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SPATIAL REDISTRIBUTION OF ORGANIC PHOSPHORUS IN HAY AND GRASS PASTURES OF EASTERN WEST VIRGINIA FOLLOWING LONGTERM ANIMAL MANURE APPLICATIONS

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ABSTRACT

Spatial Redistribution of Organic Phosphorus in Hay and Grass Pastures of Eastern West Virginia Following Long-term Animal Manure Applications

Michael B. Harman

This study was conceived to investigate the fate of organic P (P_o) in typical hay and grass pasture of eastern West Virginia. It is not uncommon in this region of West Virginia for hay and grass pastures to receive annual applications of animal manure, often on a nitrogen basis. As P management has evolved, many farms in the region have begun to use management tools such as a P index to continue use of animal manure as N fertilizer. However, many hay and grass pastures in the region already have a high degree of P saturation. There are reasonable expectations that in time the P saturation at these sites may reach thresholds whereby actions to lower P saturation become necessary. At such time it may become necessary to develop interception strategies to prevent movement of P into surface and ground water from there extremely saturated locations. While most P research focuses on P loss via surface erosion, or on tile drained land, and rarely is P_o considered. To fully evaluate the risks of P loss and develop remediation and interception strategies, data specific to P_o movement at field scales across complex landscapes is needed to increase assurances that existing research is compatible with or applicable to West Virginia pastures. To develop this data, sequentially extracted P fractions were measured in samples from both spatially-explicit locations across typical hay and grass pastures and from bench top experiments to evaluate the applicability of existing research. I examined patterns in P_o distributions to determine if P_o levels significantly exceeded what could be explained by changes in soil properties. Results support the presence of spatial structure in the variability of the $NaHCO_3$ and HCl extractable P_o fractions in some locations, but no purely spatial component is present in the variability of the NaOH and H_2O extractable fractions. Various topographic parameters were evaluated to determine their efficacy in explaining P_o variability and soil-landscape modeling techniques were successfully used to develop relatively simple models based on soil test P results and topographic data to predict the distributions of the sequential extracted P_o fractions across these landscapes. The bench top experiment indicated no significant effects from actively growing plants or P sources on the disposition of P_o. As such, the bench top results support the acceptance of existing data in decision making processes, and the field scale data supports development of soil landscape models to afford future environment professional a higher degree of understanding relative to the spatial distribution of sequentially extracted P_o fractions at a landscape scale.

DEDICATION

I dedicate my dissertation to my paternal grandmother, Eva Elizabeth Harman. She was born in 1903 and passed away in 1983. She was like a second mother to me. She was a strong and savvy woman, atypical of her era, and a remarkable person. She was kind, generous, and well-liked in the community. She was the matriarch of the Harman family as I know it. She was the best of us, and I wish she was here to witness what I have accomplished. Words cannot express the void her passing created and this void remains in our hearts to this day. I love her, I miss her, and I dedicate this dissertation in her honor.

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Introduction

Experimental Overview

This research consists of both field- and lab-scale experiments designed to examine the transformation and movement of P_o in typical hay or pasture setting on marginal soil in the poultry-producing region of West Virginia.

The selected study sites were typical of hay or grass pasture fields in eastern West Virginia. The study sites consisted of one set of two (fertilized) locations with a lengthy history (approximately 10+ yr) of annual N-based manure applications, and one set of two (unfertilized) locations with very infrequent manure applications (maximum of one application every 10 yr). All locations were as similar to each other as practically possible.

Outline of Dissertation

This dissertation is divided into six chapters, beginning with an extensive review of the literature (Chapter 1) and ending with a brief summary of the important findings of this body of work (Chapter 6). In between are four chapters that describe aspects of both lab-based experiments (Chapter 2) and field-based analysis and modeling (Chapters 3 through 5). While all of the field-based research utilized the same study sites and the same data, the results and discussion were divided into three separate chapters to more clearly organize and communicate both the methods and the results of the somewhat complex field-based research.

Chapter 1. Literature Review

The overall literature review covers the fundamental research in this field of study. This review is written to place in the proper context the issues related to this problem, the extent of research efforts to explain this phenomena, and areas in need to additional research.

Chapter 2. Lab-Scale Experiments

To understand the interactions between soils and nutrients one approach is to combine various soils and nutrients and, after an appropriate period of time, examine the changes that have occurred. The purpose of this experiment was to evaluate these changes under more realistic conditions than typically employed during such experiments. To this end, lengthy soil incubations with multiple P sources under conditions more similar to the pasture and hay land environments of the poultry producing region of West Virginia were implemented. Accordingly, operationally defined sequential extracted organic P fractions were examined in the presence of vegetation, variability in P sources, and management history of soil to determine if these variables have a significant effect on the ultimate disposition of organic P in the afore mentioned operationally defined sequential extracted P fractionations.

Chapter 3. Identifying Spatial P Patterns

In general, advanced understanding of the complex redistribution of P_o is critical for making the best decisions in terms of how to intercept P movement, how to model P behavior, and how to infer the relative value of this complex and costly data from relatively inexpensive and readily available surrogate data. To this end, the purpose of this chapter was to determine if there is an unidentified spatial component to the field-scale distribution of sequentially-extracted P_o beyond what can be explained by changes in soil properties across the landscape. Various experimental design and statistical analysis techniques were used to evaluate possible spatial relationships obvious changes in soil properties.

Chapter 4. Explaining Spatial P Patterns

This chapter describes efforts to incorporate exploratory spatial data analysis (ESDA) and geovisualization techniques while evaluating field-scale distribution of sequentially-extracted P_o in greater. Using similar techniques to chapter 3, a more detail examination was conducted to determine if the variability identified in Chapter 3 could be explained by including landscape-scale physical processes. The purpose of this inquiry was to evaluate the theoretical potential for successful soil landscape modeling applications related to the spatial distribution of sequentially extracted organic P fractions.

Chapter 5. Modeling Spatial P Patterns

This chapter was developed to construct and evaluate soil test P-based predictive models. Specifically, these models combine STP data and Landscape data to predict operationally defined sequentially-extracted P_o fractions. Successful development of such models could facilitate the accurate estimation of otherwise difficult and costly to collect data from readily available and significantly less costly inputs. This could facilitate new and exciting ways to approach P management regionally in locations like tie poultry producing region in West Virginia.

Chapter 6. Summary Review

The summary review brings together the concepts and ideas from the preceeding chapters to attempt to answer the broader questions related to P management in the poultry producing region of West Virginia.

Chapter 1. Topographic Influence on the Movement and Transformation of Organic Phosphorus in Hay and Grass Pastures of Eastern West Virginia

Introduction

Understanding the interactions between soils, fertilizers, and management practices is critical for sustainable agriculture and the protection of sensitive watersheds. Some regions of the U.S., because of the presence of high density animal agriculture production, are inherently more likely to experience environmental impacts derived from excess manure availability. The poultry-producing region of West Virginia is one such location. Grant, Hardy, and Pendleton Counties in eastern West Virginia account for about 83% of the broiler and other meat type chicken sales annually (USDA NASS, 2014). This regionally dense production generates substantial volumes of poultry manure. Given current production of approximately 78,000,000 broilers per year (USDA NASS, 2014) and an estimated 2.3 pound of poultry manure per broiler (Beegle, 2007), this region has the potential to generate in excess of 80,000 tons of poultry litter per year. Historically in this region of West Virginia as much as 90% of the poultry manure has been used as fertilizer and over 80% of that has been applied to grasslands (Basden et al., 1994). When concentrated animal agriculture and long-term N-based manure management occur together, phosphorus (P) saturation and elevated risks to water quality following are potential outcomes (Beck et al., 2004).

As concerns over pending regulation mount, a strategic long-term approach to research should become a higher priority within the poultry producing and regulatory communities. Over time more land uses will become regulated and it is reasonable to assume some of this land, currently receiving animal manure applications on a N basis or in excess of crop removal rates, will attain a degree of P saturation that will prohibit further manure application. As some lands are removed from the manure application pool, other locations within the surrounding area will be needed, or the manure resources will need to be moved greater distances. In the coming years, the effort of reduce the P saturation of the existing sites and prevent P loss from all sites will become a greater priority. Thus, some portion of current research efforts should focus on answering questions about the fate of P in these agricultural lands with extensive histories of animal manure applications.

Understanding the composition and distribution of the P pool on marginal lands could assist land managers in remediating P saturated locations, maximizing environmentally safe manure usage, reducing costly sampling, and focus the application of limited resources to maximize their affect. The utilization of a soil-landscape modelling approach could allow researchers to meet some of these goals. The soil-landscape modelling approach provides a quantitative means of estimating soil properties across an area by combining physiographic properties, and georeferenced soil sample data (McSweeney et al., 1994). In place of resource dependent sampling regimens, relatively inexpensive high-resolution surrogate data are collected and correlated with georeferenced soil sample data to develop a regression based estimate at the resolution of the surrogate data (Thompson et al., 2006).

To date, the majority of P-soil-environment research has focused on inorganic P (P_i) (Laboski and Lamb, 2003; Anderson and Magdoff, 2005). However, organic P (P_o) may be a significant part of the total soil P pool. To understand the implications of management decisions, relative to manure applications, a greater understanding of potential P_o movement and soil interactions is needed (Condron et al., 2005). Developing that understanding and generating the means of extending that knowledge is paramount. The goal of this research is evaluate the applicability of incubation studies, evaluate P_o patterns across farm fields, assess soil and topographic parameters to understand potential P_o movement, and ultimately model P_o distribution in fields. Specific hypotheses related to these individual overarching goals will follow in the individual chapters to follow.

Literature Review

The following literature review is subdivided into general topics and a summary of related research. The section on phosphorus will briefly address P in the environment, in soils, and in animal manure. The section on the phosphorus cycle will briefly discuss the P cycle, P movement, and how P was measured and defined in its various forms. The section on soil–landscape modelling will explain what soil–landscape modelling is, how it can and has been used to model soil properties. The summary of related research will examine previous P_o research in West Virginia, P_o fractionation, manure-soil incubations, and landscape modelling in the context of this research, examining what has been done and outlining what is needed.

Phosphorus

Long-term applications of plant nutrients in the form of chemical fertilizers or animal manures at rates in excess of crop or plant removal can lead to the accumulation of nutrients (Sims et al., 2002; Beck et al., 2004; Johnson et al., 2005; Miller et al., 2010). If excess nutrients are lost or transported from agricultural land to either ground or surface waters, the excess nutrients generate a degree of risk to water quality in the surrounding environment (Sharpley et al., 1992). Of particular concern are N and P because excess amounts of N and P in aquatic ecosystems can stimulate excessive algal growth, limit light penetration, and causes hypoxia as the algae decomposes (USEPA, 1996; Gachter et al., 1998; Tarkalson and Mikkelsen, 2004). The USEPA (1995) considers P among the leading causes of pollution in lakes, estuaries, and rivers. In freshwater systems, P is often the limiting nutrient for algal growth (Newton et al., 1999; Conley, 2000). Consequently, agricultural sources have been identified in some instances as contributing to algal blooms and perhaps even fish kills (Shedlock et al., 1999; Sharpley, 2000; Boesch et al., 2001).

Historically, most research on P sorption and availability has focused on inorganic sources, with significantly less attention given to organic species of P (Laboski and Lamb, 2003; Anderson and Magdoff, 2005). Some researchers hypothesize this may be due to a perception that P_i is the dominant form of P and it is the plant available form, and the analysis of organic forms was simply too problematic (Jansson et al., 1988; Turner and Haygarth, 2000; Anderson and Magdoff, 2005). However, the loss of any bioavailable P is potentially damaging to the environment (Sharpley et al., 1992).

Sorption is the generic term used to describe a series of physical (precipitation) and chemical (adsorption and absorption) processes (Bache, 1964; Sanyal and De Datta, 1991; Abekoe 1996). McGechan and Lewis (2002) stated, “Sorption is the process by which reactive chemicals become attached to surfaces, sometimes of otherwise relatively harmless solids.” Pierzynski et al (2005) likened P sorption to the generic transfer of P from the solution to the solid phase. This capacity to sorb P is typically described or defined in terms of a sorption isotherm or related function. These sorption isotherms are equations or models used to represent the sorption process (McGechan and Lewis 2002). A sorption isotherm can be used to model maximum P sorption, short-term sorption, slower long-term sorption processes, and desorption (McGechan and Lewis 2002).

The strength of the P—soil bonds affect soil solution concentrations (Brady and Weil, 2002; Blake et al., 2003; Pierzynski et al., 2005). Fundamentally, P sorption capacity is related to the soil surface chemistry, reactivity, Fe and Al content, clay content, and pH (Barrow, 1984; Fox, 1985; McGechan and Lewis, 2002). P in soils is often bound to metal oxides, carbonates, and sometimes displaces water and hydroxyl groups on surfaces (Smeck, 1985; Pierzynski et. al 2005). In solution, P tends to act like a hard Lewis base, forming inner and outer sphere complexes with hard Lewis acids such as Al^{3+}, Ca^{2+}, and Fe^{3+} (Pierzynski et. al 2005). In mineral soils, some anions of organic acids compete with the P anions for the Fe and Al cations (Struthers and Sieling, 1950). In organic soils, Gerke and Hermann (1992) found the Fe and Al compounds were crucial in creating bridges between P and the humic substances. When P forms bidentate inner sphere complexes with metal cations, he P compounds are stronger complexes and not as readily reversible as outer sphere, or monodentate, inner sphere complexes (Sims and Pierzynski 2005). The strength of these relationships with sorption capacity has allowed researchers to effectively predict the P sorption capacity of similar soils (Scheinost and Schwertmann, 1995).

In terms of P composition in soil, the P_o pool in soil is typically composed of inositol phosphates, phospholipids, nucleic acids, phosphoproteins and other unidentified P compounds (Schroeder and Kovar, 2006). P_i in soils typically comes from the weathering of apatite (Pierzynski et al., 2005). The exact speciation of these phospho-metal-oxides and hydroxides is dependent upon the available components and soil conditions (Pierzynski et al., 2005).

P within animal manure can be identified or described in a similar manner to soil. The dominant forms of P in poultry litter are orthophosphate (inorganic) and phytate (organic) and the concentrations of orthophosphate and phytate correlate well with some sequential fractionations techniques (Warren et al., 2008). The majority of the P in poultry litter is extractable with H_2O and $NaHCO_3$ (Codling, 2006; Dou et al., 2000; Dail et al., 2007). Phytate is the principal form of P in the grain-based (Maize) diets of non-ruminants (poultry) and most of it is passed along into the feces undigested (Harland and Morris, 1995; Sharpley, 2000). The P_i percentage is typically highest in the H_2O extractable fraction (Codling, 2006). However, some reports indicate that 72–83% of H_2O-extractable P in poultry manure is in an organic form (Sistani et al., 2001). A four-step fractionation (H_2O, $NaHCO_3$, NaOH, and HCl) has been shown to extract between 85 to 97% of the total P in poultry manure (Warren et al., 2008; McGrath et al. 2005) and is similar to the numerous other sequential extraction procedures used to fractionate P in soils.

Continuous applications of manures can lead to the modification of soil properties, P sorption characteristics, and perhaps increase P translocation through the soil profile (Hao et al., 2008; Harman et al., 2013). For example, by elevating the Ca content of the soil, P_o additions could alter P sorption characteristics (Robinson and Sharpley, 1996). The more labile nature of some of these Ca-P complexes following long-term manure applications could contribute to potential P translocation within the soil profile (Holford et al., 1997; Siddique and Robinson, 2003). This is supported by the research of Lehmann et al. (2005). In research on locations with long-term application of animal manure, Lehman et al. (2005) documented P leaching consistent with accumulation and translocation.

Continual additions of poultry manure shift P from binding with Fe and Al products to binding with Ca (Sharpley et al., 2004). In addition to elevating P, N, pH, and Ca, poultry manure elevates the levels of bicarbonates and of organic acids with carboxyl and phenolic hydroxyl groups (Sharpley et al., 2004). The introduction of these compounds and their associated functional groups provide new reactive surfaces within soil and change the composition of the soil solution, affecting everything from pH to ionic strength. When poultry manure is added continuously to soil, more and more P becomes contained within the most easily extractable, labile fractions (Blake et al., 2003). Ultimately, the number and type of sorbtive surfaces in the soil determine the fate of P fractions (Blake et al., 2003).

Phosphorus Cycle

The soil solution P concentration is typically very low, thus requiring a constant turnover from other pools to replace P removed from the soil solution (Pierzynski et al., 2005). This replacement or replenishment of solution P comes from the P referred to as labile P. Labile P is “soil or sediment P that rapidly equilibrates with an aqueous solution” (Pierzynski et. al 2005). Nonlabile P is the form of P in soil that is more stable and less useful in maintaining P levels in solution. This overly simplified re-allocation of P between P pools and P forms is the basis of the P cycle.

The phosphorus cycle is an extremely complex interaction of P_i, P_o, biogeochemical processes, and environmental factors, by which a semi-steady state of solution P is maintained via the transformation of P forms. It is the conceptual model of how P_i and P_o move between labile and non-labile pools through biological, chemical, and physical pathways within the environment. There are four major coupled processes within the P cycle: sorption–desorption, precipitation–dissolution, mineralization–immobilization, and input–loss.

The sorption –desorption process is a mechanism by which P can be removed from or added to the soil solution (Bunemann 2015). Sorption–desorption is a complex process of P temporarily bonding with a particle surface in the soil system. Excess P in solution becomes sorbed to the surface of primarily AL and Fe oxides (Fossard et al., 1995). When P levels in the soil solution change, P may be sorbed or desorbed from the soil particles until equilibrium between the relative attraction to the particles and the concentration in solution is achieved. Accordingly, as soils become more saturated with P, the soils have a lower ability to retain additional P (Giles et al., 2015).

Dissolution - precipitation is a very important abiotic process regulating P levels in the soil solution as labile P is only a small part of the total P pool in soil (Fossard et al., 2000). Precipitation–dissolution is the process of P forming or dissolving of primary and secondary P minerals from the soil solution. Primary P mineral generally refer to apatite, while secondary P minerals include sorbed P, as well as Al phosphate, Fe phosphates, and Ca phosphates like brushite, monetite, and octacalcium phosphate (Smeck, 1985). As soils weather and acidify, the formation of Fe and Al phosphates and P_o is favored (Smeck, 1985; Pierzynski et. al 2005). Ultimately, if the soluble pool of P becomes depleted, the encapsulation of these secondary P minerals by metal oxides and formation of occluded P becomes likely (Smeck, 1985).

The third of the major coupled process in the P cycle is mineralization - immobilization. Mineralization–immobilization is the process where P moves between organic and inorganic forms. This is the biotic phase of the P cycle. In short, organisms access P from the soil solution decreasing solution concentrations while increasing their mass or number. Over time, these organisms die and decay releasing P back into the soil solution. Thus as conditions dictate, available P can be immobilized (made unavailable) within biological entities in various states of growth or decay or mineralized (released) as various inorganic and organic P compounds.

The final phase of the P cycle includes inputs and losses. P input is typically either weathering of primary P minerals or anthropogenic additions. P loss typically takes the form of sediment mediated transport and leaching losses (Pierzynski et. al 2005). Simultaneously, sorption–desorption, precipitation–dissolution, and mineralization–immobilization reactions are actively transforming P to satisfy the plant and microbiological communities. P can change forms, shift between inorganic and organic forms, and be leached away or move with sediments and organic matter.

While, in a general sense, P is limited in its mobility, P does move in soil. In native conditions, long-term water movement can lead to slow migration of P across the landscape. Smeck (1973) and Smeck and Runge (1971) have proposed that P can move laterally within a landscape, and that P will accumulate in lower landscape positions. Measuring changes in P across a landscape can infer a reasonable approximation of how water movement occurred across and through that landscape historically (Smeck, 1973). In addition to physically moving P, water can chemically alter the soil, and this can alter the long-term fate and transport of P. For example, dryer soils found in well-drained, upland positions, rich in iron and aluminum oxides tend to have higher P sorption capacities then less oxidized soils more common in wetter landscape positions (Walker and Syers, 1976; Abekoe, 1996).

In terms of P movement, P loss from agricultural lands generally occurs via one of two mechanisms, surface and subsurface movement (King et al., 2015). When transported via subsurface flow mechanisms, P from fertilizer or manure moves through the soil into the ground or surface water.

Erosion losses occur when overland flow moves P from fertilizer, manure, or P enriched soil materials across the soil surface and or into surface waters. Historically best management practices focused on reducing or eliminating erosional losses, as erosional losses were considered the more important pathway of P loss (King et al., 2015). P leaching was generally not considered as important due to the low dissolved P concentrations in the soil solution and the sorbtive properties of the soil matrix (Baker et al., 1975). However, soils receiving large quantities of P fertilizer, soils with lower concentrations of reactive surfaces, coarser textures, pronounced structure, high concentrations of rock fragments, and extensive macropores networks pose a greater risk for P loss (Anderson and Magdoff, 2005; Harman et al., 2013). Yet, P_i is only part of the P picture. Literature indicates organic P (P_o) could be as low as 29% or as high as 65% of the total P (Harrison, 1987) and some research indicates it could be as high as 90% of P in the soil solution phase (Helal and Dressler, 1989; Shand et al., 1994; Turner and Haygarth, 2000; Anderson and Magdoff, 2005). Only considering P_i in risk assessment is overlooking the rather large remaining portion of the total P pool, or assuming it behaves proportional to the P_i component.

Measuring Soil Phosphorus

Similar to how a P extraction method is selected; how P is physically measured is equally important. Colorimetric methods of P measurement are common. However, inductively coupled plasma (ICP) spectroscopy has been the most significant development in agricultural sample analysis since the development of the atomic absorption spectroscopy (AAS) (Isaac and Johnson, 1983). The ICP has facilitated rapid and consistent measurement of many soil nutrients. However, with P there are complications when comparing colorimetric to ICP based methods. ICP P measurements can be as much as 36% higher than colorimetric methods (Ziadi et al., 2009). The obvious explanation for this increase is the ability of ICP based methods to measure P_o, polyphosphates, and orthophosphate (measured by the colorimetric methods). Identification of individual P components would require lengthy examination, and many additional analytical steps.

The most rapid, complete approach to P characterization would be ^{31}P nuclear magnetic resonance. However, it is believed the preparative steps in ^{31}P nuclear magnetic resonance can lead to partial hydrolysis of some P_o forms, thus introducing a degree of inaccuracy (He et al., 2008). The identification of P_o fractions by enzymatic hydrolysis is the more accurate method of P_o identification if identification of specific labile P_o species as opposed to the entire labile pool (He et al., 2008). Understanding the operationally defined nature of the measurement is as critical as understanding the limitations of the extraction methodology.

In general, P extracted with any hydroxide solution is assumed to be P_o and P_i typically bound to Al and Fe (Sharpley et al., 2004). P extracted with acid, is assumed to be P_o and P_i typically bound to Ca (Sharpley et al., 2004). P extracted with bicarbonate, ion exchange resin, and water-based extractions is assumed to remove only the most weakly bound most labile P components (Sharpley et al., 2004). Bicarbonate, ion exchange resin, and water-based extractions are said to remove the easily exchangeable and plant available forms of P (Sharpley et al., 2004). These examples describe parts of the pool based on the perceived role that the parts play in the P cycle (plant nutrients), how researchers extract them (ion exchange resin P), and the expected mechanism of retention in the soil (Al and Fe bound P).

Sequential P fractionation integrates a collection of chemical extractions such that the P is characterized by the type and strength of their physicochemical interactions with soil components (Bowman and Cole 1978; Hedley et al., 1982; Cross and Schlesinger, 1995). An individual fraction in a sequential fractionation is conceptually different but not necessarily pure or unique. The most common extractions used in these sequential fractionations are water (H_2O), anion exchange resin, sodium bicarbonate ($NaHCO_3$), hydrochloric acid (HCl), and sodium hydroxide (NaOH) (Guppy et al., 2000; Schroeder and Kovar, 2006). It is common to label these groups by the chemicals or chemical processes that removed them from the soil, by the believed mechanism of sorption, or the extraction’s name. Typically, total P and P_i are determined in each sequential fraction and in the residual soil following the final extraction.

Soil-Landscape Modelling

Soil-landscape modelling is an integration of Milne’s (1935) catena concept, and Jenny’s (1941) contention of topography controlling landscape scale variability in soil (Thompson et al., 2006). Terrain variables can be used to model significant variance in soil properties (Gessler et al., 2000). Soil–landscape modelling techniques were developed as a means to quantitatively predict patterns of soil properties (Gessler et al., 1995; McBratney et al., 2000; Thompson et al., 2006). In fact, in locations with uniform geology and geomorphic history, variability in topographic properties can provide appropriate means of spatial prediction of soil properties (Gessler et al., 1995). Moore et al. (1991) indicated that topography can be used as an indirect measure of the spatial processes that occur at catchment scale, thus making topography an ideal means of estimating soil properties at field scale. Terrain variables can be used to model many soil properties, such as soil organic matter, moisture content, soil depth, and erodibility (Moore et al., 1991; Bell et al., 2000; Gessler et al., 2000; Mueller and Pierce, 2003; Pei et al., 2010). Jenny (1941) cited the work of Ellis (1938) as an example of soil moisture differentiation across a landscape based on slope and landscape position. The topography factor has proven to be important in terms of the spatial distribution of soil moisture (Sorensen et.al, 2005; Thompson et a., 2012) and soil properties influenced by soil moisture content and water movement (Hall, 1983). In humid environments, water continuously moves across and through a landscape interacting with the soil influencing the physical, chemical, and biological properties of the soil and the water. This subsequent re-distribution of water within a landscape can cause changes in soil properties in multiple dimensions (Hall, 1983).

It is common for terrain attributes to correlate with soil properties (Moore et al., 1993; Bell et al., 1994; Tomer et al., 1995; Boer et al., 1996; Park et al., 2001). As water is redistributed across the landscape, any soluble or suspended material contained within the water will be redistributed as well. As sediments and any dissolved materials move, the water effectively generates a degree of soil differentiation (Girgin and Frazier, 1996; Young and Hammer, 2000; Pachepsky et al., 2001; Ziadat, 2005). Thus, the physical, chemical, biological, and development status of soil is related to the factors controlling this redistribution of materials. Cognizant of this relationship between landscape and soil properties, researchers have used numerous forms of regression and correlation procedures to study these relationships between landscape position and soil properties (Lane, 2002; Webster, 2001; Guisan et al., 2002; Park and Vlek, 2002; Ziadat, 2005).

The basis for most of these regression and correlation procedures is the digital elevation model (DEM). A DEM is a representation of the variability of the elevation across a landscape (Bishop and Minasny 2006). The elevation data from the DEM is used to create spatial explicit derivatives known as primary or secondary (sometimes called compound) terrain attributes (Bishop and Minasny 2006). These primary terrain attributes are calculated directly from DEM while the secondary or compound terrain attributes are mathematical combinations of primary terrain attributes (Moore et al., 1991). The most common primary terrain attributes are slope gradient, slope aspect, and slope curvature. The most common secondary attribute is the topographic wetness index (TWI) sometimes referred to as the compound topographic index (CTI) or wetness index (WI) (Bishop and Minasny 2006). Secondary attributes are often more useful than primary attributes for predicting soil properties as secondary attributes tend to explain more complex physical properties and may be useful for modeling nonlinear relationships (Bell et al. 1994; McBratney et al., 2000; Bishop and Minasny 2006).

Topographic wetness index has been used in one form or another in many contexts to estimate properties associated with wetness (e.g., Moore et al., 1991; Hornberger & Boyer, 1995; Iverson et al., 1997; Boerner et al., 2000; Gessler et al., 2000; Case et al., 2005). The concept of TWI was developed by Beven and Kirkby (1979) as a component in a basin hydrology model. TWI is a compound terrain attribute, as it is made up of multiple primary terrain attributes (Bishop and Minasny 2006; Murphy et al., 2009). Specifically, TWI is defined as ln (A / tan B) where A is the local upslope contributing area for that point and B is the local slope gradient (Beven and Kirkby, 1979; Bishop and Minasny 2006; Murphy et al., 2009). TWI infers relative wetness within a landform.

