN Plot= '328';
IF TubeNum= '13' THEN Plot= '330';
IF TubeNum= '18' THEN Plot= '330';
IF TubeNum= '14' THEN Plot= '335';
IF TubeNum= '19' THEN Plot= '335';
IF TubeNum= '15' THEN Plot= '336';
IF TubeNum= '20' THEN Plot= '336';
IF TubeNum= '21' THEN Plot= '302';
IF TubeNum= '26' THEN Plot= '302';
IF TubeNum= '22' THEN Plot= '303';
IF TubeNum= '27' THEN Plot= '303';
IF TubeNum= '23' THEN Plot= '307';
IF TubeNum= '28' THEN Plot= '307';
IF TubeNum= '24' THEN Plot= '309';
IF TubeNum= '29' THEN Plot= '309';
IF TubeNum= '25' THEN Plot= '311';
IF TubeNum= '30' THEN Plot= '311';
RUN;
```

```
DATA Marcie.rootsummary2016;
SET Marcie.rootsummary2016;
IF TubeNum= '01' THEN Plot= '104';
```

```
IF TubeNum= '06' THEN Plot= '104';
IF TubeNum= '02' THEN Plot= '105';
IF TubeNum= '07' THEN Plot= '105';
IF TubeNum= '03' THEN Plot= '107';
IF TubeNum= '08' THEN Plot= '107';
IF TubeNum= '04' THEN Plot= '108';
IF TubeNum= '09' THEN Plot= '108';
IF TubeNum= '05' THEN Plot= '112';
IF TubeNum= '10' THEN Plot= '112';
IF TubeNum= '11' THEN Plot= '215';
IF TubeNum= '17' THEN Plot= '215';
IF TubeNum= '12' THEN Plot= '221';
IF TubeNum= '18' THEN Plot= '221';
IF TubeNum= '13' THEN Plot= '223';
IF TubeNum= '19' THEN Plot= '223';
IF TubeNum= '14' THEN Plot= '224';
IF TubeNum= '20' THEN Plot= '224';
IF TubeNum= '15' THEN Plot= '214';
IF TubeNum= '16' THEN Plot= '214';
IF TubeNum= '21' THEN Plot= '125';
IF TubeNum= '26' THEN Plot= '125';
IF TubeNum= '22' THEN Plot= '129';
IF TubeNum= '27' THEN Plot= '129';
IF TubeNum= '23' THEN Plot= '132';
IF TubeNum= '28' THEN Plot= '132';
IF TubeNum= '24' THEN Plot= '133';
IF TubeNum= '29' THEN Plot= '133';
IF TubeNum= '25' THEN Plot= '136';
IF TubeNum= '30' THEN Plot= '136';
```

RUN;

Plot Level Data

```
PROC SORT DATA=Marcie.Rootsummary2015;
  BY Block Trt GStage Plot DepthNum;

PROC MEANS NOPRINT DATA=marcie.Rootsummary2015;
  BY Block Trt GStage Plot DepthNum;
  VAR MeanRtLength2 MeanAveDiam2 MeanArea2 MeanVolume2 RootCount
  SumRtLength;
  OUTPUT OUT =          marcie.rootsummary2015plotmeans
                        MEAN(MeanAveDiam2 MeanArea2 MeanVolume2) =
MeanDiamPL MeanAreaPL MeanVolPL
                        N(RootCount) = RootCountPL
                        SUM(SumRtLength) = SumRtLengthPL;

PROC PRINT DATA = marcie.rootsummary2015plotmeans;
  Title 'Plot Level Data';
RUN;

PROC SORT DATA=Marcie.Rootsummary2016;
  BY Block Trt GStage Plot DepthNum;

PROC MEANS NOPRINT DATA=marcie.Rootsummary2016;
  BY Block Trt GStage Plot DepthNum;
  VAR MeanRtLength2 MeanAveDiam2 MeanArea2 MeanVolume2 RootCount
  SumRtLength;
  OUTPUT OUT =          marcie.rootsummary2016plotmeans
                        MEAN(MeanAveDiam2 MeanArea2 MeanVolume2) =
MeanDiamPL MeanAreaPL MeanVolPL
                        N(RootCount) = RootCountPL
                        SUM(SumRtLength) = SumRtLengthPL;

PROC PRINT DATA = marcie.rootsummary2016plotmeans;
  Title 'Plot Level Data';
RUN;
```


Square root transformations

```
data Marcie.Rootsummary2015plotmeans;  
  set Marcie.Rootsummary2015plotmeans;  
  SumRtLengthPLsqrt=sqrt (SumRtLengthPL);  
  run;
```

```
data Marcie.Rootsummary2016plotmeans;  
  set Marcie.Rootsummary2016plotmeans;  
  SumRtLengthPLsqrt=sqrt (SumRtLengthPL);  
  run;
```

Root Length