TWI values are dependent on flow accumulation calculations (Murphy et al., 2009). Flow accumulation is contingent on first determining flow direction (Bishop and Minasny 2006). Flow direction is simply a directional assignment of likely flow for each cell. Flow accumulation is the collection of cells flowing together as defined by the flow direction layer (Murphy et al., 2009). Flow accumulation can be unidirectional, multidirectional, or dispersive; and each of these three methods of representing flow accumulation has its own specific limitations (Murphy et al., 2009). Murphy et al. (2009) contends that some TWI calculations do not adequately consider downslope topography, hydrologic conditions, and dispersive flow when modelling moisture. Others contend that the (tan B) does reflect the local drainage potential (Beven and Kirkby, 1979; Quinn et al., 1995).

Summary of Related Research

The West Virginia University Agricultural Experiment Station cataloged the amounts and types of P_o found in 17 soil series at 34 locations across WV in the 1960's (Jencks et al., 1964). This research measured total P, total P_o, phytin, available P, organic matter, and pH in these unfertilized and unlimed soils. P_o accounted for between 7 and 66% of the total P in the surface horizons, and from 13 to 55% in the subsurface horizons (Jencks et al., 1964). Phytin or phytic acid (an inositol phosphate) accounted for between 13 and 63% of the total P in the surface horizons, and from 10 to 48% in the subsurface horizons (Jencks et al., 1964). The Jencks et al. (1964) findings are corroborated by the assertions of others (Harrison, 1987; Helal and Dressler, 1989; Shand et al., 1994; Turner and Haygarth, 2000; Anderson and Magdoff, 2005) that significant portions of the total soil P pool may in fact be P_o.

There are numerous examples of research that utilized a sequential extraction process to separate the total P pool into operational defined fractions (Bowman and Cole 1978; Hedley et al., 1982; Tiessen et al., 1984; Schoenau et al., 1989; Cross and Schlesinger, 1995; Iyamuremye et al., 1996; Sui et al., 1999; Guppy et al., 2000; Qian and Schoenau, 2000; Yang et al., 2002; He et al., 2004; Schroeder and Kovar, 2006). Similarly, there are numerous examples of soil research that utilizes incubations with soil and fertilizers (Iyamuremye et al., 1996; Qian and Schoenau, 2000; Whalen et al., 2001; Maguire et al., 2001;

Yang et al., 2002; Crouse et al., 2002; Laboski and Lamb, 2003; He et al., 2004). However, in instances where researchers examine the fractions in a location under some set of conditions, it is rare that the study is accompanied by incubation experiments to further the understanding of potential sources of specific operationally defined sequentially extracted P fractions.

Historically, there have been multistep fractionation procedures developed for soil P (Chang and Jackson, 1957; Bowman and Cole 1978; Hedley et al. 1982), marine sediment P (Martin et al. 1987 and Ruttenberg, 1992), and countless modifications to these methods. The most commonly cited and modified method used in soil P research is the Hedley et al. (1982) fractionation (Guppy et al., 2000). The Hedley et al. (1982) fractionation partitions the P pool into soluble, aluminum/iron-bound, calcium-bound, and residual forms. The more recent efforts to characterize the P_o pool in soil has utilized enzymes to separate specific P_o compounds (He et al., 2004), 31P nuclear magnetic resonance analysis (Toor et al. 2003; Hill and Cade-Menun, 2009), and a sequential fractionation. Often these approaches are combined in some fashion to further differentiate P forms.

To learn how P additions change in soil over time, mixtures of fertilizer and soil are often incubated to examine changes relative to some factor or P type. This approach has been used to define changes in the soil P pool (He et al., 2004; Qian and Schoenau, 2000; Yang et al., 2002). However, long-term, field scale studies have shown P fractions do not change uniformly, and P concentrations decrease in the soil profile with depth (Hountin et al., 2000). In fact, long term applications of poultry manure (a source of P_o) can even cause the proportion of P_i in the total pool to increase (Sharply et al., 2004).

Robinson and Sharpley (1996) examined sorption and fractionation on multiple soil samples from untreated locations after adding poultry manure leachate and concluded manure fertilizers and chemical fertilizers act differently in soils. When applying deliberate P_o fractions to packed soil columns, Anderson and Magdoff (2005) found different classes of P_o fraction moved through the soil at different rates. Specifically, orthophosphate diesters are more likely to leach in soils than monoesters or P_i (Anderson and Magdoff, 2005). Other researchers have incubated poultry manure in soil and fractionated the soil, manure, and soil-manure mixture after the incubation (Warren et al., 2008). However, the incubations were not in the presence of actively growing plants. Some researchers have looked at P loss and characterized the P in manure, in farm fields, and in drainage way sediment downstream (creating a theoretical transects form source to stream) using the latest ^{31}P nuclear magnetic resonance analysis (Hill and Cade-Menun, 2009) but did not look for differentiation within a field, or examined if P_o had changed at any other scale in their theoretical transect.

A better approach to understanding the changes and movement of P_o in soils would combine aspects of these and similar studies with landscape modelling techniques. Topographic data has been shown to be a useful parameter when modelling spatial patterns in soil P. Using TWI, Moore et al. (1993) explained 48% of the variability in data from a STP extraction at landscape scale. Given, that (i) P_o fractions moved through the soil at different rates (Anderson and Magdoff, 2005), (ii) the properties that can control soil P sorption vary across the landscape (Daniels et al., 2001), and (iii) the re-distribution of water within a landscape can cause changes in soil properties (Hall, 1983), TWI is a logical choice for a high-resolution, low-cost surrogate variable to model P_o fractions at a landscape scale.

Spatially explicit sampling strategies for measuring P_o at landscape scales may be necessary due to spatial autocorrelation, and the potential effect of space on the distribution or redistribution of applied P. As Tobler (1970) put it, "Everything is related to everything else, but near things are more related than distant things." Failing to consider the potential of sample autocorrelation can jeopardize the reliability of some analysis (Fagroud and Van Meirvenne, 2002). Many statistical methodologies rely on independence within the sampling design, while in reality much of this data could be spatially autocorrelated (not independent). Sampling strategies can be devised to measure or evaluate spatial variability and autocorrelation. However, these strategies are typically resource dependent and too often are not used (Bridgham et al., 2001). In their place, researchers rely on replication, blocking, sample independence, and randomization; but these measures do not necessarily generate independent data (Fagroud and Van Meirvenne, 2002).

Acknowledging that P added from animal manures is a unique mixture of P_i and P_o with various solubility (Harland and Morris, 1995; Schroeder and Kovar, 2006; Warren et al., 2008), it seems reasonable to expect overland flow and infiltration to move these fractions at different rates, over different distances, and by potentially by different processes. Given that P forms vary in their ability to participate in the sorption–desorption process, the precipitation–dissolution process, and the mineralization–immobilization process, (Pierzynski et. al 2005) it would seem logical that these forms could accumulate or be transformed relative to their specific differences. Knowing that different organic P fractions are sorbed preferentially (Anderson and Magdoff, 2005) and P sorption capacity is dictated by soil properties (Blake et al., 2003), it would seem likely that individual P_o fractions could be partitioned in accordance with soil properties. Given the plethora of examples where landscape data has been correlated with soil properties (Moore et al., 1993; Bell et al., 1994; Tomer et al., 1995; Boer et al., 1996; Young and Hammer, 2000; Pachepsky et al., 2001; Park et al., 2001; Ziadat, 2005; and others), it would seem logical to assume this sort of data could be used for modelling P fractions at field or landscape scale.

Expected Benefits

This research should answer fundamental questions about how P from primarily poultry manure applications change within the landscape and determine if incubation with soil only is adequate for modelling P change. Additionally, the multi-dimensional distribution of P_o fractions may generate new hypothesis about P transformations, translocations, and movement, as well as potential remediation techniques that may become necessary in the years to come. Lastly, this research will develop a modeling approach that could be used to estimate a much more detailed description of the P pool in locations where site specific P distribution knowledge could be useful in designing and implementing P loss management, and remediation plans.

As concerns over regulation are becoming more common, it is reasonable to assume land currently receiving N-based animal manure applications could face P saturation. At that time, the continued application of manure will likely cease. However, that does not mean the problem will go away. Surrounding areas will become attractive destinations for excess manure. Many of these locations may be land previously unfertilized perhaps due to costs. As producers are forced to move manure off-farm, it could lead to a declining cost associated with manure. It would seem reasonable that these conditions could lead to marginally productive local farm lands seeing an increase in manure applications.

In the future there are two general problems that may need to be addressed. First, what management decisions need to be made on farmland that has become saturated with P, and second, how can resource managers maximize applications on new fields without repeating the current situation. Understanding how P_o moves at field scale could help answer these questions and more. For example, land managers could implement P remediation strategies at subfield scales to reduce cost by targeting the topographic conditions in fields where labile and possible moderately labile P accumulates. Additionally, when allocating sparse government resources to improve water quality, regional assessment of topographic data could identify farms where remediation could potential yield the most environmental impact. Lastly, the lab-scale experiment answers important questions about how different constituents of the manure respond differently; how incubation studies could be biasing the results; and how STP results correctly or incorrectly estimate risk, and determine the effect of vegetation on these transformations.

These same questions could be answered with massive field scale trials and site specific sampling in every field, but this is a poor use of resources when looking at the “big picture”. This research will provide a quantitative means of estimating P_o distribution within fields based on typical soil test results and relatively inexpensive high-resolution geospatial data. When specific questions about a field arise, additional examination would always remain an option. Voluntary precision sampling of individual fields would yield definitive answers, but would likely be too costly for many producers. This research provides valued data at a low cost and a wide scale to help solve problems of local, regional, and national importance, thus allowing limited resources to be used in such a manner as to do the most public good.

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Chapter 2. Effect of Vegetation and Phosphorus Source on Sequential-Extracted Phosphorus Fractions in Incubated Soils

Abstract

Incubation-based P transformation studies provide valuable insight into the fate of nutrients. However, the practical utility of such experiments is contingent on the characterization of the transformed P forms being consistent with characterization approaches that are used at field scales. Furthermore, this utility is limited by the ability of the incubations to mimic field conditions. This research is focused on applying experimental conditions that more closely resemble field conditions to a traditional incubation experiment, and characterize the P in a fashion that could prove useful at examining P movement and transformation at a field or landscape scale. Soil samples from locations with very high soil test P levels and locations with very low soil test P levels with and without actively growing vegetation were incubated for 16 weeks, following P additions from manure, manure leachate, leached manure, phytic acid, or $CaPO_4$. During the incubation, the samples were repeatedly wetted and allowed to dry such that the gravimetric soil moisture ranged between 80 and 105% container capacity. Consequently, the sequentially-extracted, operationally defined P fractions in the samples following the incubation were measured and examined for significant multivariate effects. The analysis identified significant differences between several fractions relative to P source and fertilization history, but no difference relative the presence of vegetation. It appears that most P additions generally end up in the NaOH extracted fractions and the presence of actively growing vegetation does not alter the fate of these P additions. Accordingly, data from other incubation studies where P is assumed to be sorbed to Fe and Al should be applicable when trying to understand P transformations. As such, the transformation seen in most of these incubations should be reflective of what would occur in field-scale situations.

Introduction

Knowledge of the interactions between soils, fertilizers, and management practices is critical for sustainable agriculture and environmental protection (Maguire and Sims, 2002; He et al., 2004; Harman et al., 2013). These concerns are most relevant in regions with extensive confined animal feeding operations or N-based manure management. When these conditions occur together, P saturation and an elevated risk to water quality become potential outcomes (Beck et al., 2004; Miller et al., 2010). In addition, the availability of P in soil can be influenced by microbial and chemical properties of the soil, manure composition, and rhizosphere processes (Waldrip et al., 2011). To better manage the relationship between soils, fertilizers, and management practices researchers must first understand how differences in P sources, P saturation, and vegetation alter the forms of P that are retained, sorbed, or transformed within the soil.

One approach to facilitate examination of the interactions between soils and nutrients is to combine them and after an appropriate period of time examine any changes that have occurred. There are numerous examples of research that utilize this approach to examine changes relative to various soil amendments (Iyamuremye et al., 1996; Qian and Schoenau, 2000; Whalen et al., 2001; Maguire et al., 2001; Yang et al., 2002; Crouse et al., 2002; Laboski and Lamb, 2003; He et al., 2004; Miller et al., 2010; Gagnon et al., 2012). These lab-scale experiments are low cost alternatives to inelegant field-scale experiments (Sharpley and Sisak, 1997). Another approach is to describe in greater detail the distribution of a nutrient, such as P, within soil. Combined, lab scale and sequential extracted spatially explicit experiments allows researchers to document the changes that occur in fertilized soil over time in greater detail.

A sequential P fractionation (one approach to describe P in soils in greater detail) is a collection of successive chemical extractions that characterize P by the type and or strength of the assumed physicochemical interactions with the soil (Bowman and Cole 1978; Hedley et al., 1982; Cross and Schlesinger, 1995; Negassa and Leinweber 2009; Gagnon et al., 2012). Some research (Qian and Schoenau, 2000; Yang et al., 2002; He et al., 2004; Warren et al., 2008; Negassa and Leinweber 2009; Gagnon et al., 2012) has used this approach to define changes in the soil P pool. Recent efforts to describe organic P (P_o) pools have included additional analytical measures such as enzymatic hydrolysis (He et al., 2008) and nuclear magnetic resonance analysis (Toor et al. 2003; Hill and Cade-Menun, 2009) in conjunction with sequential P fractionation. More advanced analytical techniques such as enzymatic hydrolysis and nuclear magnetic resonance analysis can generate a great deal of information about specific P compounds and retention mechanisms, but may not be practical for use by producers.

To improve management decisions there is a need for research that can assist in translating the existing body of knowledge into more useable formats. For example, it is known that manure application can increase soil concentrations of both total, soluble, and stable organic forms of P (Erich et al. 2002; Ylivainio et al. 2008; Waldrip-Dail et al. 2009). Research on the complex interactions between plant roots, manure, soil, and P is ongoing, but a full understanding has not yet been achieved (Waldrip et al., 2011). P_o must be mineralized into inorganic forms to fully participate in the P cycle and that ability is impacted by the biological activity within the soil matrix (Magid et al., 1996, Waldrip et al., 2011).

Specifically, insight into how manure applications change sequentially-extracted P pools in a more natural environment, with plant interactions, in a more biologically active setting is critical to updating management paradigms to reflect the latest research. Understanding the composition of the P pool relative to unique combinations of conditions could streamline research by culling ill designed and ineffective remediation strategies before research resources are consumed. Surface applications and protective berms of low cost materials with specific affinities for labile P compounds could be developed to limit P loss.

The objective of this research was to determine if the presence of vegetation, variability in P sources, and management history of soil has a significant effect on the disposition of organic P in sequential fractionations. To examine the potential P transformations, a series of laboratory incubations were performed to examined how organic P pools changed in soils given the presence of vegetation and the effect of hydrological differentiation (separation of soluble and non-soluble portions of a manure P sources) along with traditional P sources. The first research hypothesis ($H1_a$) states that an addition of P causes a significant difference in operationally defined sequentially extracted P_o fractions relative to the source of the P addition and the fertilization history of the sample. The null hypothesis ($H0_a$) states that additions of P does not cause a significant difference in operationally defined sequentially extracted P_o fractions relative to the source of the P addition and the fertilization history of the sample. The second hypothesis ($H1_b$) states that the presence of active growing vegetation can affect sequentially extracted P_o fractions in incubated soil samples. The null hypothesis ($H0_b$) states, that the presence of active growing vegetation does not affect sequentially extracted P_o fractions in incubated soil samples.

Materials & Methods

Overview

To achieve a more realistic model of typical growing conditions and evaluate change over time, manure, manure leachate, leached manure, phytic acid, and $Ca(H_2PO_4)_2$ were incubated with soil under controlled conditions more similar to the pasture and hay land environments of the poultry producing region of West Virginia. This bench top experiment includes vegetation, repeated wetting and drying, and soil from locations with very low and very high P concentrations. Under these conditions, changes in the operationally defined sequential extracted organic P pools were measured to determine what parameters, if any, altered the P pools. These considerations should place the utility of existing research in a proper context and designate a clearer direction for future research relative to both remediation and preventive strategies.

The experiment was arranged as a randomized complete block within a factorial design (Dowdy et al., 2004), with factors of management history, P sources, and vegetation. The two levels of management history are annually fertilized (Fertilized) and rarely fertilized (Unfertilized). There are six levels of the fertilizer factor (control, manure, manure leachate, leached manure, phytic acid, and Ca $(H_2PO_4)_2$). The two levels of the vegetation factor (with and without vegetation) were constructed by seeding the appropriate samples with 0.1g of tall fescue [Lolium arundinaceum (Schreb.) Darbysh. = (Schedonorus arundinaceus (Schreb.) Dumort.] seed at the onset of incubation. The experimental block was replicated three times.

The soils used in this incubation experiment came from two locations: One location with a lengthy history of poultry manure applications and one location with no know history of poultry manure fertilization. These locations have been managed as pastures in excess of 25 years. These locations are mapped as Berks-Weikert channery silt loams. The map unit composition is typically 55 % Berks and similar soils, 35 % Weikert and similar soils, and 10 % minor components. For additional details on theses soils see Table 2.1. Berks is among the most common soil series identified under hay and grass pastures in the poultry producing region of West Virginia (Harman et al. 2011). The soil used during this experiment came from Ap horizon of these locations and were air dried ground, sieved, and mixed repeatedly to create a homogenous bulk samples from each location.

Incubation

Forty grams of air dried soil were incubated 16 weeks with one of six levels of the fertilizer treatments. In the manure treatment, 1.2 mg of P (95 mg of manure) was added. In the phytic acid, and monocalcium phosphate treatments, 1.2 mg of P were added. In the other treatments 95mg of manure was leached with distilled deionized water and the leached manure added to the leached manure treatment and the leachate added to the manure leach treatment. The application 1.2mg of P per sample is roughly equal to a field scale application of 60 pound per acre. The literature indicates water-soluble P levels stabilize as quickly as 3 weeks after fertilization (Bond, et.al, 2006) or could continue to increasing in availability for up to 13 weeks before leveling off and stabilizing for up to 6 months (Gagnon and Simard, 1999). There is a great deal of variation in incubation times, from days (Warren et.al, 2008; Leytem et.al, 2004) to months (Ebeling et.al 2003; Laboski and Lamb, 2003). The 16-week incubation period exceeds the minimum time (3 weeks) for stabilization of water soluble P (Bond, et al., 2006) and the initial increase in available P reported by Gagnon and Simard (1999), while being more directly comparable to the first 16 week cycle of Ebeling et al. (2003).

Gravimetric soil moisture was adjusted based on percent container capacity (CC) (Leytem et al., 2004). CC is similar to field capacity. Gravimetric soil moisture is determined by saturating 50 g of soil with distilled deionized water (DDI) and allowing it to drain freely for 48 hr. under normal atmospheric pressure and room temperature, followed by reweighing the sample, and calculating water content per unit soil (Cassel and Nielsen, 1986). The moisture level was measured and adjusted approximately every 3-5 d. DDI was added when the sample weights fell below 80% of CC until the weight of the sample reached approximately 105% CC.

Laboratory Analysis

The chemical properties of the soils used in the experiment were characterized by a sequential extraction procedure developed to divide the P pool into operationally-defined segments. Sequential fractionation evolved from the works of Chang and Jackson (1957) and Bowman and Cole (1978), through Hedley et al. (1982). The most common extractions used in sequential fractionations are water (H_2O), anion exchange resin, sodium bicarbonate ($NaHCO_3$), hydrochloric acid (HCl), and sodium hydroxide (NaOH) (Guppy et al., 2000; Schroeder and Kovar, 2006). The P in this study was characterized with a modified Hedley fractionation as suggested by (Sui et al., 1999) and described by He et al. (2003). For each sample, approximately 1.0 g of soil and 25 mL of extractant was placed in a centrifuge tube in a reciprocal shaker at 180 oscillations per minute for 16 h at room temperature. The samples were centrifuged for 15 min at 3500 g and the supernatant filtered through a medium porosity filter paper. This process was repeated sequentially from DDI through 0.5M $NaHCO_3$, 0.1M NaOH, and 1M HCl. The extracted P from these samples were further differentiated into operationally- defined categories of organic P and inorganic P. Inorganic P includes orthophosphate plus any molybdate reactive organic and inorganic species of P. Organic P is defined as the difference between total P as measured by an ICP-OES and the molybdate reactive organic and inorganic species of P, as identified by the ammonium molybdate-ascorbic acid colorimetric method (Knudsen and Beegle, 1988).

The ammonium molybdate-ascorbic acid colorimetric method of Knudsen and Beegle (1988) is a two reagent procedure. The first reagent is the concentrated ammonium paramolybdate solution. Concentrated ammonium paramolybdate solution is made from 60.0 g of ammonium paramolybdate $(NH_4)6Mo_7O_{24}*4H_2O)$, 1.455 g antimony potassium tartrate ($KSbOC_4H_4O_6$) and 700 mL of concentrated sulfuric acid (H_2SO_4) diluted to 1 L with DDI. The second reagent is the ascorbic acid solution. It is made by dissolving 132 g of ascorbic acid in 1 L of DDI. Daily, a working reagent is made by adding 25 mL of the concentrated ammonium paramolybdate solution with 10 mL of the ascorbic acid solution and diluting to volume to 1 L with DDI. A 2 mL aliquot of soil extract is transferred in to 15 mL microcenterfuge tube and mixed with 8 mL of the working reagent. The solution is allowed to stand for 20 minutes and the color change is read at 882 nm. High pH extractants ($NaHCO_3$ and NaOH) extract some acid insoluble organic acids. These acids precipitate when pH is lowered by the ammonium molybdate-ascorbic acid colorimetric method. These organic acids were eliminated by pipetting 10 mL of these extractions into a 50 mL centrifuge tube and lowering the pH to about 1.5 by adding 6 mL to the 0.5 M $NaHCO_3$ and 1.6 mL of 0.9 M H_2SO_4 to the 0.1 M NaOH (Tiessen and Moir, 1993). The precipitated samples were centrifuged, made to volume, and analyzed accordingly.

Statistical Analysis

Prior to analysis the data were examined to evaluate univariate normality using the Ryan - Joiner method and identify potential outliers identified by the Dixon's Q test (Minitab 16, 2010). Statistical outliers were examined and considered for eliminated. With removal of outliers the data more closely fit the assumptions associated with the analysis. The experimental design dictated that the data were independent; however, the homogeneity of the covariance matrices were not examined, as the requirement for the covariance of each cell to every other cell results in numerous opportunities for the assumption of homogeneity to fail and in practice it is rarely satisfied in real research data (Lehman et al., 2005).

Data analysis began with multivariate analysis of variance (MANOVA). This looked for significant difference within the P fractions collectively relative to management history, differing sources of P used in the incubations, and the presence or absence of vegetation. Essentially, MANOVA was used to determine which independent variables had significant effects on the P fractions prior to univariate analysis. The univariate ANOVA determines if the effects of the independent variables were significant within each dependent variable. Some research assumes a significant MANOVA controls family error rate (Minitab 16 Statistical Software, 2010) while others indicate this is incorrect (Weinfurt, 1995). Multiple comparisons within the ANOVA between the levels of fertilizer treatments were examined by Tukey's Honestly Significant Differences (Tukey's HSD) test (Dowdy et al., 2004).

Results and Discussion

Poultry manure as a fertilizer has the ability to accelerate the transformation and mineralization of less labile P forms (Waldrip et al., 2011). The question is how this impacts the ultimate distribution of P under real world conditions. Samples were collected at two locations with very different histories of management but from very similar locations (Table 2.1). As expected, the results of this research indicated significant effects for main factor History P=0.000 (Table 2.2). For example, Fox and Kamprath (1970) noted increases in soluble P were related to initial soil test P levels. Specifically, Fox and Kamprath (1970) found greater increases in soils with higher initial soil test P levels. Similarly, Pote et al. (2003) and Bond et al. (2006) found higher levels of water soluble P in experimental trials was strongly correlated to the initial levels of water soluble P. This is in direct agreement the identified significance related to the factor History.

Additionally, results indicated significant effects for main factors Source P=0.005 (Table 2.2). However, there is some degree of disagreement among previously published results regarding the potential impact of P source on P distribution and fractionation. Some studies noted livestock manures were equivalent to inorganic P sources relative to their availability (Eghball et al. 2005; Sikora and Enkiri 2005; Zvomuya et al. 2006; Sneller and Laboski 2009). Conversely, others have found livestock manures and inorganic P do not have the same availability (Gracy 1984; Motavalli et al. 1989; Sharpley and Sisak 1997; Griffin et al. 2003; Miller et al. 2010), whereas these findings indicate not only a significant effect for Source, but a significant interaction between History and Source P=0.003 (Table 2.3) This data indicates that change in the distribution of P is related to the P status of soil prior to the additions. In similar work, Waldrip et al. (2011) noted increases in specific P fractions over time in soils amended with poultry manure, but did not necessarily find evidence that single applications created significant differences in specific fractions over time.

Miller et al (2010) indicated experimental conditions such as incubation periods, animal species, and inorganic P types could explain some of the conflicting findings among the literature. Similarly, Rumi et al. (2012) noted changes in soil test P was rate and time dependent. The observed interaction between Source and History means the significance of the independent variable Source is dependent upon the level of the independent variable History. Under these experimental conditions (one soil with very low STP levels vs. one with an extensive history of manure P additions and high STP levels) the ability to identify changes in soil test P levels relative to P source is related to the management history. It is reasonable to assume that the effect of that management paradigm has led to an elevated initial soil test P level (Scalenghe et al. 2002) and the changes from such management alters the soil properties and soil P dynamics (Hao et al., 2008). As a result, continual additions of poultry manure shift P from binding with Fe and Al products to binding with Ca (Sharpley et al., 2004).

No significant differences in P levels relative to the factor Vegetation P=0.494 were identified (Table 2.2). Strictly in terms of plant use, Goss and Stewart (1979) found plants grown in soil fertilized with inorganic P removed soil P at higher rates than those grown in soil fertilized with manure. Later research examined poultry liter compost and compared it to inorganic fertilizer and found no significant differences in plant uptake (Sikora and Enkiri, 2003). Yet, luxury consumption of P and elevated initial soil test P levels could be contributing factors to inconsistent plant utilization of P (Goss and Stewart, 1979; Sikora and Enkiri, 2003; Miller et al. 2010). However, one would assume the uptake of P from the plants would change P levels, leading to significant differences between samples incubated with and without vegetation. Yet in soils with significant pools of P, the transformation and redistribution of P between could buffer minimal losses during short term incubation. In this experiment P additions were nominal (equivalent to 60 pounds per acre). Typically, acidic upland soils like berks have a significant P sorption capacity (Sekhon et al., 2014) and the location with a long history of poultry manure applications should have a higher degree of P saturation. Assuming soils are capable of replenishing the labile P pools; limited change in STP levels relative to any plant uptake would be expected. Similarly, soil for the location with limited to no know history of poultry manure fertilization would have extensive unused P sorption capabilities and would retain the majority of the P applied and may not reflect change due to plant uptake. Combined the availability of P from manure applications, the effect of microbial and chemical properties of the soil and the composition of the manure (Waldrip et al. 2011) make a simple explanation of findings is unlikely. Additionally, the vegetation in this experiment was minimal given the appropriate samples were seeded with 0.1g of tall fescue and its growth did not exceed 8 cm in height. Given these conditions and factors, the lack of significance relative to the factor Vegetation was not unexpected as its inclusion as an experimental factor was not about plant uptake as much as it was to establish if the soil-root interactions significantly impacted P distribution within these operationally defined pools.

After examining the main factors and identifying a significant interaction between the dependent variables History and Treatment, (Table 2.3) the dependent variable were analyzed individually. The differences between the levels of History were significant across all dependent variables (Tables 2.4, 2.5, 2.6, and 2.7). The main factor Treatment was only significant 2 of 4 sequentially extracted fractions. A closer examination identified significant effects for treatment at the $p<0.1$ level in the H_2O-Organic (Table 2.4) and NaOH-Organic fractions (Table 2.6). Typically following brief incubations (4 weeks) the majority of the sequentially extracted P_o is found in the NaOH extractable fraction with the least extractable P_o in the most labile fractions (Waldrip et al., 2014). Following longer incubations (8 weeks) more P is retained in the NaOH extractable fraction. Likewise, Fox and Kamprath (1970) identified greater increases in soils P fractions when soils were examined with higher initial soil test P levels.

The Tukey HSD multiple comparisons procedure was applied to the H_2O-Organic (Table 2.8) and NaOH-Organic (Table 2.10) fractions, but the only significant difference identified were between the poultry manure leachate and the control in the NaOH-Organic fraction and the Organic and Inorganic P fractions and the control in the H_2O extracted fractions. In general, NaOH extracted organic fractions are considered immobile and are often phytic acid sorbed onto clay minerals or precipitated with various metals oxides (Gagnon et al. 2012). P_o can be described or assigned in to one of two generic pools (the slow cycling and the fast cycling pools) and this is related to the form of the P_o and the associated process by which the P_o is retained within the soil (Dodd and Sharpley, 2015). The more stable, slow cycling pool consists of dead microbial cells, organic matter, and plant materials where the faster cycling pool consists of P contained within the microbial biomass (Dodd and Sharpley, 2015). Others (Negassa and Leinweber, 2009) indicated that in short-term studies, changes in P fractions depended on the amount of applied P regardless of P source. Similarly, sequential-P-fractionation schemes can be insensitive to detect small changes in P fractions following incubation with little or no P additions to agricultural soils (Qian and Schoenau, 2000; Hylander and Siman, 2006). One possible explanation would be the leaching of very labile P forms and related enzymes that facilitate the transfer of sorbed P into the fast cycling P_o pool over time.

The data identified significant interactions between History and Treatment in the HCl-Inorganic (Table 2.7), and H_2O-Organic fractions (Table 2.4) at the $p<0.1$ level. The HCl extracted inorganic P is typically associated with Ca compounds and the water extractable fraction are the most labile fractions (Negassa and Leinweber 2009; Gagnon et al. 2013). Organic P when incorporated in soil can be transformed into labile inorganic P forms (Waldrip et al. 2011). Long-term additions of P even at low rates should increases the level of P_o in all extractable P o fractions (Negassa and Leinweber 2009). While in general, P additions in any form tend to follow a progression outline by Walker and Syers (1976) that predicts the gradual conversion of P additions toward occluded P, more specifically it appears over time P from labile or moderately labile fractions can be transformed into the stable more recalcitrant forms (Negassa and Leinweber 2009). The interactions seen here indicate a likely hood that the most recalcitrant pools and the most labile pools seem to be dependent upon the management history i.e. the degree of P saturation, size of the active P pool in solution, and change in organic matter, and cations associated with long-term manure applications. Clearly these changes could impact how P additions would behave and what forms of P if any would remain in solution following weeks of incubation.

Conclusions

There were no significant effects from the actively growing plants on the disposition of P within the sequentially extracted factions. While 16 week incubations may be sufficient for sorption—desorption studies, there are limits to the amount of vegetation that can be generated under these experimental conditions. It is possible more prolific vegetation and increased sample numbers could lead to statistically significant differences. However, based on the lack of effect from vegetation in this experiment, any significant differences in P distribution because of vegetation in additional research seem unlikely. As such, any previous research involving incubation of P and soil without actively growing vegetation can be assumed representative of P sorption—desorption dynamics typical of pasture settings.

It was noted that P additions contribute to P pools in somewhat predictable ways. The changes in extractable soil P pools are consistent with the basic premise of the conceptual model of Walker and Syers (1976), whereby inorganic P in the labile pool become occluded. In theory as P becomes occluded, it is replenished via P cycling between labile and non-labile and organic and inorganic forms. As such, it appears the majority the P added to these samples became sorbed or occluded to the extent that the sequential extraction failed to identify any significant differences between the various sources. The literature indicates water-soluble P levels stabilize as quickly as 3 weeks after fertilization (Bond, et.al, 2006) or could continue to increasing in availability for up to 13 weeks before leveling off and stabilizing for up to 6 month (Gagnon and Simard, 1999). The length of incubation (16 week) and the repeated wetting and drying of the soil would have facilitated P cycling and provided time for P level to equilibrate.

It is understood that additions of some P compounds such as inositol hexaphosphate (IP6) can cause other forms of sorbed P to desorb causing temporary spikes in the more labile P pools such as water soluble inorganic P (Berg and Joern, 2006). Additionally, consider the conceptual model of Walker and Syers (1976) and its' conclusion that, over time, P becomes occluded and it is known that continual additions of poultry manure shift P from binding with Fe and Al products to binding with Ca (Sharpley et al., 2004). Given the experimental design with soil from locations with extensive histories of manure application the likelihood of the soil—P dynamics changing due to shifts in binding mechanisms is high. Likewise, soil from locations with low soil test P levels should have ample sorption capacity. It is assumed these factors contribute to the modest P additions not inducing significant changes in P levels.

Combined with the fact that is not unexpected that P additions from one source may elevate seeming unrelated P pools none of these results should be viewed as entirely unexpected.

Following these lines of reasoning and these findings, it seems reasonable to support the use of existing research independent of the P source and the presence or absence of vegetation in the incubation process, for the development of P management strategies and risk assessments. However, it is also clear that long-term additions of poultry manure change the fundamental aspects of how P additions interact with the soil. It is paramount to remember that while a great deal of P is retained in these soils, the interaction of the most labile forms is fundamental affected by the management history. Moving forward much caution should be observed when anticipating the fate of new P additions with any degree of specificity. Clearly there are more opportunities to examine the impacts of management history on P and how remediation strategies could impact that relationship over time.

The first research hypothesis (H1a) states that an addition of P causes a significant difference in operationally defined sequentially extracted P_o fractions relative to the source of the P addition and the fertilization history of the sample at the $p \leq 0.1$ level. The null hypothesis (H0a) states that additions of P does not cause a significant difference in operationally defined sequentially extracted P_o fractions relative to the source of the P addition and the fertilization history of the sample at the $p \leq 0.1$ level. Results of the MANOVA identified significant difference between P levels in the samples relative to History and Treatment. A detailed examination identified that while there are differences in some instances but not for all sequentially extracted fractions but not in the samples with soils with limited histories of P additions, thus rejecting the null hypothesis.

The third hypothesis ($H1_b$) states that the presence of active growing vegetation can affect sequentially extracted P_o fractions in incubated soil samples at the $p \leq 0.1$ level. The null hypothesis ($H0_c$) states, that the presence of active growing vegetation does not affect sequentially extracted P_o fractions in incubated soil samples at the $p \leq 0.1$ level. There was no evidence that the presence of actively growing plants had any impact on the sequentially extracted P_o level. Thus the null hypothesis was confirmed.

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Tables

Table 2.1 Soil map unit information from locations where soil was collected for benchtop experiments.

Location	Map Unit Name	Size Acers
*Fertilized	Berks-Weikert channery silt loams, 8 to 15 percent slopes	4.7
**Unfertilized	Berks-Weikert channery silt loams, 15 to 25 percent slopes	1.2

*Fertilized location received annual or near annual applications of animal (poultry) manure as a source of N fertilizer for a minimum of 10 years.
**Unfertilized locations are similar but had no significant manure applications within the last 10 years.

Table 2.2 MANOVA Test Criteria and F approximations for history treatment vegetation

Effect	Test	Statistic	Approximate F statistic	Numerator DF	Denominator DF	P value
History	Wilks’	0.00384	1201.273	8	37	≤0.000*
	Lawley - Hotelling	259.73474	1201.273	8	37	≤0.000*
	Pillai’s	0.99616	1201.273	8	37	≤0.000*
			S = 1	M = 3.0	N = 17.5	
Treatment	Wilks’	0.20420	1.804	40	164	0.005*
	Lawley - Hotelling	1.99458	1.765	40	177	0.007*
	Pillai’s	1.29632	1.794	40	205	0.005*
			S = 5	M = 1.0	N = 17.5	
Vegetation	Wilks’	0.83067	0.943	8	37	0.494
	Lawley - Hotelling	0.20385	0.943	8	37	0.494
	Pillai’s	0.16933	0.943	8	37	0.494
			S = 1	M = 3.0	N = 17.5	

* indicates p value less than or equal to 0.05, Wilks' test is the most commonly used test because it was the first derived and has a well-known F approximation, the Lawley-Hotelling is based on a T statistic. Pillai's trace will give similar to the Wilks' and Lawley-Hotelling's tests. S, M, and N are used to calculate the various statistics. If S=1 or 2, the F is exact, otherwise it’s an approximation.

Table 2.3 MANOVA Test Criteria and F approximations for interactions

Effect	Test	Statistic	Approximate F statistic	Numerator DF	Denominator DF	P value
History X Treatment	Wilks’	0.19293	1.881	40	164	0.003*
	Lawley - Hotelling	2.21027	1.956	40	177	0.002*
	Pillai’s	1.28431	1.771	40	205	0.006*
			S = 5	M = 1.0	N = 17.5	
History X Vegetation	Wilks’	0.67905	2.186	8	37	0.051
	Lawley - Hotelling	0.47264	2.186	8	37	0.051
	Pillai’s	0.32095	2.186	8	37	0.051
			S = 5	M = 1.0	N = 17.5	
Treatment X Vegetation	Wilks’	0.50667	0.692	40	164	0.914
	Lawley - Hotelling	0.82902	0.734	40	177	0.876
	Pillai’s	0.57072	0.660	40	205	0.941
			S = 5	M = 1.0	N = 17.5	
History X Treatment X Vegetation	Wilks’ Lambda	0.46420	0.790	40	164	0.808
	Hotelling - Lawley	0.88306	0.782	40	177	0.820
	Pillai’s Trace	0.67342	0.798	40	205	0.801
			S = 5	M = 1.0	N = 17.5	

* indicates p value less than or equal to 0.05, Wilks' test is the most commonly used test because it was the first derived and has a well-known F approximation, the Lawley-Hotelling is based on a T statistic. Pillai's trace will give similar to the Wilks' and Lawley-Hotelling's tests. S, M, and N are used to calculate the various statistics. If S=1 or 2, the F is exact, otherwise it’s an approximation.

Table 2.4 Analysis of Variance for H_2O-Organic, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
History	1	17162.8	17173.6	17173.6	280.27	≤0.000*
Treatment	5	702.8	696.4	139.3	2.27	0.060*
History * Treatment	5	696.6	696.6	139.3	2.27	0.059*
Error	56	3431.4	3431.4	61.3		
Total	67	21993.6				

S = 7.82783 R-Sq = 84.40% R-Sq (adj) = 81.33%

* indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 2.5 Analysis of Variance for $NaHCO_3$ -Organic, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
History	1	23500.7	23913.6	23913.6	190.26	0.000*
Treatment	5	706.0	784.1	156.8	1.25	0.299

History * Treatment	5	690.0	690.0	138.0	1.10	0.372
Error	56	7038.6	7038.6	125.7		
Total	67	31935.3				

S = 11.2111 R-Sq = 77.96% R-Sq (adj) = 73.63%

* indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 2.6 Analysis of Variance for NaOH-Organic, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
History	1	469495	475973	475973	509.54	≤0.000*
Treatment	5	8620	9333	1867	2.00	0.093*
History * Treatment	5	8048	8048	1610	1.72	0.144
Error	56	52310	52310	934		
Total	67	538473				

S = 30.5633 R-Sq = 90.29% R-Sq (adj) = 88.38%

* indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 2.7 Analysis of Variance for HCl-Organic, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
History	1	24056.5	23899.5	23899.5	2662.55	≤0.000**
Treatment	5	79.9	75.4	15.1	1.68	0.154
History * Treatment	5	97.7	97.7	19.5	2.18	0.070*
Error	56	502.7	502.7	9.0		
Total	67	24736.8				

S = 2.99603 R-Sq = 97.97% R-Sq (adj) = 97.57%

* indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 2.8 Grouping Information Using Tukey Method and 90.0% Confidence for H_2O Extractable Organic P

History	Treatment	Count	Mean mg/kg	Standard Error	Standard Deviation	Grouping
Fertilized	Control	6	25.4	1.63	4.0	A
	Leached Manure	6	39.2	5.71	13.98	A B
	Manure	6	33.6	5.34	13.09	A B
	Manure Leachate	5	29.3	0.88	1.97	A B
	P_o	6	41.2	6.42	15.74	B
	P_i	6	26.0	3.29	8.06	B
Unfertilized	Control	6	0.5	0.21	0.51	C
	Leached Manure	6	0.4	0.23	0.56	C
	Manure	6	1.0	0.31	0.75	C
	Manure Leachate	6	0.9	0.31	0.75	C
	P_o	5	1.1	0.29	0.64	C
	P_i	6	1.0	0.34	0.84	C

Treatments with same grouping letter are not significantly different

Table 2.9 Grouping Information Using Tukey Method and 90.0% Confidence for $NaHCO_3$ Extractable Organic P

History	Treatment	Count	Mean mg/kg	Standard Error	Standard Deviation	Grouping
Fertilized	Control	6	48.4	7.65	18.74	A
	Leached Manure	6	50.0	5.94	13.27	A
	Manure	6	47.3	7.20	17.63	A
	Manure Leachate	5	64.5	1.74	3.88	A
	P_o	6	45.9	1.77	19.03	A
	P_i	6	46.6	5.49	13.45	A
Unfertilized	Control	6	11.5	0.30	0.75	B
	Leached Manure	6	11.7	0.26	0.64	B
	Manure	6	11.9	0.14	0.32	B
	Manure Leachate	6	13.7	1.86	4.56	B
	P_o	5	11.5	0.23	0.51	B
	P_i	6	16.6	2.14	5.25	B

Treatments with same grouping letter are not significantly different

Table 2.10 Grouping Information Using Tukey Method and 90.0% Confidence for NaOH Extractable Organic P

History	Treatment	Count	Mean mg/kg	Standard Error	Standard Deviation	Grouping
Fertilized	Manure Leachate	5	264	5.22	11.7	A
	Leached Manure	6	243	13.9	34.0	A B
	Manure	6	208	15.7	38.5	A B
	P_i	6	223	27.6	67.7	AB
	P_o	6	217	17.3	42.5	A B
	Control	6	199	11.7	28.6	B
Unfertilized	Control	6	53.6	3.60	8.81	C
	Leached Manure	6	56.7	0.98	2.41	C
	Manure	6	62.0	4.97	12.2	C
	Manure Leachate	6	58.9	4.52	11.1	C
	P_o	5	56.2	4.19	9.36	C
	P_i	6	65.6	5.73	14.0	C

Treatments with same grouping letter are not significantly different

Table 2.11 Grouping Information Using Tukey Method and 90.0% Confidence for HCl Extractable Organic P

History	Treatment	Count	Mean mg/kg	Standard Error	Standard Deviation	Grouping
Fertilized	Control	6	25.4	1.63	4.00	A
	Leached Manure	6	39.2	5.71	13.98	A
	Manure	6	33.6	5.34	13.09	A
	Manure Leachate	5	29.3	0.88	1.97	A
	P_o	6	41.2	6.42	15.75	A
	P_i	6	26.0	3.29	8.06	A
Unfertilized	Control	6	0.5	0.21	0.51	B
	Leached Manure	6	0.4	0.23	0.56	B
	Manure	6	1.0	0.31	0.75	B
	Manure Leachate	6	0.9	0.31	0.75	B
	P_o	5	1.1	0.29	0.64	B
	P_i	6	1.0	0.34	0.84	B

Treatments with same grouping letter are not significantly different

Chapter 3. Identifying the Effects of Space on the Distribution of Sequential Extracted Organic Phosphorus Fractions in Hay and Grass Pastures of Eastern West Virginia Following Long-Term Nitrogen-based Manure Applications.

Abstract

While P, in general, is strongly sorbed by soil, P applied to the soil surface may not necessarily remain in place. Not all forms of P are equal in terms of the strength with which the forms are sorbed. There is also variability in terms of ability of different soils to sorb P. Furthermore, surface applications of manure or fertilizer are not necessarily uniform across the landscape. All of these factors play some role in the physical distribution of various P compounds within any managed unit. When P at landscape scales is examined to evaluate potential movement or loss one must account for these effects prior to determining if a true pattern exists. To determine if P has moved over time, multiple P fractions were sequentially extracted and analyzed for significant spatial structure. Statistical techniques were applied to identify soil properties that could explain significant portions of the P variability while controlling for the effect of those variables while examining the residual variability for spatial structure. Thus it was concluded that in some instances the 0.5M $NaHCO_3$ extractable organic P (P_o) fraction and the 1.0M HCl extractable P fraction exhibited identifiable spatial structure (residual spatial autocorrelation) not associated with changes in soil properties. Conversely the more stable 0.1M NaOH extractable P fraction and the very transient H_2O extractable fractions did not exhibit such patterns. These results are consistent with a hypothesis of extractable P fractions behaving uniquely at landscape scales.

Introduction

Whether practicing sustainable agriculture, protecting sensitive watersheds or understanding the long-term implication of specific management paradigms is critically important. In some locations, due to capacity to generate manure and how that manure is utilized on farms, have a greater potential to influence water quality. The poultry-producing region of West Virginia is one such location. For example, Grant, Hardy, and Pendleton Counties in eastern West Virginia produce approximately 14% of the state's cattle sales and 85% of the state's meat type chicken sales (USDA-NASS, 2009). This regionally dense food animal agriculture production has the potential to generate in excess of 200,000 tons of dry manure per year (Wang et al., 2007). Significant portions of animal manure in these counties are used as fertilizers (Basden et al., 1994) on grain crops, grass hay, and pasturelands. When concentrated animal agriculture and N-based manure management occur together, P saturation and elevated risks to water quality are potential outcomes (Beck et al., 2004; Miller et al. 2010). If a given location in these counties received animal manure applications on a N basis, these locations will accumulate P as the P levels on the manures are in excess of plant removal or requirement rates when applied to meet the N needs of the crops. This should prohibit further manure applications. In the coming years the efforts to reduce the P saturation of these sites and prevent P loss will be a greater priority. As such, a greater understanding of the fate of surface applied P on these agricultural lands becomes even more critical.

Historically, most research on P sorption and availability has focused on inorganic sources, with significantly less attention given to organic species of P (Laboski and Lamb, 2003; Anderson and Magdoff, 2005). Some researchers have hypothesized that this is, in part, be due to (i) a perception that P_i is the dominant form of P, (ii) P_i is the plant available form, and (iii) the analysis of organic P is simply too problematic (Jansson et al., 1988; Turner and Haygarth, 2000; Anderson and Magdoff, 2005). Between 29 to 65% of the total P (Harrison, 1987) and perhaps greater than 90% of the soil solution phase P could be in an organic form (Helal and Dressler, 1989; Shand et al., 1994; Turner and Haygarth, 2000; Anderson and Magdoff, 2005). Only considering P_i in risk assessment is to potentially overlook a large portion of the total P pool. Such oversight could undermine the conservation and restoration efforts of regulators, the agricultural community, and conservation professionals by invalidating the basic assumptions used in developing their P loss control strategies.

In terms of P composition in soil, the P_o pool is typically composed of inositol phosphates, phospholipids, nucleic acids, phosphoproteins, and unidentified P compounds (Schroeder and Kovar, 2006). To date, the only significant examination of P_o in West Virginia soils was produced by the West Virginia University Agricultural Experiment Station in the 1960's (Jencks et al., 1964). This research measured total P, total P_o, phytin, available P, organic matter, and pH in several soils. P_o accounted for between 7 and 66% of the total P in the surface horizons, and from 13 to 55% in the subsurface horizons (Jencks et al., 1964). Phytin or phytic acid (an inositol phosphate) accounted for between 13 and 63% of the total P in the surface horizons, and from 10 to 48% in the subsurface horizons (Jencks et al., 1964). This corroborates the assertions of others (Harrison, 1987; Helal and Dressler, 1989; Shand et al., 1994; Turner and Haygarth, 2000; Anderson and Magdoff, 2005) that significant portions of the total soil P pool may, in fact, be P_o. Given any P loss is potentially damaging to the environment, resource managers need a better understanding of how P_o responds relative to management, movement, and soil interaction (Condron et al., 2005).

One way to examine differing P forms in soil is sequential extraction. There are numerous examples of research that has utilized this technique to separate the total P pool into operational defined fractions (Bowman and Cole 1978; Hedley et al., 1982; Tiessen et al., 1984; Schoenau et al., 1989; Cross and Schlesinger, 1995; Iyamuremye et al., 1996; Sui et al., 1999; Guppy et al., 2000; Qian and Schoenau, 2000; Yang et al., 2002; He et al., 2004; Schroeder and Kovar, 2006). A sequential P extraction integrates a collection of chemical extractions so as to characterize P by the type and strength of their physicochemical interactions with soil components (Bowman and Cole 1978; Hedley et al., 1982; Cross and Schlesinger, 1995). An individual fraction in a sequential extraction is conceptually different but not necessarily pure or unique. The most common extractions used in these sequential fractionations are water (H_2O), anion exchange resin, sodium bicarbonate ($NaHCO_3$), hydrochloric acid (HCl), and sodium hydroxide (NaOH) (Guppy et al., 2000; Schroeder and Kovar, 2006).

In soil, P extracted with any hydroxide solution is typically bound to Al and Fe (Sharpley et al., 2004). The P extracted with acid is typically bound to Ca (Sharpley et al., 2004). The P extracted with bicarbonate, ion exchange resin, and or H_2O are characteristically the weakest attached, most labile, and easily exchangeable or plant available forms of P (Sharpley et al., 2004). The dominant forms of P in poultry manure (the primary animal manure used in these study areas) are orthophosphate and phytate (Warren et al., 2008). The majority of the P in poultry liter can be extracted from the manure with H_2O and $NaHCO_3$ (Codling, 2006; Dou et al., 2000; Dail et al., 2007) due in part to phytate is the principal form of P in the grain-based diets of non-ruminants being passed along into the feces undigested (Harland and Morris, 1995; Sharpley, 2000). The P_i percentage is typically highest in the H_2O extractable fraction (Codling, 2006). However, some reports indicate that 72–83% of H_2O-extractable P in poultry manure is in an organic form (Sistani et al., 2001).

Poultry manure can raise P, N, and Ca levels in soil, as well as elevate the levels of bicarbonates and organic acids with carboxyl and phenolic hydroxyl groups (Sharpley et al., 2004). This provides new reactive surfaces, changes the constitution of the soil solution, and can alter basic chemical properties such as pH and ionic strength; which can, over time, shift P from binding with Fe and Al to binding with Ca (Sharpley et al., 2004). With continued manure application, more and more P ends up in the easily extractable, labile fractions (Blake et al., 2003). Ultimately, the number and type of sorbtive surfaces in the soil determines the fate of P and how it is described by a sequential extraction procedure (Blake et al., 2003). Accordingly, when analyzing spatial distributions of P across a landscape it is important to consider how changes in soils properties across the landscape could alter the distribution.

While, in a general sense, P is limited in its mobility, it can move through soil profiles (Smeck and Runge, 1971; Smeck, 1973; Harman et al. 2013) and across landscapes (Smeck, 1973). Most P loss or movement is attributed to one of two processes—leaching or erosion. Leaching occurs when P from fertilizer or manure moves through the soil into the groundwater or surface water. Erosion losses occur when overland flow moves P from fertilizer and manures or P enriched soil materials across the soil surface and into surface waters. P_i leaching is generally considered a minimal risk (Anderson and Magdoff, 2005). However, soils receiving large quantities of P fertilizer, deep sandy soils, organic soils, well structure soils, and soils with high rock fragment contents can be at risk of P leaching (Harman et al., 2013; Anderson and Magdoff, 2005). Smeck (1973) and Smeck and Runge (1971) proposed that P can move laterally in a landscape, and will accumulate in lower landscape positions. The more important questions are: do all forms of P move similarly, and are changes in P levels an indication of movement or an indication of changes in soil properties that shift or modify P retention dynamics?

Measured P levels at any location is the product of the soils properties, P additions over time, and any landscape scale process that could move P over or through the soil. Research on P sorption capacity of riparian wetlands soils has highlighted the importance of changes in soil properties relative to the soils ability to retain P (Bruland and Richardson, 2004). Bruland and Richardson (2004) found significant portion of the variability in P levels were related to changes in the P sorption capacity. In most instances, within a defined management area such as a hayfield, an attempt to achieve a somewhat uniform application of nutrients over time can be assumed. Thus if one accounts for the effect of changing soil properties across a management area and assume a uniform application of nutrients over time, any pattern in measure P at that scale must be related to the space itself, or the physical process that are dependent upon that space. Similarly, operational defined P fractions can have differing characteristics and sorb at differing rates to soil particles (Anderson and Magdoff, 2005). Thus some sequentially extracted P fractions may be more or less responsive to these spatial effects and manifest some degree of spatial dependence.

One way to measure spatial dependence is the Mantel test (Mantel, 1967). The Mantel test is a permutation-based correlations analysis where one matrix is a difference matrix and the other a distance matrix. A correlation coefficient is calculated between these matrices. The values of distance matrix are randomly reassigned to another location, and the analysis repeated. After many permutations, a distribution of the correlation coefficients is generated. Thus the significance of the correlations can be estimated from the permutated distribution (Bonnet and Van de Peer. 2002).

A further adaptation of the Mantel test is the partial Mantel test. A partial Mantel test uses three matrices. Rossi (1997) stated, “Once the existence of a relationship between two variables has been demonstrated, one can wonder if it is a true correlation or if it is only a spurious correlation due to common spatial (or temporal) pattern”. A partial Mantel test, tests the correlation of two matrices while controlling the effect of a third matrix (Bonnet and Van de Peer, 2002; Smouse et al., 1986). When looking at a P distribution across a landscape, one such spurious correlation could be changes in P sorption capacity via changes in soil properties. The partial Mantel test allows for control for the effect of changes in soil properties while determining if any remaining patterns of spatial dependence exist within the P data.

This research consists of field scale experiments designed to examine the possible movement of sequentially extracted P_o fractions in typical hay or pasture setting on marginal soil in the poultry-producing region of West Virginia. The purpose of this research is to determine if there is an unidentified spatial component to the field-scale distribution of sequentially extracted P_o beyond what can be explained by changes in soil properties across the study area.

The first research hypothesis ($H1_a$) states that over time sequentially extracted P_o fractions in typical hay or pasture setting on marginal soil in the poultry-producing region of West Virginia will exhibit spatial dependence among the sequentially extracted P_o fractions. The null hypothesis ($H0_a$) states that in typical hay or pasture settings on marginal soil in the poultry-producing region of West Virginia will not exhibit spatial dependence among the sequentially extracted P_o fractions. The second hypothesis ($H1_b$) states that there is spatial dependence among sequentially extracted P_o fractions after removing potential spatial dependence associated with changes in soil properties. The null hypothesis ($H0_b$) states, states that there is no spatial dependence among sequentially extracted P_o fractions after removing potential spatial dependence associated with changes in soil properties.

Materials & Methods

Site selection and Sampling

The selected study sites were typical hay or grass pasture fields in eastern West Virginia. The study sites consisted of one set of fertilized locations (High 1 and High 2), with lengthy histories (approximately 10+ yrs.) of annual N-based manure applications and one set of unfertilized locations (Low 1 and Low 2) with very infrequent manure applications (approximately one application every 5-10 yrs.).

Sample points sere selected in a stratified random design (Thompson et al., 2006), with the strata being the topographic wetness index (TWI) at each location. For all locations, TWI was calculated from a 3-m digital elevation model (DEM) from the WV State Address Mapping Board (SAMB) aerial imagery. All determinations were made from a DEM free of sinks or voids. TWI is defined as the ln (A / tan B) where A is the local upslope contributing area for that point and B is the local slope gradient (Beven and Kirkby, 1979). The flow directions used in the TWI calculation was the simplest design. It specifies flow direction from each raster cell into one of the eight neighboring cells based on the steepest downward slope gradient (O'Callaghan and Mark, 1984). Specific catchment area is estimated by A/L, with A being the number of pixels draining into a pixel, multiplied by the area of a pixel, and L is the pixel width (Moore et al., 1991b). At each location TWI was classified into three equal-sized classes based on the TWI score. Ten samples locations were selected at random within each class, for a total of 30 samples locations at each study area. From each sample point the first mineral horizon (surface horizon) and the 10 cm below that horizon (subsurface horizon) were sampled.

Sample Preparation

All samples were air dried, ground, sieved (2-mm sieve), and thoroughly mixed to make individual samples as homogenous as possible (Laboski and Lamb, 2003). Dried and ground samples were stored in sealed centrifuge tubes at 4°C until one day prior to analysis. All soil samples were analyzed in duplicate.

pH

The pH of the soil samples was measured as described by Eckert and Sims (1995) in the recommended soil testing procedures for the northeastern United States. A 5 cm^3 sample of dried and sieved soil was mixed with 5 mL of distilled deionized water (DDI), stirred vigorously for 15 seconds and allowed to sit for 30 minutes. The pH was measured with a calibrated pH meter. This procedure was then repeated using 0.01 M $CaCl_2$ in place of DDI.