```
PROC SORT DATA=Marcie.Rootsummary2015plotmeans;
  BY GStage Block Trt Plot DepthNum;
run;
```

```
PROC MIXED data=Marcie.Rootsummary2015plotmeans plots=all;
class GStage Block Trt Plot DepthNum;
model SumRtLengthPLsqrt = Trt|DepthNum/DDFM=SATTERTH residual;
BY GStage;
RANDOM BLOCK;
REPEATED DepthNum / subject=plot type=AR(1);
lsmeans Trt|DepthNum/ slice=(Trt DepthNum) cl diff adjust=Tukey;
run;
%include 'R:\Sindt_Thesis_Research\Marcie_SAS_Library\pdmix800-1.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
```

```
PROC SORT DATA=Marcie.Rootsummary2016plotmeans;
  BY GStage Block Trt Plot DepthNum;
run;
```

```
PROC MIXED data=Marcie.Rootsummary2016plotmeans plots=all;
class GStage Block Trt Plot DepthNum;
model SumRtLengthPLsqrt = Trt|DepthNum/DDFM=SATTERTH residual;
BY GStage;
RANDOM BLOCK;
REPEATED DepthNum / subject=plot type=AR(1);
lsmeans Trt|DepthNum/ slice=(Trt DepthNum) cl diff adjust=Tukey;
run;
%include 'R:\Sindt_Thesis_Research\Marcie_SAS_Library\pdmix800-1.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
```

Root Diameter

```
PROC SORT DATA=Marcie.Rootsummary2015plotmeans;
  BY GStage Block Trt Plot DepthNum;
  run;

PROC MIXED data=Marcie.Rootsummary2015plotmeans plots=all;
class GStage Block Trt Plot DepthNum;
model MeanDiamPL = Trt|DepthNum/DDFM=SATTERTH residual;
BY GStage;
RANDOM BLOCK;
REPEATED DepthNum / subject=plot type=AR(1);
lsmeans Trt|DepthNum/ slice=(Trt DepthNum) cl diff adjust=Tukey;
run;
%include 'R:\Sindt_Thesis_Research\Marcie_SAS_Library\pdmix800-1.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;

PROC SORT DATA=Marcie.Rootsummary2016plotmeans;
  BY GStage Block Trt Plot DepthNum;
  run;

PROC MIXED data=Marcie.Rootsummary2016plotmeans plots=all;
class GStage Block Trt Plot DepthNum;
model MeanDiamPL = Trt|DepthNum/DDFM=SATTERTH residual;
BY GStage;
RANDOM BLOCK;
REPEATED DepthNum / subject=plot type=AR(1);
lsmeans Trt|DepthNum/ slice=(Trt DepthNum) cl diff adjust=Tukey;
run;
%include 'R:\Sindt_Thesis_Research\Marcie_SAS_Library\pdmix800-1.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
```

Biomass

```
PROC SORT DATA=Marcie.rootbiomass2;
  BY Block Trt Plot Depth Loc;
  run;

PROC MEANS NOPRINT DATA=Marcie.rootbiomass2;
  BY Block Trt Plot Depth Loc;
  VAR biomasssqrt;
  OUTPUT OUT =      marcie.rootbiomassplotmeans
              MEAN(biomasssqrt) = MeanBiomassSQRT;

RUN;

Data marcie.rootbiomassplotmeans;
  SET marcie.rootbiomassplotmeans;
  If Depth= 2 THEN Depth2= 0;
  If Depth= 4 THEN Depth2= 5;
  If Depth= 6 THEN Depth2= 10;
  If Depth= 8 THEN Depth2= 15;
  If Depth= 10 THEN Depth2= 20;
  If Depth= 12 THEN Depth2= 25;
  If Depth= 18 THEN Depth2= 30;
  If Depth= 24 THEN Depth2= 45;
  If Depth= 30 THEN Depth2= 60;
  DROP Depth;
  RENAME Depth2=Depth;

RUN;
DATA marcie.rootbiomassplotmeans;
SET marcie.rootbiomassplotmeans;
  IF TubeNum= '01' THEN Plot= '201';
  IF TubeNum= '06' THEN Plot= '201';
  IF TubeNum= '02' THEN Plot= '202';
  IF TubeNum= '07' THEN Plot= '202';
  IF TubeNum= '03' THEN Plot= '206';
  IF TubeNum= '08' THEN Plot= '206';
  IF TubeNum= '04' THEN Plot= '208';
  IF TubeNum= '09' THEN Plot= '208';
  IF TubeNum= '05' THEN Plot= '211';
  IF TubeNum= '10' THEN Plot= '211';
  IF TubeNum= '11' THEN Plot= '325';
  IF TubeNum= '16' THEN Plot= '325';
  IF TubeNum= '12' THEN Plot= '328';
  IF TubeNum= '17' THEN Plot= '328';
  IF TubeNum= '13' THEN Plot= '330';
  IF TubeNum= '18' THEN Plot= '330';
  IF TubeNum= '14' THEN Plot= '335';
  IF TubeNum= '19' THEN Plot= '335';
  IF TubeNum= '15' THEN Plot= '336';
  IF TubeNum= '20' THEN Plot= '336';
  IF TubeNum= '21' THEN Plot= '302';
  IF TubeNum= '26' THEN Plot= '302';
  IF TubeNum= '22' THEN Plot= '303';
  IF TubeNum= '27' THEN Plot= '303';
  IF TubeNum= '23' THEN Plot= '307';
  IF TubeNum= '28' THEN Plot= '307';
  IF TubeNum= '24' THEN Plot= '309';
```