Sequential Fractionation

This method of sequential P fractionation is based on a suggested modification (Sui et al., 1999) of the Hedley method (Hedley et al., 1982) as described by He et al. (2003). For each sample, 1.0 g of soil and 25 mL of extractant was placed in a centrifuge tube in a reciprocal shaker at 180 oscillations per minute for 16 h at room temperature. The samples were centrifuged for 15 min at 3500 g and the supernatant filtered (Whatman No. 2 or equivalent). This process was repeated sequentially with the following extractants: distilled deionized water, 0.5M NaHCO3, 0.1M NaOH, and 1M HCl. Duplicates of each sample were fractionated in this manner. Water, 0.5M NaHCO3, and 0.1M NaOH extracts were acidified and filtered (Whatman No. 2 or equivalent) prior to analysis for P_i.

P_i was determined by the ammonium molybdate-ascorbic acid method (Knudsen and Beegle, 1988). The ammonium molybdate-ascorbic acid method is a single reagent orthophosphate colorimetric method. There are two stock solutions: the concentrated ammonium paramolybdate solution and the ascorbic acid solution. The concentrated ammonium paramolybdate solution was made by adding 60 g of ammonium paramolybdate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) to approximately 200 mL of distilled water in a 1 L volumetric flask, along with 1.455 g of antimony potassium tartrate ($KSbO\cdot C_4H_4O_6$). Then 700 mL of concentrated sulfuric acid was added and the solution was allowed to cool to room temperature, diluted to volume with distilled water, and stored in a dark glass bottle in the refrigerator. The ascorbic acid solution was made by dissolving 132 g of ascorbic acid in distilled water diluted to 1 L in a volumetric flask. The single colorimetric working solution was made daily by adding 25 mL of concentrated ammonium paramolybdate solution to approximately 800 mL distilled water, with 10 mL of the ascorbic acid solution and diluting to volume with distilled deionized water in a 1 L volumetric flask.

To determine P content, 2 mL of the sample solution was transferred to a test tube with 8 mL of the colorimetric working solution and mixed thoroughly. After 20 minutes for color development, percent transmittance was read at 882 nm. Total P (P_t) was determined with a Perkin Elmer P4000 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). P_o was calculated for each fraction as the mathematical difference between the reactive P_t and P_i.

Mehlich-1

Mehlich-1 is a soil test with a 5:1 ratio of Mehlich-1 solution (0.025 N H_2SO_4 + 0.05 N HCl) to soil. The mixture is shaken for five minutes on a reciprocating shaker set at a minimum of 180-200 oscillations per minute (Nelson et al., 1953). The extractant is filtered through a medium-porosity filter paper (Whatman No. 2 or equivalent) and analyzed for Al, Ca, Fe, and P content (Nelson et al., 1953). Total P, Fe, Al and Ca levels were determined by ICP-OES.

Total Soil Carbon

Total soil carbon was measured in a LECO TruSpec CHN elemental analyzer (LECO Corp., St Joseph, MI), where a sample was weighed into foil cups and combusted at 950°C and the CO_2 gas produced was measured by infrared gas spectrometer (Keene, 2010).

Particle Size Distribution

Particle size distribution was determined by the pipette method (Gee and Bauder, 1986), with only A horizon samples pretreated to remove organic matter. Five grams of soil and 25 ml of H_2O were placed in a tared 250 mL fleaker with 5 mL of H_2O_2. After the reaction ceased, additional H_2O_2 was added until the reactivity stopped and the mixture appeared to be fully oxidized (little reaction and a gleyed appearance). When oxidation of organic C was complete, the sample and container were oven dried at 90°C and reweighed.

The samples were dispersed in sodium hexametaphosphate solution (HMP) overnight on a mechanical shaker, in a solution at a concentration of 0.5g/L HMP. The sample was then made to volume (250 ml) shaken for 30 seconds, and positioned to allow time for differential settling of the sand and silt size particles, prior to extracting a 25 mL sample and transferring it into a tared container and dried at 90°C, cooled, and reweighed. The remaining sample was filtered through a 270-mesh sieve into a tared container and the sand size particles dried at 90°C, cooled, and reweighed. A blank of the HMP was sampled in the same manner to determine salt content from the HMP. The clay content was calculated from the sample minus the HMP blank. Sand content was calculated from the direct measurement of sieved sand. Silt will be determined mathematically as the difference between the sample mass after any pretreatments and the sum of the sand and clay (Gee and Bauder, 1986).

Statistical Analysis

The initial characterization of the soil samples from each location began by preforming a multivariate analysis of variance (MANOVA) on the soil test data from each horizon independently to determine if there were significant differences between locations, followed by a Bonferroni corrected one-way analysis of variance (ANOVA) on each soil test parameter. Spatial dependence in the sequentially extracted P_o data was assessed with Mantel and partial Mantel tests. The Mantel tests (Mantel, 1967) were used to determine if the differences between P measures and the distances between sample locations were significantly correlated, thus spatially dependent (Bonnet and Van de Peer, 2002). The Mantel test is a permutation based correlations analysis. One matrix was the difference between P_o values of each pair of points, the other a distance matrix between points. A correlation coefficient was calculated between these matrices. The values on one matrix randomly reassigned to another location, and the analysis repeated. After many permutations, a distribution of the correlation coefficients would be generated. Probabilities would then be estimated based on the data’s position within the permutated distribution (Bonnet and Van de Peer. 2002).

A stepwise regression was used to identify measured soil properties that could explain a significant portion of the variability seen in STP levels. Alpha to enter and leave values of 0.15 were selected and the Mehlich 1 extractable Fe, Al, and Ca; soil carbon; sand, silt, and clay percentages; surface horizon thickness; and pH in water and in CaCl for the surface horizon samples were examined. For the subsurface horizon samples, similarly the Mehlich 1 extractable Fe, Al, and Ca; the soil carbon; sand, silt, and clay percentages; surface horizon thickness; pH in water and in CaCl; and STP levels of the surface horizon samples were examined.

The extension of the Mantel techniques is the partial Mantel test. A partial Mantel test uses more difference or distance matrices. In a partial Mantel test, two variables are compared while fixing for the effect of a third matrix of a third variable or group of variables. Similarly, after permutation the probabilities can be estimated based on the data's position within the generated distribution (Bonnet and Van de Peer. 2002). Accordingly, a two tailed permutation Mantel and partial Mantel tests with 10,000 permutations were performed.

Results and Discussion

The Mehlich 1 soil test results, pH, particle size distribution, and surface horizon thickness were used to characterize each fertilized and unfertilized location. The MANOVA of the soil test levels was used to determine if there were significant differences between the unfertilized and fertilized locations (Table 3.1). The MANOVA results indicated significant differences ($p \leq 0.001$) between the soil test values from the surface horizons of the fertilized and unfertilized locations. Similarly, the MANOVA of the subsurface soil test values indicated significant differences ($p \leq 0.001$) between the fertilized and unfertilized locations (Table 3.2.). This result was explored in further detail by individually examining each soil test parameter via one-way ANOVA using Tukey's multiple comparison procedure to evaluate the significance of the different locations.

The one-way ANOVA of surface soil test P values indicated a significant difference between locations (Table 3.3). The mean soil test P levels ranged from 1020 mg/L at High 2 to a mean low of 12.9 mg/L at Low 2 (Table 3.4). The grouping information using Tukey's multiple comparisons procedure identified three groups: (i) High 1, (ii) High 2, and (iii) a group comprised of Low 1 and Low 2 (Table 3.4). Subsurface STP levels were significantly different between locations (Table 3.3), with values ranging from a high of 982 mg/L at High 2 to a low of 4.9 mg/L at Low 1 (Table 3.4). The grouping Information from the Tukey's multiple comparisons procedure followed the same pattern as the STP levels of the surface horizon (Table 3.5). The elevated STP levels at the fertilized locations are consistent with N-based manure applications (Beck et al., 2004) and P translocation into the soil profile (Harman et al., 2013).

The one-way ANOVA of surface and subsurface soil test Ca (STCa) levels indicated a significant difference between locations (see Table 3.3). The mean surface horizon STCa levels ranged from a high of 4170 mg/L at High 2 to a low of 745 at Low 2, while mean subsurface STCa levels ranged from 1620 mg/L at High 1 to 365 mg/L at Low 2 (Table 3.4). The grouping information for the subsurface STCa levels using the Tukey's multiple comparisons procedure identified three groups: (i) High 1, (ii) High 2, and (iii) a group comprised of Low 1 and Low 2 (Table 3.5) following the same pattern as the surface and subsurface STP levels, and surface STCa levels. Poultry manure can raise P, N, and Ca levels in soil, as well as elevate the levels of bicarbonates and organic acids with carboxyl and phenolic hydroxyl groups (Sharpley et al., 2004). Based on STP levels, the effects of long term N-based poultry manure applications, and the Tukey groupings it appears the High fertilization locations were managed significantly different from each other, with one receiving more frequent applications leading to higher STP levels.

Soil test Fe and Al (STFe and STAl) results do not follow this pattern. The one-way ANOVA of STAl indicated a significant difference between locations (see Table 3.3). The mean STAl levels ranged from a high of 277 mg/L at High 2 to a low of 120 at Low 1 (Table 3.4). The grouping information using Tukey multiple comparisons procedure identified three groups. STAl levels at High 1 and Low 2 were not significantly different forming one group while High 2 and Low 1 were significantly different from each other and from High 1 and Low 2 (Table 3.5). The one-way ANOVA of STFe indicated a significant difference between locations (see Table 3.3). The mean soil test Fe levels ranged from a high of 57.2 mg/L at Low 1 to a low of 16.1 at High 2 (Table 3.4). The grouping Information from the Tukey multiple comparisons procedure identified two groups. Only Low 1 was significantly different from the other locations (see Table 3.5). This indicates differences in STFe and STAl are not related to management practices, i.e., the differences are pedogenic. However, these differences are potentially important because as soils weather and acidify, the formation of Fe and Al phosphates and P_o is favored (Smech, 1985; Pierzynski et. al 2005). Thus, differences in concentrations of extractable Fe and Al could impact retention mechanisms.

Changes in Fe, Al, and Ca concentrations and soil texture have the potential to be important factors in the translocation of P across a landscape. Specifically, Sharpley et al. (2004) described sequentially extracted P as typically bound to Al, Fe, and Ca, or weakly attached, most labile, and easily exchangeable. Blake et al. (2003) stated that continued additions of poultry manure would increase the more easily extractable labile fraction of soil P. Additionally it is known that concentrations of various P fractions can be affected by slope position (Heilmann et al., 2005, Kistner et al., 2013). Thus it seems likely that, even if applied uniformly over a lengthy period of time, P accumulation and distribution would not remain uniform. As such, there could be patterns in the P distributions related to changes in these properties or changes dependent on the landscape itself. When combined, the physical distances between locations and the significant differences in STP, STFe, STAl, and STCa justifies examining each location independently.

The Mantel test for correlation between dissimilarity matrices was applied to each location for each sequential fraction. In brief, this test identifies significant correlation between dissimilarity matrices of data. The partial Mantel test was applied to examine the data in greater detail. The partial mantel test fixes for the effect of selected data while comparing the correlation of dissimilarity matrices of the other data sets. The identification of a significant correlation between the dissimilarity matrices is an indication of an underlying spatial structure within the data.

To identify other factors that could explain the distribution of P across the landscape a stepwise regression of the response STP was used for the surface and subsurface horizons and identified multiple properties related to STP levels (Tables 3.6 and 3.7). These properties were fixed while evaluating the correlation between the dissimilarity matrices in sequentially extracted P fractions using the partial Mantel test. This approach is similar to Bruland and Richardson (2004), who used these techniques to measure partial correlations between soil properties and P sorption while controlling for the effect of spatial autocorrelation. In this instance the procedure controlled for the effects of soil properties when evaluating the probability of spatial structure in the data. In this example each sequential fraction at each location was examined for spatial autocorrelation. The Mantel tests identified spatial structure among multiple P_o fractions (Table 3.8). For any location-sequential fraction combination with identified spatial structure the effects of the select soil properties as identified via stepwise regression were held constant and the remaining variability was examined via the partial Mantel test to determine if the underlying spatial structure was intact. Initial observation of the data seemed to indicate that the locations with the higher STP and the lower STP levels do not behave consistently. Specifically, locations High 1 and High 2 each have P_o fractions exhibited spatial structure after fixing for the effects of changing soil properties, but not the same fractions. Management can be an important factor in how P is distributed between sequentially extracted pools. P availability post manure application can be influenced by microbial and chemical properties of the soil, the makeup of the manure, and the complex interactions between these components (Waldrip et al. 2011). Lilientein et al. (2000) determined that changes in landuse strongly influence available P fractions relative to Ca, Fe, and Al-bounded P fractions. The location with the highest STP levels shows spatial dependence in the HCl extracted fraction. Low 2 has a lower mean STP level (approximately 20% of High 1). This location has a significant spatially autocorrelated $NaHCO_3$ extracted fraction. It is not uncommon for P additions on fertilized pastures to lead to elevated $NaHCO_3$ and NaOH fractions (Haynes and Williams, 1992) and it is possible due to the higher STP levels at High 1 that the HCl extractable fraction is behaving as a sink for the more labile P forms. Specifically, Sharpley et al. (2004) found that long term applications of manures shifted P fractions into the HCl extractable fractions. Thus it seems likely that there are differing mechanisms of retention between High 1 and High 2.

Similarly, the location Low 1 also exhibited spatial dependence in the$NaHCO_3$ fraction. Waldrip et al. (2011) hypothesized that stable P forms from poultry manure would steadily replenish plant available forms of P in the soil solution. Additionally, it is known that $NaHCO_3$ extractable P_o is labile and in some instances is considered plant available (Johnson et al. 2003, Dieter et al. 2010). As a labile to moderately labile fraction it is not unexpected to find spatial differentiation of this P fraction. However, while there was no statistical difference between the STP levels of the unfertilized plots, there were differences in terms of which extractable fractions exhibited spatial dependence after fixing for the effects of soil properties. At location Low 1 the $NaHCO_3$ extracted fraction was spatially autocorrelated and at Low 2 the HCl extracted fraction was spatially autocorrelated. This finding is inconsistent with what is expected.

When examining the subsurface horizons data there were no spatially autocorrelated fractions in the High locations. Given the frequency and pedologiclly diverse conditions where preferential flow occurs in pasture of this region (Harman et al., 2011) one would expect to find a great deal of variability in the subsurface data. However, given the elevated STP level of the fertilized sites and the modest depths of the subsurface samples the soil could be more fully saturated than the subsurface samples from the unfertilized locations. Likewise, the number and diversity of fractions exhibiting spatial dependence in the unfertilized location could be a product of preferential flow. In similar pasture soils, Harman et al. (2013) identified P translocation through multiple soil profiles.

Conclusions

P availability following manure application can be influenced by the microbial communities and chemical properties of the soil, the makeup of the manure, and any number of complex interactions between these components (Waldrip et al. 2011). Among the chemical properties influencing P availability is soil surface chemistry, reactivity, Fe and Al content, clay content, and pH (Barrow, 1984; Fox, 1985; McGechan and Lewis, 2002). As these soil properties change, the strength of the interactions between P and the soil changes. The changes in these interactions affects soil solution P concentrations (Brady and Weill, 2002; Blake et al., 2003; Pierzynski et al., 2005). Knowing that P from animal manures is a mixture of P forms of various solubility (Harland and Morris, 1995; Schroeder and Kovar, 2006; Warren et al., 2008), not all P forms are equally labile (Pierzynski et. al 2005), and concentrations of various P fractions can be affected by slope position (Heilmann et al., 2005; Kistner et al., 2013). The logical conclusion is a differential distribution of all P forms across a landscape.

However, if inferring risk of P_o loss from over-fertilized landscapes based in patterns seen in the P distribution, the first step in determining if P_o loss and leaching is occurring is identifying how much of the changes in P_o concentrations are related to soil properties and how much is related to the landscape. Water movement in a landscape is often an indication of the potential P distribution (Smeck and Runge, 1971).

Given the dynamic relationships between the soil, the fertilizers, and the landscape one would assume several specific occurrences within the spatial distribution of P_o. The first logical assumption would be to expect relatively low and or constant levels of water soluble P_o due to the transient nature of the pool and constant replenishment from other P fractions. A second assumption would be the labile but more recalcitrant $NaHCO_3$ extractable P fractions would potentially be mobile enough to redistribute yet recalcitrant enough to accumulate. As metal oxide sorption sites become saturated with P, the NaOH extractable fraction would essentially mimic the distribution of metal oxides and be explained by the changes in particle size distribution and the Fe and Al concentrations. Lastly, one would expect the HCl extractable P_o fractions should be closely matched to the concentrations of Ca in the soil.

The data collected was consistent with a sequential saturation of progressively stronger sorption sites. At the fertilized location with the highest STP levels, the variability in the transient and labile fractions were fully explained by the changes in soil properties (Table 3.8). However, the HCl extracted fraction in this location continued to exhibit spatial dependence beyond that explained by changes in soil properties (Table 3.8). It is not uncommon for soils with high levels of P to show evidence of P movement even if the respective P levels in deeper horizons are very low (Nelson, et al., 2005). Similarly, at the fertilized location with lower STP levels, there was variability unexplained by changes in soil properties but it was identified in the more labile fractions (Table 3.8). The location Low 1 also exhibited spatial dependence in theNaHCO3 fraction (Table 3.8). Waldrip et al. (2011) hypothesized that stable P forms from poultry manure would steadily replenish plant available forms of P in the soil solution. Additionally, it is known that NaHCO3 extractable P_o is labile and in some instances is considered as plant available (Johnson et al. 2003, Dieter et al. 2010). While there was no statistical difference between the STP levels of the unfertilized plots (Table 3.5), there were differences in terms of which extractable fractions exhibited spatial dependence after fixing for the effects of soil properties (Table 3.8). At location Low 1 the NaHCO3 extracted fraction was spatially autocorrelated and at Low 2 the HCl extracted fraction was spatially autocorrelated (Table 3.8).

At Low 2 several fractions in the subsurface exhibit spatial dependence (Table 3.8). While somewhat unclear as to why, there is obvious evidence of landscape factors leading to P_o redistribution. As such it is very apparent there is in fact an unidentified spatial component to the field scale distribution of sequentially extracted P_o beyond what can be explained by changes in soil properties. In the future, detailed exploratory spatial data analysis and geovisualization techniques could be applied to formulate specific hypotheses related to the apparent spatial structure within this data. While the likely cause of the variability is the landform itself, by examining the data and its structure, it should be possible to develop specific hypothesis related to how pedogenic processes and management factors could contribute to controlling the ultimate distribution of the itinerant P_o fractions. These hypotheses, when tested, should be the next step in the progression of identifying spatial significance, determining the cause of that significance, and using that understanding to more efficiently model P_o distributions across landscapes.

After reviewing the data collected it became apparent spatial dependence among some sequentially extracted P_o fractions was confirmed at multiple locations, conforming ($H1_a$). This outcome was in contradiction to ($H0_a$). $H1_b$ states that there is spatial dependence among sequentially extracted P_o fractions after removing potential spatial dependence associated with changes in soil properties. Likewise $H1_b$ was confirmed.

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Tables

Table 3.1 MANOVA for fertility levels surface samples

Criterion	Test Statistic	F	Numerator	Denominator	P
			Degree Freedom		
Wilks’	0.43631	37.143	4	115	≤0.000*
Lawley-Hotelling	1.29193	37.143	4	115	≤0.000*
Pillai’s	0.56369	37.143	4	115	≤0.000*
Roy’s	1.29193				
s = 1		m = 1.0		N = 56.5	

* indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 3.2 MANOVA for fertility levels subsurface samples

Criterion	Test Statistic	F	Numerator	Denominator	P
			Degree Freedom		
Wilks’	0.41976	39.742	4	115	≤0.000*
Lawley-Hotelling	1.38234	39.742	4	115	≤0.000*
Pillai’s	0.58024	39.742	4	115	0.000*
Roy’s	1.38234				
s = 1		m = 1.0		N = 56.5	

* indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 3.3 Summary of Bonferroni corrected one-way ANOVA of soil test values

Horizon	STP	STCa	STAl	STFe
Surface	≤0.005	≤0.005	≤0.005	≤0.005*
Subsurface	≤0.005	≤0.005	≤0.005	≤0.005*

* indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 3.4 Descriptive statistics

Variable	Location	N	Mean	SE Mean	St Dev	Median
Surface Horizon Thickness	High 1	30	5.85	0.342	1.87	5
	High 2	30	9.58	0.833	4.56	9
	Low 1	30	7.17	0.254	1.39	7
	Low 2	30	4.72	0.203	1.11	4.75
Surface Horizon STAl	High 1	30	277	8.41	46.1	272
	High 2	30	152	6.18	33.8	158
	Low 1	30	120	4.54	24.9	117
	Low 2	30	168	7.48	41.0	163
Surface Horizon STCa	High 1	30	4170	229	1260	3750
	High 2	30	1980	66.3	363	1940
	Low 1	30	837	46.0	252	788
	Low 2	30	743	35.3	194	715
Surface Horizon STP	High 1	30	1020	58.4	320	962
	High 2	30	229	13.4	73.6	228
	Low 1	30	15.2	1.92	10.5	12.7
	Low 2	30	12.9	0.985	5.39	12.1
Surface Horizon % Sand	High 1	30	0.248	0.005	0.0290	0.250
	High 2	30	0.258	0.009	0.051	0.252
	Low 1	30	0.412	0.026	0.1402	0.430
	Low 2	30	0.193	0.006	0.032	0.195
Surface Horizon STFe	High 1	30	16.1	0.577	3.16	15.7
	High 2	30	29.4	4.90	26.8	17.6
	Low 1	30	16.2	1.52	8.32	14.7
	Low 2	30	57.2	7.29	38.6	45.3
Subsurface Horizon STCa	High 1	30	1620	62.8	344	1630
	High 2	30	3680	257	1410	3020
	Low 1	30	523	35.2	193	482
	Low 2	30	365	40.9	224	308
Subsurface Horizon pH ($CaCl_2$)	High 1	30	5.7	0.059	0.323	5.70
	High 2	30	5.97	0.060	0.326	6.04
	Low 1	30	4.97	0.043	0.234	4.98
	Low 2	30	4.51	0.053	0.292	4.52
Subsurface Horizon STAl	High 1	30	162	8.03	43.96	148
	High 2	30	329	12.8	70.0	332
	Low 1	30	136	6.54	35.8	126
	Low 2	30	221	18.3	100	199
Subsurface Horizon % Sand	High 1	30	0.259	0.011	0.059	0.271
	High 2	30	0.278	0.008	0.046	0.278
	Low 1	30	0.428	0.021	0.115	0.475
	Low 2	30	0.208	0.007	0.038	0.211
Subsurface	High 1	30	18.7	0.931	5.10	18.0

Variable	Location	N	Mean	SE Mean	St Dev	Median
Horizon STFe	High 2	30	26.4	5.12	28.1	15.1
	Low 1	30	12.0	1.29	7.04	9.48
	Low 2	30	43.6	5.02	26.5	35.8
Subsurface Horizon % Carbon	High 1	30	2.06	0.111	0.608	2.04
	High 2	30	2.61	0.141	0.774	2.60
	Low 1	30	1.83	0.117	0.642	1.66
	Low 2	30	1.50	0.103	0.564	1.36

Table 3.5 Summary groupings from one-way ANOVA of soil test values

Horizon	Location	STP	STCa	STAl	STFe
		Group	Group	Group	Group
Surface	High 1	B	B	B	B
	High 2	A	A	A	B
	Low 1	C	C	C	A
	Low 2	C	C	B	B
Subsurface	High 1	B	B	C	B
	High 2	A	A	A	BC
	Low 1	C	C	C	C
	Low 2	C	C	B	A

Treatments with same grouping letter are not significantly different

Table 3.6 Stepwise regression of soil properties for surface samples with STP as the response, and Mehlich 1 extractable Fe, Al, and Ca; % soil carbon; % sand, silt, and clay; surface horizon thickness; and pH in water and in CaCl as the predictors

Alpha-to-enter: 0.15 Alpha-to-remove: 0.15 Response is STP, 10 predictors, n = 120

Step	1	2	3	4
Constant	-215.2	-349.5	-268.7	-337.6
STCa	0.2762	0.2398	0.2464	0.2467
T-Value	34.90	23.64	23.94	24.31
P-Value	≤0.000**	0.000	0.000	0.000
STAl		1.14	0.96	1.04
T-Value		5.08	4.13	4.48
P-Value		≤0.000**	0.000	0.000
Thickness			-8.9	-9.4
T-Value			-2.46	-2.63
P-Value			0.015**	0.010
% Sand				207
T-Value				2.09
P-Value				0.038**
S	133	121	118	116
R-Sq	91.17	92.77	93.13	93.38
R-Sq (adj)	91.09	92.64	92.95	93.15

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10. The adjusted R-squared compares the explanatory power of regression models. The predicted R-squared indicates how well a regression model predicts responses for new observations. S represents the average distance that the observed values fall from the regression line

Table 3.7 Stepwise regression of soil properties for subsurface samples with STP as the response, and Mehlich 1 extractable Fe, Al, and Ca; the soil carbon; sand, silt, and clay percentages; surface horizon thickness; pH in water and in CaCl; and STP levels of the surface horizon as predictors

Alpha-to-enter: 0.15 Alpha-to-remove: 0.15 Response is STP, 11 predictors, n = 120

Step	1	2	3	4	5	6	7
Constant	-18.97	-56.69	367.23	187.45	139.52	80.09	98.01
Surface STP	0.977	0.736	0.687	0.577	0.561	0.537	0.515
T-Value	56.00	13.43	13.82	10.69	10.89	10.42	9.87
P-Value	≤0.000**	≤0.000**	≤0.000**	≤0.000**	≤0.000**	≤0.000**	≤0.000**
STCa		0.074	0.118	0.129	0.135	0.136	0.146
T-Value		4.60	7.20	8.26	9.06	9.28	9.50
P-Value		≤0.000**	≤0.000**	≤0.000**	≤0.000**	≤0.000**	≤0.000**
pH in $CaCl_2$			-90	-67	-71	-51	-50
T-Value			-5.56	-4.11	-4.60	-2.92	2.90
P-Value			≤0.000**	≤0.000**	≤0.000**	0.004**	0.005**
STAl				0.353	0.393	0.379	0.411
T-Value				4.03	4.69	4.59	4.95
P-Value				≤0.000**	≤0.000**	≤0.000**	≤0.000**
% Sand					199	189	204
T-Value					3.67	3.56	3.84
P-Value					≤0.000**	0.001**	≤0.000**
Thickness						-5.2	-6.1
T-Value						-2.35	-2.74
P-Value						0.021**	0.007**
% Carbon							-18.5
T-Value							-1.95
P-Value							0.053*
S	84.6	78.2	69.8	65.6	62.3	61.1	60.3
R-Sq	96.37	96.93	97.58	97.88	98.10	98.19	98.25
R-Sq (adj)	96.34	96.88	97.51	97.80	98.02	98.09	98.14

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10. The adjusted R-squared compares the explanatory power of regression models. The predicted R-squared indicates how well a regression model predicts responses for new observations. S represents the average distance that the observed values fall from the regression line.

Table 3.8 Mantel and Partial Mantel tests for spatial structure in surface samples

	High 1		High 2		Low 1		Low 2	
	Surface	Subsurface	Surface	Subsurface	Surface	Subsurface	Surface	Subsurface
DDI H_2O P_o	0.28047	0.65123	0.78742	0.53695	0.89601	0.61504	0.68513	0.33227
0.5 M $NaHCO_3$ P_o	0.41676	0.63874	**0.02040** 0.01940 **	0.36716	**0.00060** 0.00170 **	**0.00030** 0.00910 **	0.75682	0.64454
0.1 M NaOH P_o	0.76712	0.15368	**0.02620** 0.48215	0.41696	0.69003	**0.00010** 0.02640 **	0.95960	0.68943
1.0 M HCl P_o	**0.03920** 0.03790 **	0.57274	0.10739	0.13719	**0.05859** 0.26907	**0.00290** 0.01270 **	**0.01560** 0.01550 **	**0.05749** 0.36746

Values in bold indicate significant mantel correlations, values identified with ** indicate a significant ($P \leq 0.05$) after controlling for the contribution of soil properties identified by stepwise regression.

Chapter 4. Topographic Influence on the Movement and Transformation of Organic Phosphorus in Hay and Grass Pastures of Eastern West Virginia

Abstract

This study was conceived to investigate the fate of organic P (P_o) in typical hay and grass pasture of eastern West Virginia following long-term annual applications of animal manure, often on a N basis. Over the past decade, P management has evolved on many West Virginia farms from N-based manure management towards using tools such as a P index. At present, many hay and grass pastures in the region have a high degree of P saturation and, at some locations, additional application may be occurring. As environmental regulations tighten there are expectations that remediation and interception strategies at some locations may be needed. Given that most P research focuses on P loss via surface erosion, on tile drained land, and rarely on P_o there is a clear deficit in knowledge. The capacity to predict how P_o moves is a potentially useful tool in evaluating risk of loss and for developing remediating strategies. Sequentially extracted P fractions derived from spatially explicit samples can be used to better understand P_o movement at field scales. In this research a Pearson's product-moment correlation matrix was used to determine what, if any, topographic variables were significantly correlated to selected sequentially extracted P_o fractions. Stepwise regression was further used to identify variables specific to each location and each fraction. Partial Mantel tests were then used to determine if the remaining variability could be explained. Specifically, it was hypothesized that the pure spatial portion of the variability in sequentially extracted P_o fractions could be explained by topographic variables. This only proved to be the case in locations with histories of long-term N-based manure applications. However, identifying explanatory topographic variables can still be a strategic component in developing field-scale predictive models of P_o distributions that could prove invaluable in risk assessment, remediation, and model development.

Introduction

Plant nutrients applied in excess of crop removal rates allow some nutrients to accumulate (Sims et al., 2002; Johnson et al., 2005). When concentrated animal agriculture occurs and P is applied at rates in excess of plant needs, the soil becomes saturated with P and may pose a risk to water quality (Beck et al., 2004). As more fields become saturated with P, efforts of lower saturation and prevent P loss may become a greater priority. To effectively address this situation, it will require strategies that incorporate topographic data to effectively model field scale P movement.

Conceptually, the idea of relating soil properties to terrain can be traced to the catena concept (Milne 1935) and the belief that soils differentiate in predictable ways along toposequences. This concept was refined by Jenny (1941) who developed the idea of factors of soil formation. Jenny (1941) concluded that soil properties were related to a series of factors: climate, organisms, relief, parent material, and time. In particular, topography directs the movement of water. Jenny (1941) believed relief or topography was responsible the majority of the variability seen in soils at landscape scale. Today, many studies have included topographic elements when modeling soil moisture and other properties (Moore et al., 1991; Hornberger & Boyer, 1995; Iverson et al., 1997; Famiglietti et al. 1998; Boerner et al., 2000; Gessler et al., 2000; Western et al. 2001; Mohanty and Skaggs 2001; Case et al., 2005). Accordingly, there is reasonable expectation of changes in soil properties directly related to the changes in the mechanistic process that occur differentially across the landform.

When examining P distributions one must consider the possible effects of changing soil properties. If the ability of the soil profile to retain P is, in part, related to changes in soil properties, then as these properties change so changes the P exchange dynamics of the soil. A soil’s ability to retain P is related to the soil surface chemistry, reactivity, Fe and Al content, clay content, and pH (Barrow, 1984; Fox, 1985; McGechan and Lewis, 2002). Over time, management can modify soil properties and, by extension, P sorption characteristics, which may increase P translocation, through the soil profile (Hao et al., 2008). Specifically, the continual additions of poultry manure shifts P from binding with Fe and Al products to binding with Ca (Sharpley et al., 2004). The soluble nature of some of these Ca-P complexes under some conditions could contribute to potential P translocation within the soil profile (Holford et al., 1997; Siddique and Robinson, 2003). As such, it is critical to consider the variability in soil properties within a management unit when evaluating the mobility of any portion of the P pool.

Much of the P-soil-environment research has focused on P_i or total P (Laboski, and Lamb, 2003; Anderson and Magdoff, 2005; Loria and Sawyer, 2005; Casson, et al., 2006; Haden et al., 2007). However, P_o is a significant part of the total soil P pool. For example, Jencks et al. (1964) examined various P fractions in a selection of soils across West Virginia and found P_o accounted for between 7 and 66% of the total P in the surface horizons, and from 13 to 55% in the subsurface horizons. While P_i leaching is generally considered of minimal risk, soils receiving large quantities of P fertilizer, sandy soils, organic soils, well structure soils, and soils with high rock fragment content can be at risk of P leaching

(Anderson and Magdoff, 2005; Harman et al., 2013). A greater understanding of potential P_o movement and soil interactions is needed (Condron et al., 2005).

In terms of water movement, it is known that soils with high percentages of rock fragments can rapidly infiltrate to some depth via preferential flow processes (Harman et al. 2011). Likewise, research has shown that in this environment (hay and pasture lands of eastern West Virginia) there is P translocation within the soil profile (Harman et al. 2013). The literature indicates that downslope receiving positions can in some conditions soils test considerably higher for P than stable upland positions due to the movement of P downslope (Porder et al. 2005). This is consistent with the assertions of Smeck (1973) and Smeck and Runge (1971) when they proposed that P can move latterly within a landscape, and will accumulate in lower landscape positions. This is an indication of the importance of understanding the landscape, water movement, and topographic data.

Quantitative topographic data for use in soil-landscape analysis and modeling is most often obtained from digital elevation models (DEM). These DEM-derived land surface parameters can be classified as primary or secondary (sometimes called compound) terrain attributes (Moore et al., 1991; Thompson et al., 1997; Bishop and Minasny 2006). Using a computer, the most easily estimated primary attributes would include slope gradient, slope aspect, slope curvature, drainage direction, and drainage area (Moore at al. 1991). A complete list of primary terrain attributes was published by Speight (1974, 1980). The most common secondary attribute is the topographic wetness index (TWI) (Bishop and Minasny 2006; Grundwald 2006). Secondary attributes are often more useful than primary attributes for predicting soil properties (Bell et al. 1994; Gessler et al., 1995; McBratney et al., 2000; Bishop and Minasny 2006). TWI describes the tendency of a cell to accumulate water (Gruber and Peckham, 2009). TWI is defined as the ln (A / tan B) where A is the local upslope contributing area for that point and B is the local slope (Beven and Kirkby, 1979). Up slope contributing area is estimated by a/L, with a being the number of pixels draining into a pixel, multiplied by the area of a pixel, and L as the pixel width (Moore et al., 1991). A similar secondary terrain attribute, stream power index, was developed to be used to describe erosion and related landscape processes, and is defined as A × (tan B) (Moore et al., 1991).

There are many techniques that can be used to determine flow direction and each has the potential to calculate a unique outcome. Flow direction determines flow accumulation, which establishes the upslope contributing area. The earliest and simplest flow direction calculation is the deterministic 8 (D8) of O’Callaghan and Mark (1984). D8 specifies a single flow direction from each raster cell into one of the eight neighboring cells based on the steepest downward slope (O'Callaghan and Mark, 1984). Another method is the multiple flow direction (MFD) method (Quinn et al., 1991). MFD divides flow between all down slope cells based on slope gradient (Tarbonton, 1997). A third method, which is a compromise between D8 and MFD, is the deterministic infinity method ($D\infty$) of Tarboton (1997). The $D\infty$ method divides a 3×3 grid into eight triangular facets, and allocates flow to the steepest direction, where by allocating flow solely to one cell or dividing it based on slope gradient between the cells that define the downslope facet (Tarbonton, 1997).

P_o fractions move through the soil at different rates (Anderson and Magdoff, 2005), and soil P fixation varies with changes in soil properties as soils vary across the landscape (Daniels et al., 2001; Borling et al., 2004; Herlihy and McGrath, 2007). Thus P_o levels at any location are the product of the soils properties, P_o additions, P_o transformations and any landscape-scale process that move P or soil over or through the landscape. In fact, compound topographic variables have been successfully used to explaining variability in STP data at field scale (Moore et al., 1993). If accepted that water can move P_o and erode surface soils while statistically accounting for the variability in the P_o levels associated with changes in soil properties, any residual pattern must be related to space or a physical process dependent upon the configuration of that space such as the movement of water. This necessitates not only the consideration of topographic variables when modeling P_o fractions across a landform or management unit, but closer attention to the location of high and low values during data processing.

It is assumed that spatial dependence identified in selected P_o fractions not explained by changes in management practices, soil properties or sub field level management units are primarily due to water movement. Thus the research hypothesis (Ha) states: Spatial dependence in sequentially extracted P_o fractions can be sufficiently explained via compound topographic variables, TWI and SPI such that the remaining variability will be randomly distributed across management units. The null hypothesis (Ho) states with the inclusion of compound topographic variables, TWI and SPI will not render the remaining spatial variability randomly distributed across the management units.

Materials and Methods

Site Selection

The study sites were hay or grass pasture fields typical of eastern West Virginia. There were two fertilized locations with histories of annual N-based manure applications (High 1 and High 2), and two unfertilized locations with very infrequent manure applications (Low 1 and Low 2). The unfertilized locations typically received manure application approximately once every 5 to 10 yr. For more information on the composition of these locations see Table 4.1.

Compound Topographic Indices

All terrain attributes were calculated from 3-m resolution (DEM) data from United States Geologic Survey (USGS) using SAGA GIS (Bock et al., 2008). The DEM was extracted for each field, and was preprocessed to remove any sinks or voids (Wang and Liu, 2006). From these DEM, three flow direction grids were calculated: A D8 grid (O'Callaghan & Mark, 1984), a MFD grid (Quinn et al., 1991), and a D∞ grid (Tarboton, 1997). From each of these grids, upslope contributing area grids were developed using the recursive upslope method and then both TWI and SPI grids were created. In addition to these six compound indices (MFD TWI, D8 TWI, D∞ TWI, MFD SPI, D8 SPI, and D∞ SPI), two additional indices were constructed, SAGA wetness index (TWI_s) and modified SPI (SPI_m). TWI_s uses a modified catchment area calculation to better represent water dispersions in low slope areas (Boehner et al., 2002). SPI_m is the same SPI calculation but it is generated using the modified catchment area calculation of the TWI_s.

Sample Locations

A stratified random design was used to select sample points (Thompson et al., 2006). The stratification variable was the most basic TWI the D8-based TWI (Moore et al., 1991). Three equal sized TWI classes were specified and ten samples locations were selected randomly within each class. In the field, two soil samples were collected at each sample location: one sample the surface A or Ap horizon and one from the 10 cm immediately below. Samples were air dried, ground, sieved (2-mm sieve), and thoroughly mixed (Laboski and Lamb, 2003).

Laboratory Methods

The pH of the soil samples was measured in distilled deionized water (DDI) and 0.01 M $CaCl_2$ (Eckert and Sims, 1995). Samples were sequential fractioned based on a suggested modification (Sui et al., 1999) of the Hedley method (Hedley, et al., 1982) as described by He et al (2003). Mehlich-1 extractable Al, Ca, Fe, and P content (Nelson et al., 1953) were determined with a Perkin Elmer P4000 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Particle size distribution was determined by the pipette method (Gee and Bauder, 1986). Total soil carbon was measured in a LECO TruSpec CHN elemental analyzer (LECO Corp., St Joseph, MI), where soil carbon total is measured by dry combustion (Keene, 2010).

Data Analysis

Values for all terrain attributes were extracted for each of the sample locations. The combined data was than exported as .DBF files for analysis in Minitab version 16 (Minitab Inc. 2012) and PASSaGes version 2.0 (Rosenburg and Anderson, 2011).

Unforeseen spatial factors can contribute to the data variability. To reduce these likelihood elements of exploratory spatial data analysis (ESDA) and geovisualization (GV) techniques were incorporated to identify spatial outliers and enhance the understanding of this data (ESRI, 2011). ESDA was used to visualize spatial distributions of data and identify potential unforeseen spatial factors leading to atypical locations, spatial clusters, spatial regions, or forms of spatial instability or non-stationary within the data (Anselin 1996, 1998a, 1998b). In brief, the STP levels were analyzed for spatial clusters, particularly hotspots (locations with clusters of high values) using the Getis-Ord Gi* statistic (Getis and Ord, 1992). This analysis examines the Z-scores of the variables relative to the surrounding data points and compared to the entire data set (Mitchell, 2005). When hotspots were identified the aerial images of the locations were reviewed along with the history of the sample locations. If there was a possibility of an external factor contributing to the elevated values, the data in the hot spot was considered a spatial outlier and excluded. Additionally, each location was examined in 2D to determine if there was a need to be subdivided or separated based on the complexity of the landform. Accordingly, the sample points were categorized (as landform units) within each location by any landscape position or change in slope aspect that dramatically inhibits uniform downslope movement of nutrients.

Following the ESDA, any sequentially extracted fraction-horizon-location combinations with spatial outliers were re-examined. Specifically, stepwise regression was used to identify measured soil properties that could explain a significant portion of the variability. Using alpha to enter and leave values of 0.15 the Mehlich 1 extractable Fe, Al, and Ca; soil carbon; sand, silt, and clay percentages; surface horizon thickness; and pH in water and in CaCl of the surface horizon samples were examined. For the subsurface horizon samples, the Mehlich 1 extractable Fe, Al, and Ca; the soil carbon; sand, silt, and clay percentages; surface horizon thickness; pH in water and in CaCl; and STP levels of the surface horizon samples were examined. Next the data was examined to determine if the data retained a purely spatial component in the data variability. Subsequently all reaming sequentially extracted fraction-horizon-location combinations with a purely spatial component in their data variability were examined in detail. Specifically, compound topographic variables were selected to include in a stepwise regression based on a Pearson's product-moment correlation matrix. S selected soil properties and the compound topographic variable with the strongest correlation were entered into a stepwise regression with p values to enter and to remove of 0.05 to determine what if any soil and compound topographic properties explained a significant portion of the data variability.

To examine the role of space in the structure of the data variability, Mantel and partial Mantel tests were used to fix for the effects of soil and topographic variables. The Mantel test is a permutation based correlations analysis with one matrix being the difference between P_o values of each pair of points, the other a distance matrix between points. A correlation coefficient is calculated between these matrices. The values of one matrix are randomly reassigned to another spatial location, and the analysis repeated. After 10,000 permutations, a distribution of correlation coefficients is generated. Probabilities can be estimated, based on the data's position within the permutated distribution (Bonnet and Van de Peer. 2002). One extension of the Mantel techniques is the partial Mantel test. A partial Mantel test uses more difference or distance matrices. In a partial Mantel test, two variables are compared while fixing for the effect of a third matrices of a third variable or group of variables. Similarly, after permutation the probabilities can be estimated, based on the data's position within the generated distribution (Bonnet and Van de Peer. 2002). Selected sequentially extracted fraction-horizon-location combinations were examined with a series of Mantel tests to determine if the selected sequentially extracted fraction-horizon-location combinations exhibited spatial structure in the data variability after fixing for the effect of soil properties, compound topographic indices, and sub field level delineations.

Results and Discussion

Of the 32 possible combinations of horizon, location, and sequential extracted P_o fraction examined in chapter 3, seven had significant partial mantel correlations indicating spatial structure beyond that explained by changes in soil properties identified as explaining a significant portion of the variability in STP data (Table 4.1). The next analysis included a review of the data using ESDA techniques. Next each location was examined using the Getis-Ord Gi* technique (Figs. 4.1, 4.2, 4.3 and 4.4), which identified multiple hotspots among the locations. Each hotspot was examined in detail to determine if the hotspots could be attributed to additional management factors.

At location Low 2, it was determined that three sample points (Fig. 4.5) near the entrance to the pasture formed a hotspot potentially associated with land use and management. When viewed against the field imagery, it seemed apparent the two data points located closest to the entrance were potentially spatial outliers. Two of the three data points were very near the entrance. It is possible this location was a natural bottleneck in cattle traffic and this location has been used as a winter feeding area. The third data point that made up the hotspot while having a higher STP value, it was farther away from the entrance to the pasture. Thus the two data points nearest the entrance were considered spatial outliers and excluded them from the analysis. When the location was reexamined, it no longer exhibited the underlying spatial dependence noted in chapter 3. Hence moving forward only the remaining six possible combinations of horizon, location, and sequential extracted P_o fraction were examined.

When reviewed it became apparent there was a need for additional division of the management units (the fields) at each location. At location High 1, it became apparent the field had three distinct regions, a summit position, and a sub field drainage exiting the field midslope. (Fig. 4.6). Sample points were categorized accordingly. Location High 2 also had a complex configuration. At High 2 the field was composed of two basic elements, a very flat footslope and a series of convex and concave backslopes. Most of the data points appeared hydrologically connected (based on the topography) however a sub set of the data that appear to drain away from the majority of the data. Accordingly, the field was into two groups (Fig. 4.7). At location Low 1 the field was composed of four basic elements, a summit, two backslopes and a footslope position. The sample points in the summit drained into one of two backslopes, which converged at the footslope of the landscape. The field was divided into three parts. The first two parts were the two backslopes with the associated points at the summit/shoulder area. The remaining data points at the footslope were grouped together (Fig. 4.8).

Initially, the Pearson product-moment correlations were calculated between the compound topographic indices (Table 4.3) and each of the six unique combination (UC) of horizon–location–P_o fraction identified in chapter 3 (Table 4.2). Stepwise regression of each UC was used to identify the variables that best explained the P_o data. Next, partial Mantel tests were used to determine if the soil variables explained all the spatial variability in the data to an extent that it eliminated the correlation of the dissimilarity matrices and void of identifiable spatial structure. The partial Mantel test was performed again fixing for the effect of soil properties and the compound topographic indices with the strongest linear relationship to each UC. Similarly, partial Mantel tests were calculated including a variable of in-field division (IFD), and with the combination of compound topographic indices and IFD.

Location Fertilized 1 is a pasture and hay field that has received annual poultry manure applications on a N-basis for more than 30 yr. Long-term manure application has been documented to cause total P concentration in the top 5 cm to increase as much as 2.8 to 5.5 times (Koopmans et al., 2007). Location Fertilized 1-surface horizon-1.0 M HCl extractable P_o fraction has a significant partial Mantel correlation indicating an underlying spatial structure within the data (Table 4.1). P fertilization mostly increases the labile and moderately labile inorganic soil P fractions (Oniani et al., 1973; Blake et al., 2003) with minimal effect on P_o levels except the most labile P_o fraction, such as the $NaHCO_3$ extractable P_o fraction (Pätzold 2013). Pedogenic transport processes can govern the distribution and forms of P at field scale (Heilmann et al., 2005). As such, recognizing connectivity within a complex landform is important given significance of movement mechanisms when evaluating P loss and movement (Davies et al 2006). As such the subdivision of data points in to three subsets for analysis seems prudent.

The Mantel correlation from the 1.0 M HCl extractable P_o fraction of Fertilized 1 was p = 0.038 (Table 4.2). The Pearson's product-moment correlation analysis identified TWI_s as the topographic index with the strongest linear relationship to the 1.0 M HCl extractable P_o fraction (see Table 4.3), with a correlation of -0.576 and a p value = 0.001. The stepwise regression of 11 predictors (TWI_s, horizon thickness, STAl, STFe, STCa, % Carbon, % Sand, % Silt, % Clay, pH in H_2O and pH in 0.01M $CaCl_2$) for 1.0M HCl extracted P_o identified STCa and TWI_s as the only predictors that explained a significant portion of the variability (Table 4.4). Poultry manure can raise P, N, and Ca, levels in soil, as well as elevate the levels of bicarbonates and organic acids with carboxyl and phenolic hydroxyl groups (Sharpley et al., 2004). P bound to Ca can be a significant fraction within some soil (Amaizah et al., 2012). STCa explained 55.9% of the variability. Together, STCa and TWI_s explained 61. 6% of the variability in the data. The partial Mantel tests indicated significant spatial structure (Table 4.5) in the 1.0 M HCl extractable P_o fraction when fixing for the effects of soil properties (p=0.038), soil properties with TWI_s (p=0.037), and soil properties with IFD (p=0.090). When fixed for the effect of STCa, TWI_s, and IFD, the residuals were no longer spatially autocorrelated (p=0.133) indicating the identifiable patterns of P distribution were likely related to changes in soil properties and water movement / soil moister conditions.

Location Fertilized 2 is a pasture and hay field that has received annual poultry manure applications on a N-basis for more than 30 yr. Location Fertilized 2-surface horizon-0.5 M $NaHCO_3$ extractable P_o fraction has a significant partial Mantel correlation indicating an underlying spatial structure within the data (Table 4.2). P losses are often driven by fast transport processes such as surface runoff, shallow interflow, and macropore flow in close interaction with P enriched topsoil layers resulting in high P concentrations in along these rapid pathways, particularly in permanent grassland with histories of P accumulation (Schärer et al. 2007). P extracted with $NaHCO_3$ is a labile P fraction can contribute to the nutrient supply to plants and can be transferred to the surrounding environment by moving through the soil profile (Pizzeghello et al., 2011; Schmitt et al., 2014).

The Mantel correlation from the 0.5 M $NaHCO_3$ extractable P_o fraction from the surface horizon of Fertilized 2 was p = 0.019 (Table 4.2). The Pearson's product-moment correlation analysis identified TWI_s as the topographic index with the strongest linear relationship to the 0.5 M $NaHCO_3$ extractable P_o fraction (Table 4.6) with a correlation of 0.450 and a p value = 0.014. The stepwise regression of 11 predictors (TWI_s, horizon thickness, STAl, STFe, STCa, % Carbon, % Sand, % Silt, % Clay, pH in H_2O, and pH in 0.01M $CaCl_2$) for the 0.5 M $NaHCO_3$ extractable P_o fraction identified TWI_s as the only predictor that explained a significant portion of the variability, with TWI_s explaining 17.26% of the variability in the data (Table 4.7). While 17.26% would seem to be a small percentage of the total variability, the compound topographic variable in location High 1 only accounted for 5.7%. While that model accounted for 61.4%. The partial Mantel tests indicated a significant correlation between the dissimilarity matrices (Table 4.5) in the 0.5 M $NaHCO_3$ extractable P_o fraction when fixing for the effects of TWI_s (p=0.01350), and for IFD (p=0.09749). When fixed for the effect of TWI_s and IFD the residuals were no longer exhibited spatial structure (p=0.12479). Any number of factors (slope position, soil order, management, and weather condition) can affect the concentration and proportions of P fractions (Wagar et al., 1986; Heilmann et al., 2005; Negassa and Leinweber, 2009).

The Mantel correlation from the 0.5 M $NaHCO_3$ extractable P_o fraction from the surface horizon of Unfertilized 1 was p = 0.002 (Table 4.2). The Pearson's product-moment correlation analysis identified D8 SPI as the topographic index with the strongest linear relationship to the 0.5 M $NaHCO_3$ extractable P_o fraction (Table 4.8) with a correlation of 0.341 and p value = 0.065. The Mantel correlation from the 0.5 M $NaHCO_3$ extractable P_o fraction from the subsurface horizon of Unfertilized 1 was p = 0.00910 (Table 4.2). In general it is expected to find elevated NaHCO3 extractable P levels when soils are fertilized (Haynes and Williams, 1992) and the NaHCO3 fraction is labile and under some conditions can be considered plant available (Johnson et al. 2003, Dieter et al. 2010).The Pearson's product-moment correlation analysis identified MFD SPI and SPI_m as the topographic indices with the strongest linear relationships to the 0.5 M $NaHCO_3$ extractable P_o fraction (Table 4.8) with a correlation of 0.451 and p value = 0.012. Given the labile nature of NaHCO3 fraction this was not unexpected.

The Mantel correlation from the 0.1 M NaOH extractable P_o fraction from the subsurface horizon of Unfertilized 1 was p = 0.026 (Table 4.1). The Pearson's product-moment correlation analysis identified TWI_s as the topographic index with the strongest linear relationship to the 0.1 M NaOH extractable P_o fraction (Table 4.8) with a correlation of 0.290 and p value = 0.121. In general, NaOH extracted organic fractions are immobile sorbed onto clay minerals or precipitated with metals oxides (Gagnon et al.

2012). As such, under conditions with minimal P saturation, it is less likely the NaOH extractable fractions would be strongly correlated with metrics for water movement. However, the Mantel correlation from the 1.0 M HCl extractable P_o fraction from the subsurface horizon of Unfertilized 1 was p = 0.013 (Table 4.1). The Pearson's product-moment correlation analysis identified D8 SPI as the topographic index with the strongest linear relationship to the 1.0 M HCl extractable P_o fraction (Table 4.8) with a correlation of 0.540 and p value = 0.004.

P availability following manure application can also be influenced by microbial properties of the soil, the makeup of the manure, and any number of complex interactions between these components (Waldrip et al. 2011). Stepwise regression was used to determine the variables that explained a significant portion of the variability in each fraction–horizon combination with known spatial correlation. The stepwise regression of 11 predictors (TWI_s, horizon thickness, STAl, STFe, STCa, % Carbon, % Sand, % Silt, % Clay, pH in H_2O and pH in 0.01M $CaCl_2$) identified horizon thickness as significant for the 0.5 M $NaHCO_3$ extractable P_o fraction from the surface (Table 4.9) and subsurface (Table 4.10) horizons of Unfertilized 1. Likewise, via regression depth of horizon, % carbon, STAl, STFe, % silt, and pH that explained significant portions of the variability in the 0.1 M NaOH extractable P_o fraction from the subsurface horizon of Unfertilized 1 (Table 4.11) were determined. The more labile nature of some of these Ca-P complexes following long-term manure applications could contribute to potential P translocation within the soil profile (Holford et al., 1997; Siddique and Robinson, 2003). It is believed this is likely due in part to the elevated organic matter concentration, the formation of dissolved organic P species, and colloid mediated transport facilitated by association dissolved organic carbon (Gerke, 1992; Dolfing et al., 1999; Ilg et al., 2005; Koopmans et al 2007). Similarly, horizon depth was the only variable that explained a significant portions of the variability 1.0 M HCl extractable P_o fraction from the subsurface horizon of Unfertilized 1 (Table 4.12).

Conclusions

Understanding how topographic variables and sequential extracted P_o fractions relate is important to the understanding of landscape-scape scale P movement. While P is generally relatively insoluble in soil, considerable movement can occur over time (Smeck, 1973). P inputs elevate labile organic P pools regardless of the type of P input (Guggenberger et al) and P_o fractions can be as much as 20–30% of the total P (Amaizah et al., 2012). The primary objective of this research was to identify an optimal topographic variable to explain the variability in P_o levels not explained by management practices, changes in soil properties, or sub field level delineations. The relationship between P fractions and environmental loss is related to the susceptibility to runoff and the distance to waterways (Negassa and Leinweber 2009), and reducing elevated P concentrations to more environmentally acceptable levels by cession of P applications could take decades or more (Dodd et al., 2012). To facilitate an immediate response or reduce imminent loss, the use of these topographic variables could be fundamental to the predictive capabilities needed to identify potential points of P egress.

The results from the locations with lengthy histories of poultry manure applications were different but explainable. Smeck and Runge (1971) indicated that the distribution of P across a landscape was a good indicator of past water movement. Logically this relationship should prove useful when looking for patterns in P distribution. Very different behaviors between TWI and the 1.0 M HCl extractable P_o fractions (a negative correlation) and the more labile 0.5M NaHCO3 P_o fraction (positive correlation) at locations with histories of N-based manure applications were identified. One would expect to find the labile P_o fractions will be highest in the lower slope positions (Heilmann et al., 2005) and following continual additions of poultry manure one would expect P to shift from binding with Fe and Al to binding with Ca (Sharpley et al., 2004). Additionally, one would expect the soluble nature of some of these Ca-P complexes to be subject to loss relative to water movement (Holford et al., 1997; Siddique and Robinson, 2003). Overall this is what was confirmed. Topography is guiding the distribution and to some extent the composition of the P_o pool and the determining threshold appears to be relative P status.