```

    IF TubeNum= '29' THEN Plot= '309';
    IF TubeNum= '25' THEN Plot= '311';
    IF TubeNum= '30' THEN Plot= '311';
RUN;

PROC SORT DATA=Marcie.rootbiomassplotmeans;
  BY Loc Block Trt Depth Plot;
  run;

PROC MIXED data=Marcie.rootbiomassplotmeans plots=all;
class Loc Block Trt Depth Plot ;
model MeanBiomassSqrt = Trt|Depth/DDFM=SATTERTH residual;
By Loc;
RANDOM BLOCK;
REPEATED Depth/ subject=plot type=AR(1);
lsmeans Trt|Depth/ slice=(Trt Depth) cl diff adjust=Tukey;
run;

%include 'R:\Sindt_Thesis_Research\Marcie_SAS_Library\pdmix800-1.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;

```

Soil Test Phosphorus

```
PROC MEANS NOPRINT DATA=Marcie.soiltestp;
  BY Block Trt Plot Depth Loc;
  VAR P;
  OUTPUT OUT =      marcie.soiltestpplotmeans
                MEAN(P) = MeanP;
RUN;
Data marcie.soiltestpplotmeans ;
SET marcie.soiltestpplotmeans ;
If Depth= 2 THEN Depth2= 0;
If Depth= 4 THEN Depth2= 5;
If Depth= 6 THEN Depth2= 10;
If Depth= 8 THEN Depth2= 15;
If Depth= 10 THEN Depth2= 20;
If Depth= 12 THEN Depth2= 25;
If Depth= 18 THEN Depth2= 30;
If Depth= 24 THEN Depth2= 45;
If Depth= 30 THEN Depth2= 60;
DROP Depth;
RENAME Depth2=Depth;
RUN;

PROC SORT DATA=marcie.soiltestpplotmeans;
  BY Loc Block Trt Depth Plot;
  run;

PROC MIXED data=marcie.soiltestpplotmeans plots=all;
class Loc Block Trt Depth Plot ;
model MeanP = Trt|Depth/DDFM=SATTERTH residual;
By Loc;
RANDOM BLOCK;
REPEATED Depth/ subject=plot type=AR(1);
lsmeans Trt|Depth/ slice=(Trt Depth) cl diff adjust=Tukey;
run;

PROC SORT DATA=marcie.soiltestp;
  BY Block Trt Plot Depth Loc;
  run;

PROC MIXED data=Marcie.soiltestp plots=all;
class Block Trt Plot Depth Loc;
model P = Trt|Depth|Loc/DDFM=SATTERTH residual;
/*BY Loc;*/
RANDOM BLOCK;
REPEATED Depth/ subject=plot type=AR(1);
lsmeans Trt|Depth|Loc/ slice=(Trt Depth Loc) cl diff adjust=Tukey;
run;
%include 'R:\Sindt_Thesis_Research\Marcie_SAS_Library\pdmix800-1.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
```

Root Summary by Tube

```
PROC SORT DATA=Marcie.Rootsummary2015;
  BY Block Trt GStage Plot TubeNum;

PROC MEANS NOPRINT DATA=marcie.Rootsummary2015;
  BY Block Trt GStage Plot TubeNum;
  VAR RootCount SumRtLength;
  OUTPUT OUT =      marcie.rootsummary2015_ByTube
                 MEAN(MeanAveDiam $\bar{2}$ ) = MeanDiam
                 N(RootCount) = RootCount
                 SUM(SumRtLength RootCount) = SumRtLength
SumRootCount;
PROC PRINT DATA = marcie.rootsummary2015_ByTube;
  Title 'Summary by Tube';
RUN;

PROC SORT DATA=marcie.rootsummary2015_ByTube;
  BY GStage Block Trt Plot TubeNum;
  run;
ODS GRAPHICS ON;
PROC MIXED DATA=marcie.rootsummary2015_ByTube;
  CLASS GStage Block Trt Plot TubeNum;
  MODEL SumRtLength = GStage|Trt/DDFM=SATTERTH residual;
  BY GStage;
  RANDOM BLOCK;
  REPEATED TubeNum / subject=plot type=AR(1);
  lsmeans Trt/ slice=(Trt) cl diff adjust=Tukey;
Run;
ODS GRAPHICS OFF;
```