The unfertilized location was very different. The unfertilized location had multiple fractions across the surface and subsurface horizons that contained a pure spatial component in their data structure. The same fractions exhibited differing behaviors between horizons and overall there seems to be no definite trend. Still it is interesting that in situations where P is scarce and sorption and consumption is expected to render the collective P distribution somewhat fixed in place, yet some patterns, particularly at depth were still identified. The analysis was able to explain some of these patterns via topographic means. As the unfertilized locations have had poultry manure applications in the past, it could lead one to speculate that these locations may have had significantly higher ST levels at some point in time causing these relict spatial patterns that are not easily interpreted.

Specifically, the research hypothesis (Ha) states: Spatial dependence in sequentially extracted P_o fractions can be sufficiently explained via compound topographic variables, TWI and SPI such that the remaining variability will be randomly distributed across management units. This only proved to be the case in locations with histories of long-term N-based P applications. Dieter et al. (2010) pointed out that the interpretation of all P fractionation results is complicated and of limited practical utility, however there may be significant utility in some fractions at some locations. The null hypothesis (Ho) states with the inclusion of compound topographic variables, TWI and SPI will not render the remaining spatial variability randomly distributed across the management units. Thus Ha is accepted and Ho rejected.

Ultimately it appears the determining factor in which extractable P fraction is subject to movement and redistribution in accordance with spatially predictable parameters is the degree or level of P at the location. Additionally, in several instances a purely spatial component in the data variability was identified. These insights lend themselves to speculation about field scale process of P_o movement. Many soil properties like soil carbon, clay content, and STP have been shown to be strongly correlated with TWI, in particular. As such, it is very likely that TWI alone or in conjunction with a metric for local field-scale P status could directly—and through collinearity with other important variables—explains a significant portion of the spatial variability seen in sequentially extracted P_o fractions.

Unlike the initial analysis in chapter 3 that sought to identify spatial patterns in sequentially extracted P fractions, this chapter's intent was to go one step further and look for the specific soil properties that explain variability in each of the fraction-location combinations and fully describe the sources of the variability. In doing so it became obvious, water related metrics were capable of modeling the spatial variability in the soils with lengthy histories of fertilization with animal manures, primarily poultry manure. This distinction indicates that these terms could be beneficial for modeling these relationships.

Future research should focus on additional compound topographic variables that could prove useful for modeling P_o movement, determining the threshold where P makes the transition from spatial relationships between 0.5$NaHCO_3$ extractable P_o and soil properties, to 1.0M HCl extractable P_o fractions and Ca levels in the soil, and selecting optimal metrics for local field scale P assessment. As agricultural fields in similar landscape to those investigated become saturated with P, the ability to predict or model P_o movement will become critically important, particularly to the poultry producing region of West Virginia. At a minimum, this research supports the use of TWI as a risk assessment tool, and as supporting information for focused remediation. With TWI resource managers could select fields that pose a greater risk for P_o leaching or movement, and target funding for conservation or remediation efforts. In the years to come as environmental regulations tighten and policies change, the ability to effect meaningful change with minimal resources on an immediate time scale will become a necessity. This sort of soil landscape modeling technique could allow a greater portion of the limited financial resources available to be focused on locations with the greatest potential to pollute and by extension generate the greatest savings per unit of funding.

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Tables

Table 4.1Soil Map Units by Study Site

Location	Map Unit Name	Slope Range	Acres	Percent Total
High 1	Berks-Weikert channery silt loams	8 – 15 %	4.7	44.9
	Berks-Weikert channery silt loams	15 – 25 %	5.5	51.8
	Berks-Weikert channery silt loams	25 – 55 %	0.3	3.3
High 2	Berks channery silt loam	8 – 15 %	1.4	21.4
	Berks-Weikert channery silt loams	22 – 55 %	1.1	17.3
	Ernest silt loam	3 – 8 %	2.9	46.1
	Lobdell loam	0 – 3 %	1.0	15.2
Low 1	Blackthorn channery sandy loam	8 – 15 %	8.6	85.8
	Toms silt loam	3 – 8 %	1.4	14.2
Low 2	Berks-Weikert channery silt loams	15 – 25 %	1.2	23.1
	Berks-Weikert channery silt loams	25 – 55%	4.2	76.6

Table 4.2 Location – horizon – fraction combinations with a significant spatial component to the data variability

Location & Horizon*	Fertilized 1 Surface	Fertilized 2 Surface	Unfertilized 1 Surface	Unfertilized 1 Subsurface	Unfertilized 1 Subsurface	Unfertilized 1 Subsurface	Unfertilized 2 Surface
P_o Fraction	1.0 M HCl	0.5 M NaHCO3	0.5 M NaHCO3	0.5 M NaHCO3	0.1 M NaOH	1.0 M HCl	1.0 M HCl
Two-tailed p	0.038	0.019	0.002	0.009	0.026	0.013	0.016**

These combinations were previous identified in chapters 3 as having a purely spatial portion to their variability after fixing for the effects of soil properties significantly related to STP data. ** After removing spatial outliers this location – horizon – fraction combination was no longer spatially significant.

Table 4.3 Pearson's product-moment correlation matrix for sequential extracted P_o fractions from location Fertilized 1

	1.0M HCl	MFD TWI	MFD SPI	D∞ TWI	D∞ SPI	D8 TWI	D8 SPI	TWI$_s$
MFD TWI	0.071							
	0.710							
MFD SPI	-0.345	-1.519						
	0.062**	0.003**						
D∞ TWI	0.054	0.997	-0.505					
	0.778	0.000**	0.004**					
D∞ SPI	-0.338	-0.490	0.988	-0.472				
	0.068*	0.006**	0.000**	0.008**				
D8 TWI	0.060	0.973	-0.468	0.971	-0.441			
	0.752	0.000**	0.009**	0.000**	0.015**			
D8 SPI	-0.374	-0.482	0.990	-0.467	0.989	-0.427		
	0.042	0.007**	0.000**	0.009**	0.000**	0.019**		
TWI$_s$	-0.576	-0.020	0.522	-0.001	0.504	0.012	0.509	
	0.001**	0.914	0.003**	0.994	0.005**	0.950	0.004**	
SPI$_m$	-0.345	-0.521	1.000	-0.507	0.988	-0.470	0.990	0.524
	0.062*	0.003**	0.000**	0.004**	0.000**	0.009**	0.000**	0.003**

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 4.4 Stepwise regression of surface horizon from location Fertilized 1 with 1.0M HCl extracted P_o as the response and Mehlich 1 extractable Fe, Al, and Ca; the soil carbon; sand, silt, and clay percentages; surface horizon thickness; pH in water and in CaCl; and TWI_s as the predictors

Step	1	2
Constant	-259	2900
STCa	0.504	0.370
T-Value	6.14	3.83
P-Value	≤0.000**	0.001**
TWI_s		-505
T-Value		-2.27
P-Value		0.031**
S	560	518
R-Sq	57.4	64.2
R-Sq (adj)	55.9	61.6

Alpha to enter or remove = P≤ 0.05 Response = 1.0M HCl extracted P_o, predictors = 11, n = 30

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10. The adjusted R-squared compares the explanatory power of regression models. The predicted R-squared indicates how well a regression model predicts responses for new observations. S represents the average distance that the observed values fall from the regression line.

Table 4.5 Mantel and partial Mantel correlations all unique combinations with a spatial component in the data variability

Location & Horizon		Fertilized 1 Surface	Fertilized 2 Surface	Unfertilized 1 Surface	Unfertilized 1 Subsurface		Unfertilized 1 Subsurface	Unfertilized 1 Subsurface
P_o Fraction		1.0 M HCl	0.5 M NaHCO3	0.5 M NaHCO3	0.5 M NaHCO3		0.1 M NaOH	1.0 M HCl
Stepwise selected variables (P≤0.05)		STCa TWI_s	TWI_s	Horizon Thickness	Depth to Horizon		Depth to Horizon, %C, STAl, STFe, %Silt, pH	Depth to Horizon
Partial Mantel test Soil properties	correlation	0.174	-na-	0.254	0.184		0.163	0.235
	t	2.21	-na-	3.30	2.472		2.43985	2.95
	Two-tailed p	0.038**	-na-	0.003**	0.012**		0.01570**	0.010**
Partial Mantel test Soil and TI	correlation	0.176	0.168	0.205	0.094	0.093	0.16661	0.123
	t	2.20	2.68	2.98	1.46	1.44	2.48767	1.842
	Two-tailed p	0.037**	0.014**	0.007**	0.155	0.157	0.01601**	0.094*
Partial Mantel test Soil and IFD	correlation	0.136	0.104	0.262	0.233		0.20830	0.301
	t	1.78	1.66	2.84	2.63		2.75	3.14
	Two-tailed p	0.090*	0.097*	0.007**	0.009**		0.008**	0.007**
Partial Mantel test Soil, TI, and IFD	correlation	0.120	0.102	0.198	0.121	0.119	0.206	0.157
	t	1.55	1.57	2.38	1.58	1.56	2.73	1.98
	Two-tailed p	0.133	0.125	0.022**	0.135	0.138	0.010**	0.081*

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 4.6 Pearson's product-moment correlation matrix for sequential extracted P_o fractions from location Fertilized 2

	0.5 M NaHCO3	MFD TWI	MFD SPI	D∞ TWI	D∞ SPI	D8 TWI	D8 SPI	$\mathrm{TWI_s}$
MFD TWI	0.155							
	0.421							
MFD SPI	0.195	-0.201						
	0.311	0.295						
D∞ TWI	0.142	0.994	-0.210					
	0.464	0.000**	0.275					
D∞ SPI	0.198	-0.213	0.999	-0.220				
	0.304	0.266	0.000**	0.251				
D8 TWI	0.169	0.956	-0.155	0.952	-0.164			
	0.380	0.000**	0.423	0.000**	0.394			
D8 SPI	0.201	-0.189	0.999	-0.196	0.999	-0.141		
	0.297	0.327	0.000**	0.309	0.000**	0.467		
$\mathrm{TWI_s}$	**0.450**	0.330	0.575	0.319	0.569	0.338	0.576	
	0.014*	0.080*	0.001**	0.092*	0.001**	0.072*	0.001**	
$\mathrm{SPI_m}$	0.193	-0.232	0.999	-0.240	0.990	-0.184	0.997	0.565
	0.315	0.226	0.000**	0.210	0.000**	0.339	0.000**	0.001**

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 4.7 Stepwise regression of soil properties for surface horizons of location fertilized 2 with 0.5M $NaHCO_3$ as the response and Mehlich 1 extractable Fe, Al, and Ca; the soil carbon; sand, silt, and clay percentages; surface horizon thickness; pH in water and in CaCl; and TWI_s as the predictors

Step	1
Constant	133
TWI_s	20.9
T-Value	2.62
P-Value	0.014
S	43.8
R-Sq	20.2
R-Sq (adj)	17.3

Alpha to enter or remove = P≤ 0.05 Response = 0.5M $NaHCO_3$ extracted P_o, predictors = 11, n = 29

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10. The adjusted R-squared compares the explanatory power of regression models. The predicted R-squared indicates how well a regression model predicts responses for new observations. S represents the average distance that the observed values fall from the regression line.

Table 4.8 Pearson's product-moment correlation matrix for sequential extracted P_o fractions from location Unfertilized 1

	0.5 M NaHCO3	0.5 M NaHCO3	0.1 M NaOH	1.0 M HCl	MFD SPI	MFD TWI	D∞ TWI	D∞ SPI	D8 TWI	D8 SPI	TWI_s
0.5 M NaHCO3	0.467										
	0.009*										
0.1 M NaOH	0.543	0.726									
	0.002*	0.000*									
1.0 M HCl	0.300	0.410	0.585								
	0.107	0.024**	0.001**								
MFD SPI	0.339	**0.451**	0.240	0.479							
	0.067zxt	**0.012****	0.201	0.007**							
MFD TWI	-0.298	-0.205	-0.197	-0.266	-0.477						
	0.109	0.277	0.297	0.156	0.008**						
D∞ TWI	-0.294	-0.209	-0.194	-0.286	-0.444	-0.992					
	0.115	0.268	0.305	0.126	0.014**	0.000**					
D∞ SPI	0.336	0.440	0.229	0.484	0.998	-0.449	-0.415				
	0.070zxt	0.015**	0.224	0.007**	0.000**	0.013**	0.023**				
D8 TWI	-0.202	-0.066	-0.104	-0.097	-0.439	0.926	0.905	-0.412			
	0.285	0.730	0.585	0.610	0.015**	0.000**	0.000**	0.024**			
D8 SPI	**0.341**	0.438	0.198	**0.504**	0.959	-0.445	-0.415	0.967	-0.352		
	0.065zxt	0.015**	0.294	**0.004****	0.000**	0.014**	0.023**	0.000**	0.056zxt		
TWI_s	-0.127	0.118	**0.290**	0.004	0.083	0.260	0.304	0.081	0.212	0.087	
	0.503	0.536	**0.121**	0.983	0.664	0.165	0.102	0.670	0.260	0.648	
SPI_m	0.340	**0.451**	0.238	0.482	1.000	-0.466	-0.433	0.999	-0.427	0.963	0.080
	0.066zxt	**0.012****	0.206	0.007**	0.000**	0.009**	0.017**	0.000**	0.019**	0.000**	0.673

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10

Table 4.9 Stepwise regression of soil properties for surface horizon of location Unfertilized 1 with 0.5M $NaHCO_3$ as the response and Mehlich 1 extractable Fe, Al, and Ca; the soil carbon; sand, silt, and clay percentages; surface horizon thickness; pH in water and in CaCl; and TWI_s as the predictors

Step	1
Constant	129
Horizon Thickness	-4.7
T-Value	-2.06
P-Value	0.049
S	17.0
R-Sq	13.1
R-Sq (adj)	10.0

Alpha to enter or remove = P≤ 0.05 Response = 0.5M $NaHCO_3$ extracted P_o, predictors = 11, n = 30

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10. The adjusted R-squared compares the explanatory power of regression models. The predicted R-squared indicates how well a regression model predicts responses for new observations. S represents the average distance that the observed values fall from the regression line.

Table 4.10 Stepwise regression of soil properties for subsurface horizon of location low one with Mehlich 1 extractable Fe, Al, and Ca; the soil carbon; sand, silt, and clay percentages; surface horizon thickness; pH in water and in CaCl; and STP levels of the surface horizon as predictors

Step	1
Constant	132
Depth to Horizon	-12.3
T-Value	-5.38
P-Value	≤0.000**
S	17.2
R-Sq	50.8
R-Sq (adj)	49.1

Alpha to enter or remove = P≤ 0.05 Response is 0.5M $NaHCO_3$ predictors = 12, n = 30

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10. The adjusted R-squared compares the explanatory power of regression models. The predicted R-squared indicates how well a regression model predicts responses for new observations. S represents the average distance that the observed values fall from the regression line.

Table 4.11 Stepwise regression of soil properties for subsurface horizon of location Unfertilized 1 with Mehlich 1 extractable Fe, Al, and Ca; the soil carbon; sand, silt, and clay percentages; surface horizon thickness; pH in water and in CaCl; TWI_s; and STP levels of the surface horizon as predictors

Step	1	2	3	4	5	6
Constant	820	649	427	354	114	726
Depth	-63	-53	-42	-38	-49	-39
T-Value	-5.61	-4.50	-3.42	-3.40	-4.72	-3.75
P-Value	0.000	0.000	0.002	0.002	0.000	0.001
% Carbon		53	65	95	97	95
T-Value		2.09	2.63	3.81	4.50	4.86
P-Value		0.046	0.014	0.001	0.000	0.000
STAl			0.90	1.31	1.76	1.74
T-Value			2.07	3.13	4.48	4.86
P-Value			0.048	0.004	0.000	0.000
STFe				-5.6	-6.9	-7.3
T-Value				-2.68	-3.70	-4.24
P-Value				0.013	0.001	0.000
%Silt					581	478
T-Value					3.01	2.66
P-Value					0.006	0.014
pH						-114
T-Value						-2.46
p-Value						0.022
S	84.4	79.8	75.3	67.7	58.8	53.5
R-Sq	52.9	59.5	65.2	73.0	80.4	84.5
R-Sq (adj)	51.2	56.5	61.2	68.7	76.3	80.4

Alpha to enter or remove = $P \leq 0.05$ Response = 0.1M NaOH extracted P_o, predictors = 12, n = 30

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10. The adjusted R-squared compares the explanatory power of regression models. The predicted R-squared indicates how well a regression model predicts responses for new observations. S represents the average distance that the observed values fall from the regression line.

Table 4.12 Stepwise regression of soil properties for subsurface horizon of location low one

Step	1
Constant	88.8
Depth to Horizon	-9.2
T-Value	-2.38
P-Value	0.024**
S	29.1
R-Sq	16.9
R-Sq (adj)	13.9

A-to-enter: 0.15 A-to-remove: 0.15 Response is 1.0M HCl extracted P_o, 11 predictors, n = 30

** indicates p value less than or equal to 0.05, * indicates p value less than or equal to 0.10. The adjusted R-squared compares the explanatory power of regression models. The predicted R-squared indicates how well a regression model predicts responses for new observations. S represents the average distance that the observed values fall from the regression line.

Figures

Figure 4.1 Hot spot analysis of Mehlich 1 soil test phosphorus data at location fertilized 1where locations with high value, surrounded by other features with high values are identified and hot spots and locations with low value, surrounded by other features with low values are identified and cold spots

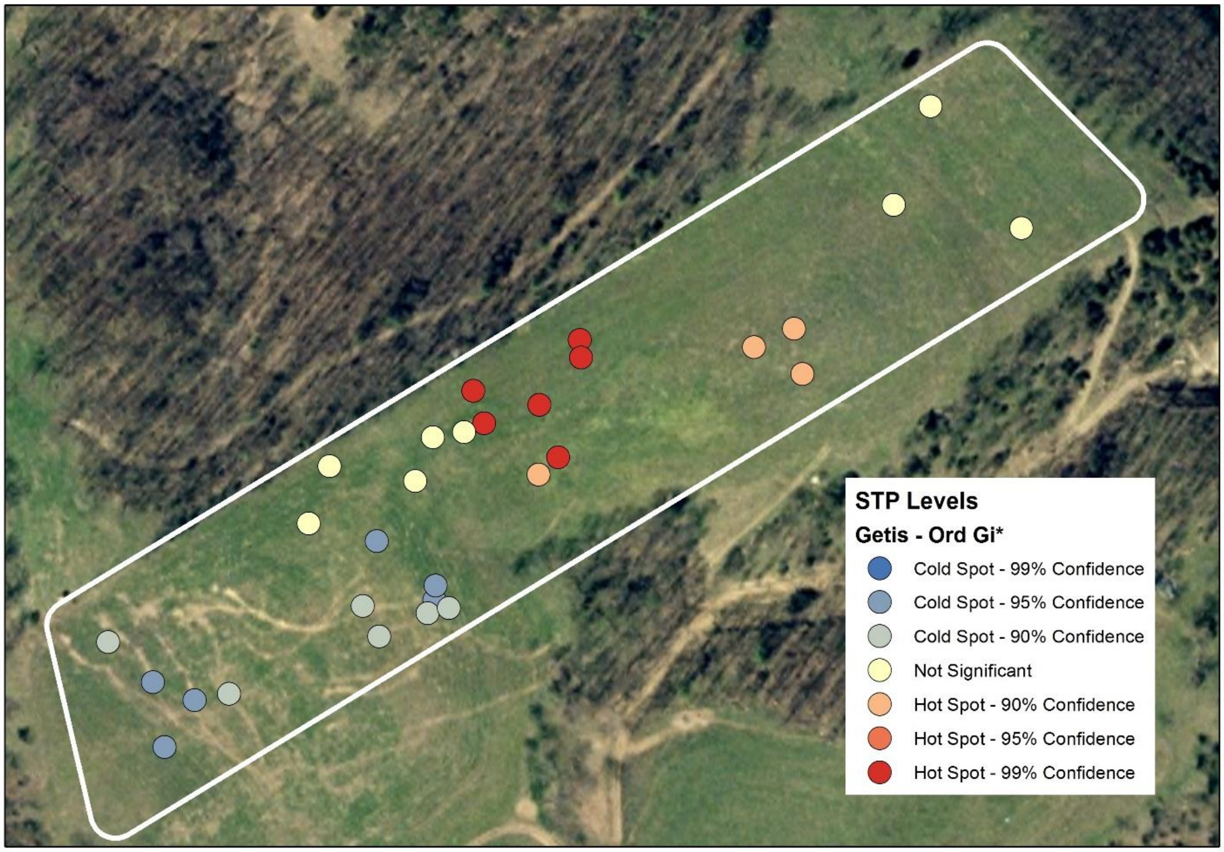


Figure 4.2 Hot spot analysis of Mehlich 1 soil test phosphorus data at location fertilized 2where locations with high value, surrounded by other features with high values are identified and hot spots and locations with low value, surrounded by other features with low values are identified and cold spots

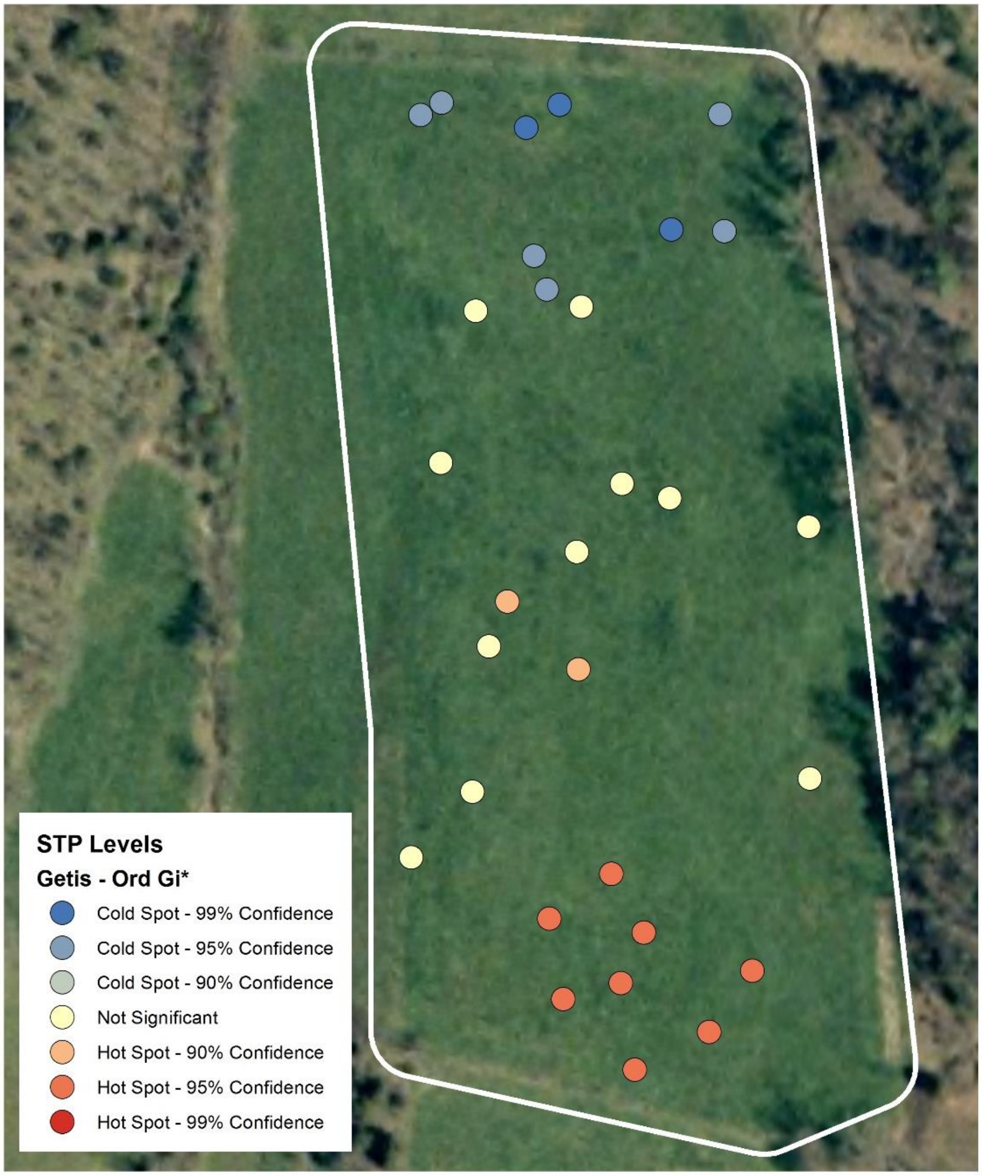


Figure 4.3 Hot spot analysis of Mehlich 1 soil test phosphorus data at location unfertilized 1where locations with high value, surrounded by other features with high values are identified and hot spots and locations with low value, surrounded by other features with low values are identified and cold spots

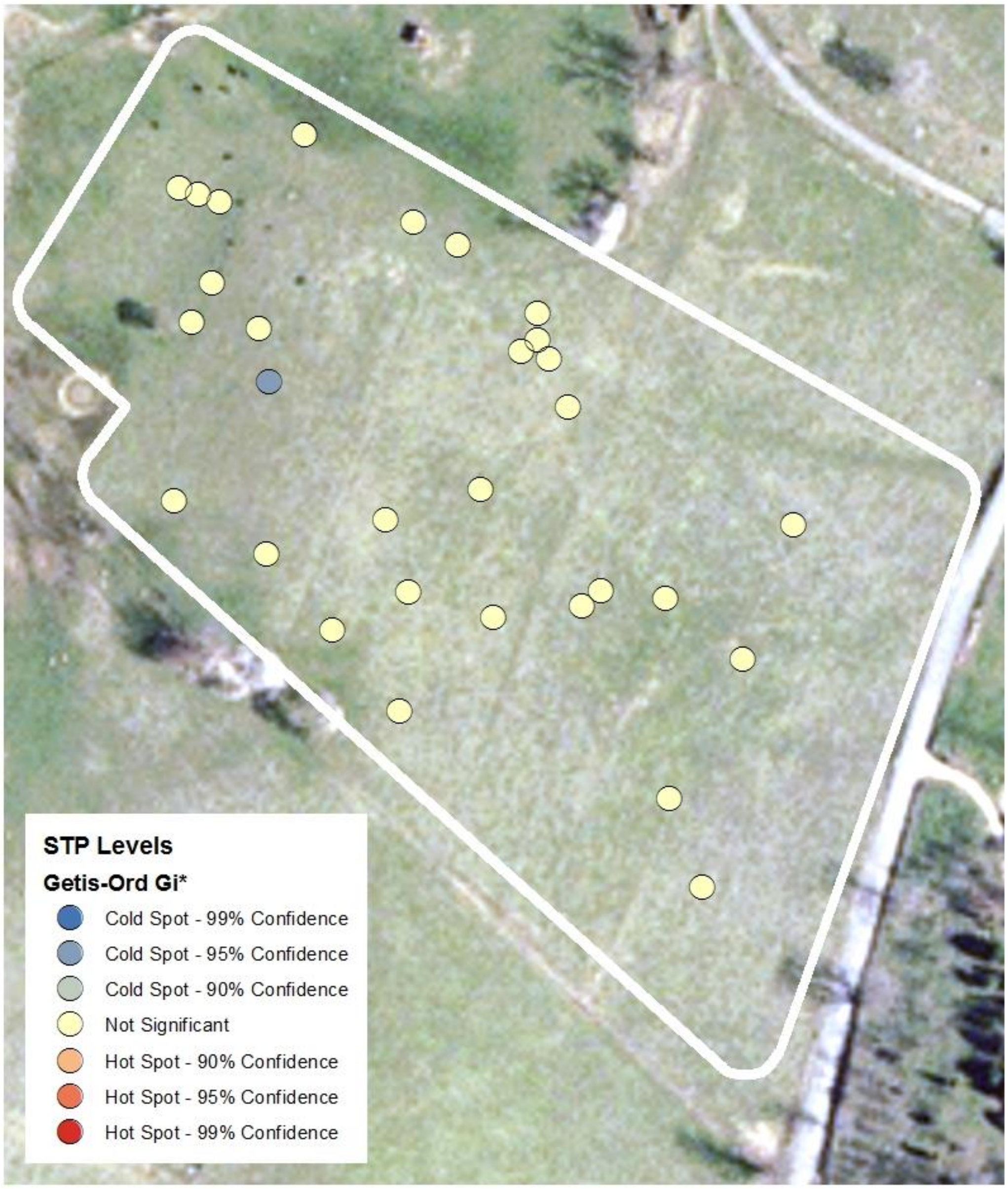


Figure 4.4 Hot spot analysis of Mehlich 1 soil test phosphorus data at location unfertilized 2 where locations with high value, surrounded by other features with high values are identified and hot spots and locations with low value, surrounded by other features with low values are identified and cold spots

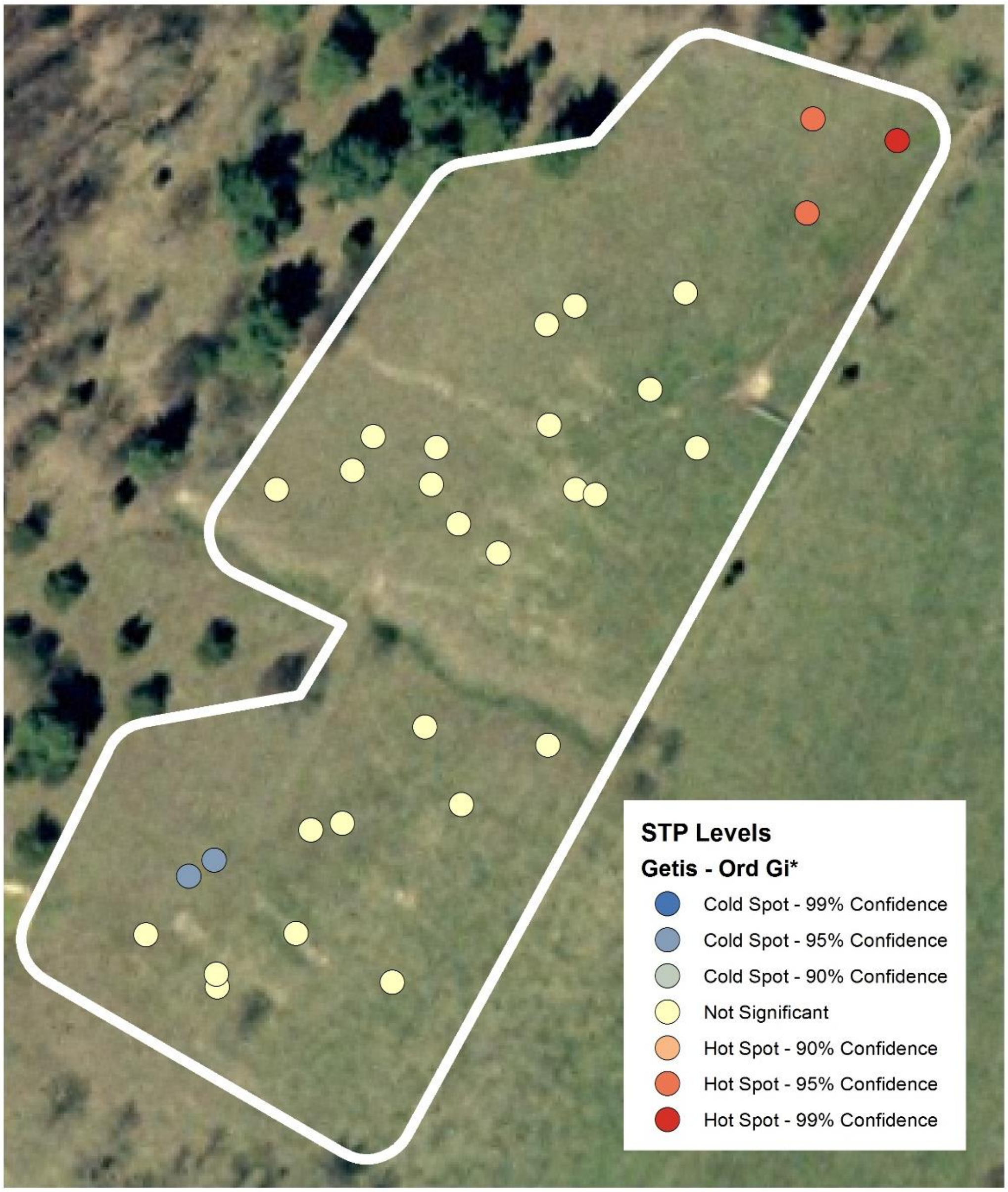
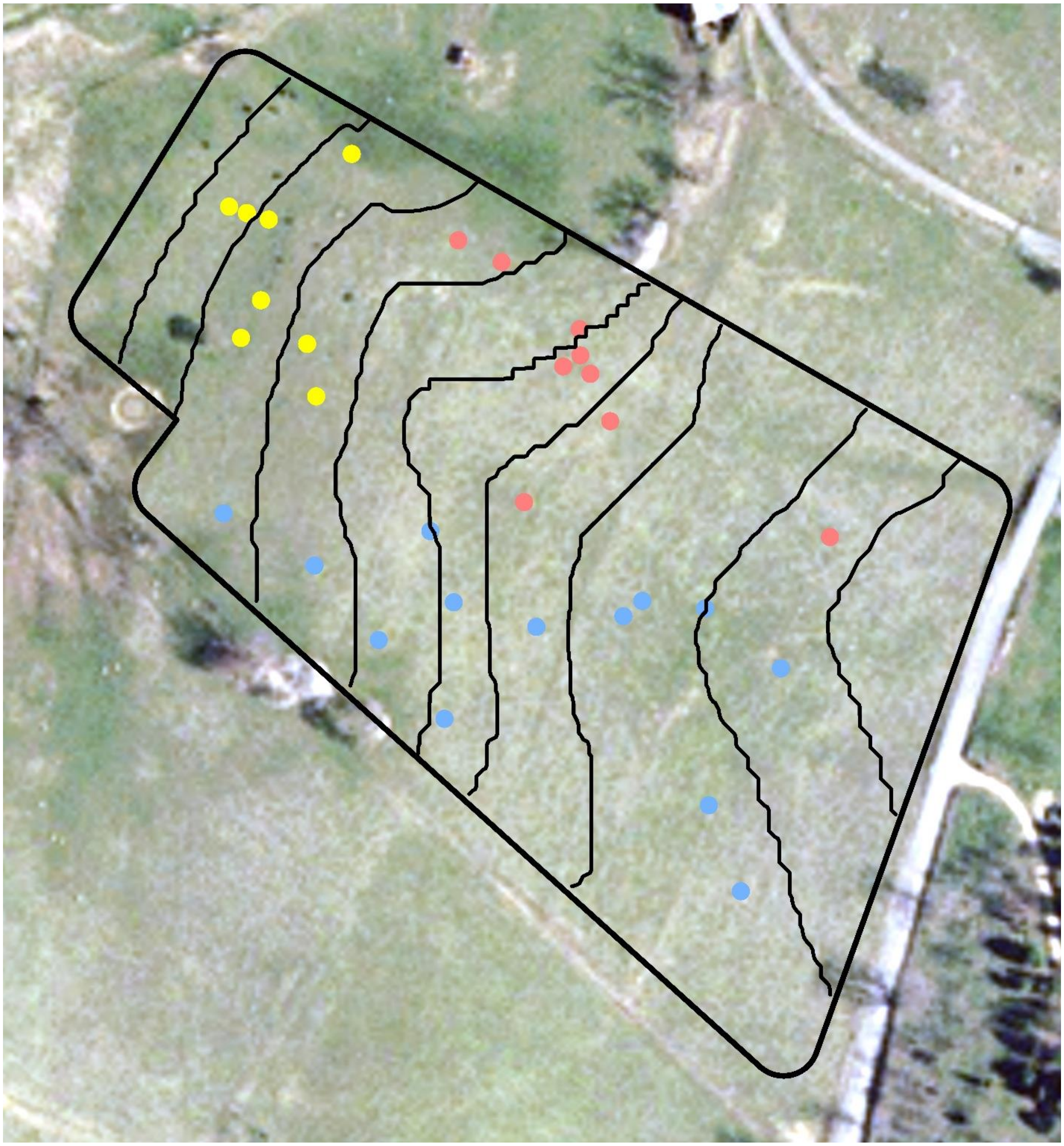


Figure 4.5 Hot spots near former gated entrance and winter feeding area at entrance of the pasture area at location unfertilized 2 where locations with high value, surrounded by other features with high values are identified and hot spots and locations with low value, surrounded by other features with low values are identified and cold spots

Figure 4.6 Isolines of location fertilized 1 with sample points divided into three distinct subsets with the sample points located in the summit position (blue), the sample points contributing to a midfield concave feature draining out of the field (yellow), and the remaining points (red).

Figure 4.7 Isolines of location fertilized 2 with sample points divided into two subsets: a limited subset of sample points located on the higher landscape positons in the foot slope that appear to be hydrologically isolated from the remaining data points (blue) and the remaining data points (yellow).

Figure 4.8 Isolines of location unfertilized 1 is composed of four basic elements, a summit, two back slopes and a foot slope position. The sample points in the summit drained into one of two back slopes, who converged at the foot slope of the landscape. The data points are divided into three parts: the two back slopes (red and blue) and data points at the foot slope position (yellow).



Chapter 5. Modeling Sequentially Extracted Organic Phosphorus Fractions from Soil Test Phosphorus and Topographic Data

Abstract

In locations with significant food animal agriculture and historic N-based manure applications, P accumulation is a significant environmental concern. As efforts to evaluate risk associated with additional manure applications evolve, tools to improve the ability of conservation professionals to make such determinations become more critical. When locations are identified with a degree of P saturation that poses a risk of environmental loss, any tools that assist conservation professionals in developing remediation strategies are equally utilitarian. However, the resources to manage risk and develop remediation strategies are often at a premium. By utilizing readily available topographic data and routine soil test data, P distributions in locations with lengthy histories of animal manure applications can be modeled without the costs associated with additional sampling and elaborate multi-step laboratory procedures. As such, when sequential extracted P fractions were modeled using Mehlich 1 (M1) and Mehlich 3 (M3) soil test data and various topographic data it explained between 43.2 and 97.9 % of the variability in the data. This has the potential to allow resource managers to utilize basic soil test results and topographic data to predict multiple organic and inorganic P fractions providing a useful tool for risk assessment and remediation at a fraction of normal cost.

Introduction

Historically, there have been multi-step fractionation procedures developed to describe and categorize soil P (Chang and Jackson, 1957; Bowman and Cole 1978; Hedley et al. 1982) and countless modifications to these methods. The most commonly cited and modified method in soil P research is the Hedley et al. (1982) fractionation (Guppy et al., 2000). The Hedley et al. (1982) fractionation partitions the P pool into soluble, aluminum/iron-bound, calcium-bound, and residual forms. Most research on P has focused on inorganic P (P_i), with significantly less attention given to organic species of P (Laboski, and Lamb, 2003; Anderson and Magdoff, 2005). Some researchers hypothesize that this is in part due to a perception that P_i is the dominant form of P, it is the plant available form, and the analysis of organic forms was simply too problematic (Jansson et al., 1988; Turner and Haygarth, 2000; Anderson and Magdoff, 2005).

In general, P mobility is dependent upon hydrological and chemical processes (Galeone, 1996; Easton et al., 2009). P movement follows the direction of surface and subsurface water movement (Smeck and

Runge, 1971). The research of Smeck (1973) and Smeck and Runge (1971) documented lateral P movement and accumulate in lower landscape positions. Smeck (1985) identified multiple examples where soils sampled from lower positions in a toposequence had higher total P levels.

Multiple researchers have reported subsurface transport of P (Turner and Haygarth, 2000; Kleinman et al., 2004; Nelson et al., 2005; Andersen and Kronvang, 2006). Using a topographic wetness index (TWI) Moore et al. (1993) explained 48% of the variability within STP extractions at a field scale. Similarly, McKenzie and Ryan (1999) found climate, terrain, and parent material to explain as much as 78% of total P variation within a catchment. As sediments and any dissolved materials move with water, it effectively generates vertical and lateral differentiation in the soil or its properties (Ziadat, 2005; Pachepsky et al., 2001; Young and Hammer, 2000; Girgin and Frazier, 1996). Water and material movement at field scale is strongly influenced by slope configuration (Huggett, 1975). However, the specific factors that control surface and subsurface hydrology may change with scale (Park and Vlek, 2002; Kirkby et al., 1996).

Phosphorus fractionation procedures can provide insight into how P exists within a soil and across a landscape. The strength of the P-soil bonds affects soil solution concentrations (Brady and Weil, 2002; Blake et al., 2003; Pierzynski et al., 2005). In terms of P composition in soil, the P_o pool in soil is typical composed of inositol phosphates, phospholipids, nucleic acids, phosphoproteins and other unidentified P compounds (Schroeder and Kovar, 2006). P_i in soils typically comes from the weathering of apatite (Pierzynski et. al 2005). If multiple P_o fractions are applied to packed soil columns, different classes of P_o fractions will move through the soil at different rates (Anderson and Magdoff, 2005). For example, orthophosphate diesters are more likely to leach in soils than monoesters or P_i (Anderson and Magdoff, 2005). Based on the principals of soil landscape modeling, if one can model the water movement within a landscape and that water moves one or more fractions of P, one could develop a quantitative method to predict the redistribution of P across the same landscape.

While effective at describing the distribution of P within a sample, sequential extractions are time consuming and may not be practical for routine soil analysis. Yet at a landscape scale, knowledge of the labile P fractions is of importance in assessing risk of P loss (Negassa and Leinweber, 2009). The proposed solution is to predict P fractions from more readily available data. In recent years there has been research on predicting and assessing the spatial distribution of soil P (Wang et al., 2009; Liu et al., 2013; Rubaek et al., 2013; Roger et al., 2014), but little effort to predict spatially explicit sequentially extracted P fractions from landscape data. This may be in part related to the perception that terrain attributes do not adequately explain the variability seen in P distributions. Roger et al. (2014) noted the poor performance of terrain attributes at spatially predicting various P forms and the overwhelming influence of land use and management on P levels. Similarly, Sarmadian et al. (2014) indicated only a moderate influence of terrain attributes on P levels in Iran. However, if landscape variables are only part of the model and landscape variables are combined with other metrics for P status, field level spatial prediction is not unreasonable.

As concerns over pending P regulation mount, strategic long-term solutions to localized P saturation will become more necessary. Locations currently applying animal manure on a N basis or locations subject to new more restrictive P indices will soon be prohibited further P application. In the coming years, the efforts of reduce the P saturation at these sites and prevent P loss will become more critical. Similarly, the ability to model P movement within these sites and develop spatially-based P interception strategies will be dependent on basic, accurate, and cost-effective modeling of P distributions at the field level. Soil-landscape modeling can provide that capability.

There are two primary objectives of this research. The first is to establish a series of statistical soil-landscape models that best explain the spatial distributions of sequential extracted P fractions across hay and grass pastures in West Virginia. The second is to determine which soil test extraction (Mehlich 1 or Mehlich 3) is best suited for modeling sequential extracted P fractions. As such, these models could serve as a guide for resource allocations, as a component in remediation strategies, and have the potential to improve regional risk assessments of P loss.

The first research hypothesis (Ha_1) states that soil landscape data and soil test P data will be significant ($p \leq 0.05$) terms in sequentially extracted P_o models at landscape scales. The null hypothesis (Ho_1) states that soil landscape data and soil test P data will not be significant ($p \leq 0.05$) terms in sequentially extracted P_o models at landscape scales. The second research hypothesis (Ha_2) states Mehlich 1 soil test phosphorus data based models will have higher R^2 values than Mehlich 3 soil test phosphorus data based models. The second null hypothesis (Ho_2) states Mehlich 3 soil test phosphorus data based models will have higher R^2 values than Mehlich 1 soil test phosphorus data based models.

Materials and Methods

Study Sites and Sample Locations

The selected study sites were typical hay or grass pasture fields in eastern West Virginia. The study sites consisted of four fields. Two fields had lengthy histories (approximately 10+ yrs.) of annual N-based manure applications. Two fields had very infrequent applications of animal manure (once every 4 or 5 yrs.). Within each field a stratified random sampling design (Thompson et al., 2006) was used to select sample points. The topographic wetness index (TWI) of each location was used as the basis for the stratification. TWI was calculated from a 3-m DEM (SAMB) made free of sinks or voids. TWI is defined as the ln (A / tan B) where A is the local upslope contributing area for that point and B is the local slope gradient (Beven and Kirkby, 1979). Flow direction was calculated for TWI by determining the specific flow direction from each cell into one of the eight neighboring cells based on the steepest downward slope (O'Callaghan and Mark, 1984). Specific catchment area is estimated by A/L, with A being the number of pixels draining into a pixel multiplied by the area of a pixel, and L as the pixel width (Moore et al., 1991). TWI was grouped into three classes of equal area. Ten samples locations were selected at random within each class. From each sample point the first mineral horizon (surface horizon) and the 10 cm below that horizon (subsurface horizon) were sampled.

Sample Preparation

All samples were air dried, ground, sieved (2-mm sieve), and thoroughly mixed to make individual samples as homogenous as possible (Laboski and Lamb, 2003). Dried and ground samples were stored in sealed centrifuge tubes at 4°C until 1 day prior to analysis.

Sequential Fractionation

The method of sequential P fractionation is based on a suggested modification (Sui et al., 1999) of the Hedley method (Hedley et al., 1982) as described by He et al. (2003). From each sample, 1.0 g of soil and 25 mL of extractant was placed in a centrifuge tube in a reciprocal shaker at 180 oscillations per minute for 16 h at room temperature. The samples were centrifuged for 15 min at 2,800 X g and the supernatant filtered (Whatman No. 2 or equivalent). This process was repeated sequentially with the following extractants: (i) distilled deionized water, (ii) 0.5M $NaHCO_3$, (iii) 0.1M NaOH, and (iv) 1.0M HCl. Duplicates of each sample were fractionated in this manner. Water, 0.5M $NaHCO_3$, and 0.1M NaOH extracts were acidified and filtered prior to analysis for P_i. P_i was determined by the ammonium molybdate-ascorbic acid method (Knudsen and Beegle, 1988). The ammonium molybdate-ascorbic acid method is a single reagent orthophosphate colorimetric method with two stock solutions, the concentrated ammonium paramolybdate solution and the ascorbic acid solution. The concentrated ammonium paramolybdate solution consists of 60 g of ammonium paramolybdate $(NH_4)6Mo_7O_{24}\cdot 4H_20)$ and approximately 200 mL of distilled water in a 1 L volumetric flask, along with 1.455 g of antimony potassium tartrate K_2Sb_2 $(C_4H_2O_6)_2$. These compounds are added to 700 mL of concentrated sulfuric acid and allowed to cool to room temperature, diluted to volume with distilled water, and stored in a dark glass bottle in the refrigerator. The ascorbic acid solution was made by dissolving 132 g of ascorbic acid in distilled water and diluted to 1 L in a volumetric flask. The single colorimetric working solution was made daily by adding 25 mL of concentrated ammonium paramolybdate solution to approximately 800 mL distilled water, with 10 mL of the ascorbic acid solution and diluting to volume with distilled deionized water in a 1 L volumetric flask. To determine P content, 2 mL of the soil extract or P standard was transferred to a test tube with 8 mL of the colorimetric working solution and mixed thoroughly. After 20 minutes for color development, the percent transmittance was read at 882 nm. Total P (P_t) was determined with a Perkin Elmer P4000 Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). P_o was calculated for each fraction as the mathematical difference between the reactive P_i and P_t.

Mehlich-1

Mehlich-1 (M1) is a soil test with a 5:1 ratio of Mehlich-1 solution (0.025 N H_2SO_4 + 0.05 N HCl) to soil. The mixture is shaken for five minutes on a reciprocating shaker set at a minimum of 180-200 oscillations per minute (Nelson et al., 1953). The extractant is filtered through a medium-porosity filter paper (Whatman No. 2 or equivalent) and analyzed for P content (Nelson et al., 1953). P_t was determined with a Perkin Elmer P4000 Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). For modeling purposes, a mean M1 P levels was calculated for each field.

Mehlich-3

Mehlich-3 (M3) is a 10:1 ratio of Mehlich-3 solution (0.2 N CH_3COOH + 0.25 N NH_4NO_3 + 0.015 N NH_4F + 0.013 N HNO_3 + 0.001 M EDTA) to soil. The mixture is shaken for five minutes on a reciprocating shaker set at a minimum of 180-200 oscillations per minute (Mehlich, 1984). The extractant is filtered through a medium-porosity filter paper (Whatman No. 2 or equivalent) and analyzed for P content (Mehlich, 1984). P_t level was determined with a Perkin Elmer P4000 ICP-OES. For modeling purposes, a mean M3 P levels was calculated for each field.

Modeling Parameters

Terrain attributes from 3-m resolution United States Geologic Survey (USGS) DEM were calculated. The elevation model was converted and exported as an ASCII files using ArcGIS 10.3. The ASCII files were imported into SAGA GIS (Bock et al., 2008). In SAGA, each DEM was examined for and filled any sinks or voids (Wang and Liu, 2006). Primary terrain attributes slope gradient, profile curvature, plan curvature, and tangential curvature were calculated (SAGA). Based on multiple flow direction (MFD), deterministic eight (D8) and deterministic infinity (D∞) calculation methods, the upslope contributing areas using the recursive upslope method, were calculated for use when calculating the compound topographic indices TWI and SPI. In addition to these six compound indices three additional indices, a SAGA wetness index (TWI_s), modified SPI (SPI_m) and convergence index (CI) were constructed. CI is a terrain parameter that examines the aspect of surrounding cells and determines the degree to which those aspects point toward or away from a given cell (Koethe and Lehmeier, 1996). TWI_s uses a modified catchment area calculation to better represent water dispersions in low slope areas (Boehner et al., 2002). The SPI_m is the same SPI calculation but it is generated using the modified catchment area calculation of the TWI_s. All compound indices were exported as ESRI Grid files using SAGA 2.0. Lastly, the data was imported into Arc Map 10.2 and the respective TWI and SPI values attached to the sample point data. The combined data was than exported as .DBF files for analysis in Minitab version 17 (Minitab, 2012).

While the 3M DEM was the basis for all topographic data in this research, it is likely there is a local optimal resolution. Clearly there is some potential for a variation on the modifiable areal unit problem related to the artificial selection of 30 meter dimensionality. Specifically a DEM at another resolution or scale could generate different results. However a multi scale assessment to optimize DEM resolution to the study sites or the region is beyond the scope of this research. Additionally 30 meters is a resolution that is available statewide and is less resource dependent in terms of processing the DEM.

Statistical Analysis and Modeling

Surface and subsurface models were developed. All models included a STP term (individual data points or field averages). Stepwise regression was used to identify topographic variables that explain a significant portion of the variability in the sequential extracted P fractions across all study sites. Alpha to enter and leave values of 0.05 were selected. Each P_o and P_i fraction for the surface and subsurface horizons relative to the modeling parameters S TWI, CI, ELE, MFD, Z Slope, D8 SPI, D8 TWI, DINF SPI, PRC, and TANC were examined. To determine final model parameters, the initial models with multiple landscape variables were reexamined. Where models were generated with multiple terms measuring the same phenomena and or spatial model terms were significantly correlated ($p \leq 0.05$), selected model terms were removed. Model terms selected for removal were based on variance inflation factors, p values, frequency of occurrence in other models, and expert knowledge.

Randomly 30% of the data was selected for the purpose of model validation. After selecting model terms, the regression equations were applied to the validation data, and modeled the predicted values against the real data and measured the fit (r^2) for each equation. Next field level averages were calculated from the sample data for M1 and M3 STP values in place of individual sample STP values and evaluated these predicted values against validation data and measured the fit (R^2) for each equation. Lastly the fit of the M1 and M3 STP models was measured across all samples to determine which STP method explained the greatest portion of variability in the test data. This step was developed to determine how well each soil test data set did respectively when modeling the sequentially extracted P fractions over the landscape.

Results and Discussion

To facilitate the development of these models an extensive set spatially explicit sequentially extracted P data was used to develop and test a series of multiple regression models based on initial stepwise regression models. When the stepwise procedure was applied to the model dataset the predicted $\mathbf{r^2}$ ranged from a low of .528 for the subsurface P_i M3-0.5M $NaHCO_3$ model to a high of .962 for the surface P_o M1-1.0M HCl model (Table 5.1). However, when examined closer, several models contained terms that were potentially collinear and the lack of fit with some models could be an indication of inaccuracy and bias in the models (Minitab, 2012). Of the 18 initial landscape variables considered by the stepwise procedure, ten variables were included in at least one model (Table 5.2). The Pearson product moment correlation (Table 5.3) identified 16 significant ($p \leq 0.05$) correlations between various landscape terms. Inclusion of multiple collinear model terms violates the basic assumptions inherent with regression models and thereby reducing the predictive utility of the model (Thompson et al., 1997). Two of more landscape variables were included in 14 of the regression models. In some instances, (Surface P_i M1-DDI H_2O and Subsurface P_o M1-0.5M $NaHCO_3$) models had Mallows' Cp values more than double the number of model terms (Table 5.1) an indication of collinearity. In other instances, potential collinearity was identified when models contained compound terrain variables as well as some of their component primary terrain variable components. Thompson et al. (1997) point out how several of the models generated by Moore et al. (1993) used regression models with slope gradient and TWI to predict soil properties. The primary terrain variable slope gradient is a component in the TWI and using both has the potential to reduce the predictive utility of the model (Thompson et al., 1997).

To view selected model terms, see (Table 5.4 & 5.5). In general, model terms were selected that had lower variance inflation factors, lower p values, and terms that occurred frequently in other models

(Table 5.6). For example, the model for the surface inorganic P fraction extracted with 0.5M $NaHCO_3$, contained a slope gradient term and a compound terrain variable in which slope is a component (Table 5.4), and the subsurface inorganic P fraction extracted with DDI H_2O, contained another compound terrain variable, and upslope contributing area term (Table 5.5). While not a component of the compound variable, the up slope contributing area term is another calculation of a component term. In both instance, there were significant correlations between the terms (Table 5.3), similar variance inflation factors (Table 5.4 & 5.5). The complete list of final regression models is provided in Table 5.7.

Among the models for the sequentially extracted P_i fractions from the surface horizon samples, the term TWI_s was included in six of eight models (Table 5.6). Among compound terrain variables, TWI is a good indicator of soil moisture (Pei et al., 2010) and TWI has been suggested as a tool to identify critical source areas (CSA) (Endreny and Wood 2003; Page et al., 2005). “The strong influence of terrain parameters on the soil spatial variation is now a well-known principle” (Park and Vlek 2002). Overall, in seven of eight models the terrain parameter model term (TWI or CI) was selected. Terrain parameters tend to be very effective soil predictors at the hillslope or field scale (Huggett, 1975; McKenzie and Austin, 1993; Gessler et al., 1995; Thompson et al., 1997; McBratney et al., 2000; Park and Vlek 2002). This is consistent with the way P movement was described by Smeck and Runge (1971) when they indicated P movement follows the direction of surface and subsurface water movement. The frequent inclusion of these terms reinforces the notion that hydrologic data needs to be integrated when identifying CSA, predicting P loss, and developing P management strategies (Collick et al., 2015).

The sequentially extracted P_o fractions from the surface horizon samples behaved somewhat similar to the P_i fractions. However, unlike the inorganic fractions where only M3 models did not contain the term TWI_s, the two models without TWI_s in this group were in the same extractable fraction in both the M1 and M3 STP models. It would appear that 1.0M HCl extracted P_o fractions may be controlled by other factors. For example, as pH and other the concentrations of other ions change the forms of soil phosphorus also change (Smeck, 1973). In general, as pH drops less soluble and more occluded forms of P dominate (Smeck, 1973). In general, the HCl extractable pools are the non-available and or recalcitrant P pools (Ziadi et al 2013). It may be the relative stability of this fraction does not lend to downslope movement as seen in the other fractions. Similarly, others have indicated the HCl extractable fraction may be a sink for P (Haynes and Williams, 1992; Patzold et al. 2013). The continual and excessive additions of P to soils in temperate climates causes elevated levels of the most labile P fractions (Negassa and Leinweber 2009). Perhaps over time these mobile fraction satiate the sorption sites typically extracted by the stronger components in the sequential extraction and the spatial variability of that factor is adequately expressed by the variability in the routine soil test data.

The subsurface sequentially extracted inorganic P fractions did not display the consistency in selected model terms found in the surface samples. Traditionally P transfer by subsurface pathways has been perceived as negligible (Turner and Haygarth, 2000). However, P does bypass much of the soil matrix via preferential flow (Kleinman et al., 2004; Harman et al., 20130. Clearly subsurface P movement is a less understood transport mechanism (Turner and Haygarth, 2000). As such, unlike the majority of the surface samples, within the inorganic fractions, only two of the eight models used the combination of TWI_s and STP. Three models include other topographic variables (profile curvature, elevation, and SPI). This is consistent with the idea that topography can have an indirect effect on soil properties, and the distribution of P (Seibert et al. 2007; Vasques et al. 2010), but as a whole, the more mobile fraction (DDI H_2O, and 0.5M $NaHCO_3$) models tend to not have topographic components while the models for the less mobile fractions do include topographic components. This is consistent with mobile fractions moving rapidly away via subsurface flow and less mobile fractions persisting. As Rittenburger et al. (2015) pointed out, in subsurface pathways, strongly adsorbed chemicals tend to adhere to the soil matrix and become somewhat immobile. In the literature there are ample examples of P accumulating in the sub surface horizons of agricultural soils (Eghball et al., 1996; Hountin et al., 1997; Oehl et al., 2002; Stephenson and Chapman, 1931; Harman et al., 2013).

In the subsurface horizon, sequentially extracted P_o fractions behaved in a pattern more consistent with the surface organic fractions. TWI_s was a model tem in six of eight models. P applied with animal manure is generally more prone to leaching than inorganic fertilizer (Chardon et al., 1997; Eghball et al., 1996; Glæsner et al., 2011). As such the ability to model these fractions similarly to their surface counterparts is not unexpected. The only sequentially extracted fraction models not to include the term TWI_s were the 1.0M HCl fractions with the M1 and M3 model terms. Given the similarity of extraction mechanisms (strong acid and dilute double acid) it is it is understandable that such a model could explain 94.26% of the variability. The M3 model did not include a spatial term similar to its corresponding surface model. The models for the 1.0M HCl fraction in the M1 P_o subsurface, M1 P_i subsurface, and the M1 P_o surface models all contained the same model terms (list those model terms in parentheses here).

Overall, M1 models explained 89.35% of the variability in the validation data set, while the M3 models only explained 69.08% (Table 5.8). This may be an important distinction, as terrain variables are poorly correlated to various P forms across multiple land uses (Roger et al., 2014; Sarmadian et al., 2014), but have proven highly useful at identifying CSA and predicting P loss at field and sub field levels. While P variability can be substantial between fields, among land uses, and within fields (Page et al., 2005), the combination of actual soil test P data and topographic data can adequately describe said variability and generate useful knowledge. When examined by soil test type (M1 vs. M3) some broad generalizations about the appropriateness of each soil test for the purpose of modeling sequentially extracted P fractions could be made. For example, the M1 and M3 models were examined across all sample data by various grouping intervals (surface, subsurface, P_o and P_i) to determine which soil test had the best overall fit. Among the surface samples, point value M1 STP data had an overall fit (R^2) of 92.44% compared to the M3 STP data fit of 84.56%. Among the subsurface models those numbers dropped to 84.13% and 54.56%, respectively. Accordingly, it is critical to utilize a soil test parameter that is the best compromise between availability of data and simple fit of the predictive model. Overall, the M1 STP models yielded a higher R^2, but in many instances both models have the ability to improve understanding and enhance the decision making processes.

Given the global frequency of P application in excess of removal (Kronvang et al., 2009), the acceleration of P redistribution by agricultural practices (Rubaek et al., 2013) and need for models that reflect P fate and transport (Kleinman et al., 2015) it is reasonable to expect such models may find practical application. Given this, it is prudent to consider how a model may be used as opposed to how it was intended to be used. While terrain variables are extensive in number and scope, and while terrain variables provide a great deal of important information about the various physical process that occur at a given location (Pei et al., 2010), discrete STP data for any or all fields or sub-field units of management in a region are generally not available. Often in agriculture producers sample at coarser scales than researchers may prefer for modeling P fate and or transport. Thus the best available STP data may at times be a single field value from a composite sample.

If applied in practice regionally in the poultry producing region of West Virginia, a single field average is likely the only data that would be available. While not specifically modeled for this use, it is likely that would be how these models would be applied. To understand the impact of using the incorrect data for these models, the field average STP data was utilized in place of the point data values. When this substitution was made for the surface models the R^2 values dropped (Table 5.8). However, when the same substitution was applied to the subsurface models the M3 model dropped but the M1 model improved slightly. Overall, the point data out performed field averages, but in many instances both models have the ability to improve understanding and decision making.

Conclusions

The goal of these models was to develop a simple, affordable, and utilitarian approach for predicting sequentially extracted P fractions across complex landforms utilizing the best available data. Such models could facilitate frugality and equitableness in allocation of resources to identify locations for targeted mediation. Historically soil-landscape relationships have been used successfully at numerus scales to model many soil properties and characteristics (Moore et al. 1993; Gessler et al., 1995; Gessler et al., 2000; Omran, 2012). However, for such models to be successfully applied to the management of P, soil-P- landscape models must accurately capture the impact of the various important processes controlling the distribution of P at a given scale (Kleinman et al., 2015). In the context of these models, that means not only selecting the proper terrain variables, but also selecting the STP method that yields the greatest predictive power. The data is suggestive of an ability to adequately model the spatial distribution of sequentially extracted P data within individual fields from routine soil test data and readily available terrain data. Thus confirming Ha_1 as soil landscape data and soil test P data were successfully used to model sequentially extracted P_o models at landscape scales thus rejecting the Ho_1. Likewise when the Mehlich 1 and Mehlich 3 soil test P levels were used to model sequentially extracted P_o fraction the Mehlich 1 models generally yielded models with higher R^2 vales, thus rejecting Ho_2.

In most cases a single field average from a typical agronomic soil test and the associated TWI_s for that field would generate a map of highly labile, labile, moderately labile, and somewhat non-labile P for the field in question. From this data, conservation professionals could evaluate the infield area that is most likely to contribute significant P loss. In addition to a basic evaluation of potential loss, environmental and conservation professionals could in theory apply some sort of economic metric in terms of P loss prevented per dollar spent and spatially model competing scenarios for how to allocate resources. Clearly this model may not have adequate exportability to be useful at larger national scales, but in terms of grassland in the poultry producing region of West Virginia this may prove to be very useful.

Given the political climate in the Chesapeake Bay watershed, some locations with significant P accumulation could ultimately face mandatory mitigation. At that time P loss assessment tools will be needed to implement mediation strategies. It is safe to assume there will never be adequate funding to pay for full implementation, and it is very unlikely that all producers would voluntarily make every desired remediation effort given the elevated P levels may have occurred while operating under previously acceptable management practices. This makes targeted remediation using available funding not only sensible, but a reasonable first step in a long term plan to eliminate much of the current P loss.

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Tables

Table 5.1 Initial regression models using stepwise procedure with adjusted R^2 - a modified version of R-squared that has been adjusted for the number of predictors in the model, Mallow's CP - when close to the number of predictors in a model indicates unbiased in estimating the true regression coefficients and predicting future responses and S the standard error of the regression

Model	Stepwise regression equation	Model Adj. R^2	Mallow's CP	S
Surface P_i M1 DDI H2O	Y = -40.0 + 21.89 Z Slope + 16.03 S TWI + 0.12151 M1S	85.04	15.56	22.3
Surface P_i M1 0.5M NaHCO3	Y = -150.1 + 40.4 Z Slope + 68.63 S TWI + 0.2866 M1S	85.60	16.48	48.6
Surface P_i M1 0.1M NaOH	Y = -388.2 - 0.4325 MFD + 230.5 S TWI - 8.65 D8 TWI - 0.0696 DINF SPI + 0.6462 M1S	87.26	1.77	110
Surface P_i M1 1.0M HCl	Y = -105.6 - 7663 TANC + 60.2 S TWI + 1.5076 M1S	95.52	1.19	140
Surface P_i M3 DDI H2O	Y = 8.65 + 1.743 m3s	60.20	7.94	34.7
Surface P_i M3 0.5M NaHCO3	Y = -91.1 + 4.348 m3s + 48.5 S TWI	63.50	9.54	77.4
Surface P_i M3 0.1M NaOH	Y = -438.3 + 11.157 m3s - 0.224 MFD + 202.6 S TWI	78.23	4.25	144
Surface P_i M3 1.0M HCl	Y = 28.0 + 25.80 m3s - 7.71 CI	76.25	0.14	322
Surface P_o M1 DDI H2O	Y = -24.2 + 22.60 S TWI + 0.18152 M1S	85.23	-1.0	32.9
Surface P_o M1 0.5M NaHCO3	Y = -46.3 + 1.668 CI + 59.2 S TWI + 0.2812 M1S	73.76	-4.30	75.5
Surface P_o M1 0.1M NaOH	Y = -263 + 7.53 CI + 319.4 S TWI + 0.9823 M1S	69.58	8.34	299
Surface P_o M1 1.0M HCl	Y = -21.2 - 44.2 Z_ELEVATIO + 1.8775 M1S	96.18	3.50	166
Surface P_o M3 DDI H2O	Y = -12.5 + 2.986 m3s + 17.02 S TWI	72.38	3.92	43.7
Surface P_o M3 0.5M NaHCO3	Y = -4.9 + 5.380 m3s + 38.8 S TWI	74.22	-3.58	74.9
Surface P_o M3 0.1M NaOH	Y = -50 + 18.67 m3s + 227.3 S TWI	65.87	3.05	316
Surface P_o M3 1.0M HCl	Y = -57.4 + 30.89 m3s	80.03	-1.35	379
Subsurface P_i M1 DDI H2O	Y = 0.50 + 0.08461 M1S	86.94	-3.70	14.5
Subsurface P_i M1 0.5M NaHCO3	Y = 42.8 - 4359 PRC + 0.2665 M1S	70.07	-1.70	74.6
Subsurface P_i M1 0.1M NaOH	Y = -378.2 + 4.00 CI + 162.9 S TWI + 0.5942 M1S	83.5	1.96	120
Subsurface P_i M1 1.0M HCl	Y = 4.9 - 37.3 Z_ELEVATIO + 1.2702 M1S	94.26	-2.02	139
Subsurface P_i M3 DDI H2O	Y = 0.01 + 1.324 m3s	65.70	1.49	23.5
Subsurface P_i M3 0.5M NaHCO3	Y = 43.6 + 4.054 m3s	52.83	0.61	93.7
Subsurface P_i M3 0.1M NaOH	Y = -254.2 + 10.985 m3s + 110.5 S TWI	76.43	2.10	144
Subsurface P_i M3 1.0M HCl	Y = -36.3 + 22.36 m3s - 5.72 CI + 0.1441 D8 SPI	77.63	-1.28	274
Subsurface P_o M1 DDI H2O	Y = -36.7 - 0.0440 MFD + 24.69 S TWI + 0.15711 M1S	86.98	6.57	26.1
Subsurface P_o M1 0.5M	Y = -101.4 + 2.175 CI + 54.7 S TWI + 0.3218 M1S	84.62	-6.81	63.2
Subsurface P_o M1 0.1M NaOH	Y = -587 + 11.00 CI + 339.6 S TWI + 0.8370 M1S	71.85	3.23	261
Subsurface P_o M1 1.0M HCl	Y = -42.8 - 48.0 Z_ELEVATIO + 1.3785 M1S	90.99	-5.66	193
Subsurface P_o M3 DDI H2O	Y = -43.2 + 2.739 m3s + 32.3 Z Slope + 21.65 S TWI	75.76	5.94	35.6
Subsurface P_o M3 0.5M NaHCO3	Y = -52.8 + 6.134 m3s + 30.9 S TWI + 0.0297 D8 SPI	74.22	3.00	67.0
Subsurface P_o M3 0.1M NaOH	Y = -304 + 17.24 m3s + 221.5 S TWI	68.31	6.52	277
Subsurface P_o M3 1.0M HCl	Y = -69.3 + 22.80 m3s	76.21	0.15	313

Table 5.2 All topographic variables considered & selected by the initial stepwise regression procedure

Potential model terms	Selected	Variable type
Deterministic 8 up slope contributing area (D8)		primary terrain variable
Deterministic infinity up slope contributing area (INF)		primary terrain variable
Multiple flow direction up slope contributing area (MFD)	•	primary terrain variable
Profile curvature (PRC)	•	primary terrain variable
Plan curvature (PLC)		primary terrain variable
Tangential curvature (TANC)	•	primary terrain variable
Z score of individual study site aspects (Z Aspect)		primary terrain variable
Z score of individual study site aspects slope (Z Slope)	•	primary terrain variable
Z score individual study site aspects (Z Elevation)	•	primary terrain variable
Convergence index (CI)	•	Compound terrain variable
SPI from D8 up slope contributing area (D8 SPI)	•	Compound terrain variable
TWI from D8 up slope contributing area (D8 TWI)	•	Compound terrain variable
SPI from INF up slope contributing area (DINF SPI)	•	Compound terrain variable
TWI from INF up slope contributing area (DINF TWI)		Compound terrain variable
SPI from saga modified contributing area (MC SPI)		Compound terrain variable
SPI from MFD up slope contributing area (MFD SPI)		Compound terrain variable
TWI from MFD up slope contributing area MFD TWI)		Compound terrain variable
Saga topographic wetness index (S TWI)	•	Compound terrain variable

Table 5.3 Pearson product moment correlation of topographic model terms selected by initial stepwise regression procedure.

	Z Elevation	Z Slope	PRC	TANC	MFD	CI	S TWI	D8 TWI	DINF SPI
Z Slope	0.221								
	0.052								
PRC	-0.027	-0.063							
	0.816	0.582							
TANC	0.007	-0.067	**0.465**						
	0.955	0.561	**0.000**						
MFD	-0.176	**-0.289**	0.012	**-0.356**					
	0.123	**0.010**	0.981	**0.001**					
CI	**0.236**	-0.030	0.167	**0.453**	**-0.597**				
	0.038	0.792	0.144	**0.000**	**0.000**				
S TWI	-0.198	**-0.395**	-0.084	**-0.379**	**0.483**	**-0.554**			
	0.083	**0.000**	0.465	**0.001**	**0.000**	**0.000**			
D8 TWI	0.034	-0.033	-0.027	-0.043	0.193	-0.102	-0.001		
	0.767	0.774	0.816	0.712	0.091	0.376	0.993		
DINF SPI	0.076	0.151	0.011	0.153	**-0.514**	0.104	-0.171	**-0.491**	
	0.509	0.186	0.927	0.181	**0.000**	0.367	0.135	**0.000**	
D8 SPI	0.061	0.113	0.019	0.111	**-0.363**	0.049	-0.120	**-0.559**	**0.822**
	0.599	0.322	0.868	0.335	**0.001**	0.671	0.296	**0.000**	**0.000**

Table 5.4 Summary model data for surface stepwise regression models with potentially collinear model terms

Surface P_i M1 DDI H_2O

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-40.0	12.9	-3.11	0.003	
Z Slope	21.89	8.20	2.67	0.009	1.24
M1S	16.03	4.12	3.89	0.000	1.15
S TWI	0.12151	0.00588	20.67	0.000	1.35

Surface P_i M1 0.5M NaHCO3

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-150.0	29.4	-5.11	0.000	
Z Slope	40.4	18.7	2.16	0.034	1.24
S TWI	68.63	9.42	7.28	0.000	1.35
M1S	0.2866	0.0134	21.34	0.000	1.15

Surface P_i M1 0.1M NaOH

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-388.2	70.2	-5.53	0.000	
MFD	-0.4325	0.0881	-4.91	0.000	1.76
S TWI	230.5	21.7	10.63	0.000	1.39
D8 TWI	-8.65	3.89	-2.22	0.029	1.33
DINF SPI	-0.0696	0.0199	-3.50	0.001	1.75
M1S	0.6462	0.0300	21.56	0.000	1.11

Surface P_i M1 1.0M HCl

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-105.6	80.5	-1.31	0.194	
TANC	-7663	3614	-2.12	0.037	1.21
S TWI	60.2	25.8	2.34	0.022	1.23
M1S	1.5076	0.0384	39.22	0.000	1.14

Surface P_i M3 0.1M NaOH

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-483.3	80.0	-5.48	0.000	
M3S	11.157	0.701	15.91	0.000	1.10
MFD	-0.224	0.100	-2.24	0.028	1.33
S TWI	202.6	27.9	7.27	0.000	1.35

Table 5.5 Summary model data for subsurface stepwise regression models with potentially collinear model terms

Subsurface P_i M1 0.1M NaOH

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-378.2	73.7	-5.13	0.000	
CI	4.00	1.24	3.22	0.002	1.67
S TWI	162.9	24.1	6.77	0.000	1.45
M1S	0.5942	0.0349	17.00	0.000	1.27

Subsurface P_i M3 1.0M HCl

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-36.3	42.8	-0.85	0.400	
M3S	22.36	1.54	14.48	0.000	1.48
CI	-5.72	2.67	-2.14	0.036	1.48
D8 SPI	0.1441	0.0578	2.49	0.015	1.00

Subsurface P_o M1 DDI H_2O

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-36.7	14.5	-2.52	0.014	
MFD	-0.0440	0.0180	-2.44	0.017	1.31
S TWI	24.69	5.10	4.84	0.000	1.38
M1S	0.15711	0.00709	22.17	0.000	1.11

Subsurface P_o M1 0.5M NaHCO3

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-101.4	38.8	-2.61	0.011	
CI	2.175	0.653	3.33	0.001	1.67
S TWI	54.7	12.7	4.32	0.000	1.45
M1S	0.3218	0.0184	17.50	0.000	1.27

Subsurface P_o M1 0.1M NaOH

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-587	160	-3.66	0.000	
CI	11.00	2.70	4.08	0.000	1.67
S TWI	339.6	52.3	6.49	0.000	1.45
M1S	0.8370	0.0759	11.02	0.000	1.27

Subsurface P_o M3 DDI H_2O

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-43.2	21.8	-1.98	0.052	
M3M	2.739	0.178	15.38	0.000	1.16
Z Slope	32.3	13.9	2.31	0.023	1.28
S TWI	21.65	6.92	3.13	0.002	1.36

Subsurface P_o M3 0.5M NaHCO3

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-52.8	36.9	-1.43	0.156	
M3M	6.134	0.323	18.98	0.000	1.08
S TWI	30.9	11.7	2.64	0.010	1.10
D8 SPI	0.0297	0.0142	2.09	0.040	1.02

Table 5.6 Frequency of terms across all initial stepwise regression models

Model Terms	Number of Occurrences	% of models including term
Saga topographic wetness index (S TWI)	20	62.5
Convergence index (CI)	7	21.9
Z score individual study site elevation (ELE)	3	9.4
Multiple flow direction up slope contributing area (MFD)	3	9.4
Z score of individual study site slope (Z Slope)	3	9.4
SPI from D8 up slope contributing area (D8 SPI)	2	6.3
TWI from D8 up slope contributing area (D8 TWI)	1	3.1
SPI from INF up slope contributing area (DINF SPI)	1	3.1
Profile curvature (PRC)	1	3.1
Tangential curvature (TANC)	1	3.1

Table 5.7 Final regression models for sequentially extracted P fractions with adjusted R^2 - a modified version of R-squared that has been adjusted for the number of predictors in the model, R^2 fit 1 - fit against the validation set using point STP data, R^2 fit 2 - fit against the validation set using field average STP data and S the standard error of the regression

Model	Stepwise regression equation	Model Adj. R2	R2 fit 1 *	R2 fit 2 *	S
Surface P_i M1 DDI H2O	Y = -25.1 + 0.11832 M1S + 11.25 S TWI	83.8%	73.4%	78.3%	22.1
Surface P_i M1 0.5M NaHCO3	Y = -122.6 + 0.2807 M1S + 59.81 S TWI	84.9%	89.0%	81.2%	49.8
Surface P_i M1 0.1M NaOH	Y = -422.3 + 0.6617 M1S + 190.4 S TWI	82.9%	78.0%	81.3%	128
Surface P_i M1 1.0M HCl	Y = -161.6 + 1.4928 M1S + 77.9 S TWI	95.3%	95.7%	89.3%	143
Surface P_i M3 DDI H2O	Y = 8.65 + 1.743 m3s	60.2%	55.6%	63.7%	34.7
Surface P_i M3 .5M NaHCO3	Y = -91.1 + 4.348 m3s + 48.5 S TWI	63.5%	74.0%	69.1%	77.4
Surface P_i M3 0.1M NaOH	Y = -404.2 + 11.371 m3s + 174.8 S TWI	77.1%	69.9%	79.3%	148
Surface P_i M3 1.0M HCl	Y = 28.0 + 25.80 m3s - 7.71 CI	76.3%	81.1%	82.2%	322
Surface P_o M1 DDI H2O	Y = -24.2 + 0.18152 M1S + 22.60 S TWI	85.2%	75.0%	80.1%	32.0
Surface P_o M1 0.5M NaHCO3	Y = -0.6 + 0.2985 M1S + 43.3 S TWI	72.5%	78.6%	66.6%	77.3
Surface P_o M1 0.1M NaOH	Y = -56 + 1.0604 M1S + 247.7 S TWI	67.6%	61.3%	62.9%	308
Surface P_o M1 1.0M HCl	Y = -21.2 + 1.8775 M1S - 44.2 Z Elevation	96.2%	95.5%	77.5%	166
Surface P_o M3 DDI H2O	Y = -12.5 + 2.986 m3s + 17.02 S TWI	72.4%	77.1%	78.4%	43.7
Surface P_o M3 0.5M NaHCO3	Y = -4.9 + 5.380 m3s + 38.8 S TWI	74.2%	84.3%	66.0%	74.9
Surface P_o M3 0.1M NaOH	Y = -50 + 18.67 m3s + 227.3 S TWI	65.9%	61.1%	61.8%	316
Surface P_o M3 1.0M HCl	Y = -57.4 + 30.89 m3s	80.0%	80.9%	67.2%	379
Subsurface P_i M1 DDI H2O	Y = 0.50 + 0.08461 M1S	86.9%	58.4%	72.0%	14.5
Subsurface P_i M1 0.5M NaHCO3	Y = 42.8 + 0.2665 M1S - 4359 PRC	70.1%	75.8%	71.0%	74.6
Subsurface P_i M1 0.1M NaOH	Y = -268.6 + 0.6357 M1S + 124.8 S TWI	81.5%	65.3%	75.9%	128
Subsurface P_i M1 1.0M HCl	Y = 4.9 + 1.2702 M1S - 37.3 Z Elevation	94.3%	95.3%	92.6%	139
Subsurface P_i M3 DDI H2O	Y = 0.01 + 1.324 m3s	65.7%	43.7%	63.9%	23.5
Subsurface P_i M3 0.5M NaHCO3	Y = 43.6 + 4.054 m3s	52.8%	64.4%	62.6%	93.7
Subsurface P_i M3 0.1M NaOH	Y = -254.2 + 10.985 m3s + 110.5 S TWI	76.4%	62.4%	75.4%	144
Subsurface P_i M3 1.0M HCl	Y = -36.3 + 22.36 m3s - 5.72 CI	77.6%	37.0%	32.3%	274
Subsurface P_o M1 DDI H2O	Y = -29.2 + 0.15856 M1S + 19.10 S TWI	86.1%	65.6%	76.0%	27.0
Subsurface P_o M1 0.5M NaHCO3	Y = -41.8 + 0.3444 M1S + 34.0 S TWI	82.6%	76.2%	80.8%	67.3
Subsurface P_o M1 0.1M NaOH	Y = -285 + 0.9512 M1S + 235.0 S TWI	66.0%	32.5%	48.1%	287
Subsurface P_o M1 1.0M HCl	Y = -42.8 + 1.3785 M1S -	91.0%	93.5%	81.8%	192
Subsurface P_o M3 DDI H2O	Y = -20.0 + 2.629 m3s + 14.44 S TWI	74.4%	64.1%	72.6%	36.6
Subsurface P_o M3 0.5M NaHCO3	Y = -42.2 + 6.114 m3s + 27.8 S TWI	81.9%	81.1%	80.2%	68.5
Subsurface P_o M3 0.1M NaOH	Y = -304 + 17.24 m3s + 221.5 S TWI	68.3%	34.2%	48.2%	277
Subsurface P_o M3 1.0M HCl	Y = -69.3 + 22.80 m3s	76.2%	84.5%	75.5%	313

Table 5.8 Mean fit of Mehlich 1 & 3 models using point and field average soil test P values for surface, subsurface, and all data

Modeled P fractions	Soil test model term	R^2
Surface	Mehlich 1 point test values	92.4 %
	Mehlich 1 field average	85.2 %
	Mehlich 3 point test values	84.6 %
	Mehlich 3 field average	81.4 %
Subsurface	Mehlich 1 point test values	84.1 %
	Mehlich 1 field average	84.6 %
	Mehlich 3 point test values	54.6 %
	Mehlich 3 field average	52.4 %
All	Mehlich 1 point test values	89.4 %
	Mehlich 1 field average	85.1 %
	Mehlich 3 point test values	69.1 %
	Mehlich 3 field average	66.0 %

Chapter 6. Summary

Summary of Findings

P management is more than a scientific endeavor; it is an issue of science, economics, and politics. As such, evolving technology, changing socioeconomic factors and the current political will generates an elastic target in terms of what is or is not an acceptable level of P saturation in soils and dissolved P in surface waters. This evolving target does place some agriculturalists in some locations between the proverbial rock and a hard place. The availability of low cost P-rich sources of N places economic pressures to apply said N sources liberally at the expenses of over application of P. As the impacts of excessive P accumulation become more apparent, the availability of low-cost animal manure fertilizers decline, and the political will to regulate agriculture increases, acceptable levels of P will likely be lowered. It is very likely producers and conservation professionals will soon face difficult management decisions. How scientists, environmental mangers, conservationists, and members of the production agriculture community deal with this will become critical in the near future. Thus, the issues which have the most relevance not only in terms of best management practices moving forward but in terms of how environmental professionals address the problems associated with management strategies from the past must be addressed. This research in part addresses several of these issues.

Bench Top Experiments

Understanding the interactions between soils, fertilizers, and management practices is critical for sustainable agriculture and environmental protection (Harman et al., 2013). However, much of what is known in terms of such interactions comes from controlled laboratory experiments and field trials. Furthermore, the conditions in the lab experiments are often different from the conditions in the field. In particular, field trials lack the controls in place in bench top experiments. One aspect of this research focused on applying experimental conditions that more closely resemble field conditions to a traditional incubation experiment, and then characterizing the fate of that P via a sequential P extraction procedure.

A sequential P fractionation is a series of chemical extractions to characterize P by the type and/or strength of the assumed physicochemical interactions with the soil (Bowman and Cole 1978; Hedley et al., 1982; Cross and Schlesinger, 1995; Negassa and Leinweber 2009; Gagnon et al., 2012). Soil from locations with differing management histories were incubated over a period of time following one of several P treatments both with and without the presence of actively growing plants. During the course of this research it was determined there were significant differences between several fractions relative to P source and fertilization history, but no difference relative the presence of vegetation. This is a somewhat common approach to define changes in soil P pools (Warren et al., 2008; He et al., 2004; Qian and Schoenau, 2000; Yang et al., 2002; Negassa and Leinweber 2009; Gagnon et al., 2012). In general, the results of this research agree with other results in terms of the effect of management history (Fox and Kamprath, 1970; Pote et al., 2003; Bond et al., 2006). In other aspects (impact of P source on P distribution and fractionation) there are conflicting results. One collection of papers (Eghball et al. 2005; Sikora and Enkiri 2005; Zvomuya et al. 2006; Sneller and Laboski 2009) indicate source of P was not important in terms of P distribution and fractionation while another (Gracy 1984; Motavalli et al. 1989; Sharpley and Sisak 1997; Griffin et al. 2003; Miller et al. 2010) indicated it was. These results concur with the second group. However, results did not indicate and effect from actively growing vegetation.

Identifying Actual Patterns in Sequentially Extracted P Fractions

Organic P (P_o) in soil is the lesser studied part of the total P pool. Understanding P_o is critical in managing potential P loss to the environment. If one were to assume P_o moves across and within landscapes, it would be expected that evidence of that movement would be identified when the sequentially extracted P_o fractions are examined across management units. However, a pattern is not always indicative of what is assumed to be causing it. Thus, to make that distinction clearer, it requires a deeper examination of the data. To determine if P_o has moved over time on these research sites, sequentially extracted P fractions were analyzed for spatial significance (an indication that the P_o levels seen across the landscape were not random). Next statistical techniques were used to identify soil properties that could explain some of the patterns seen in the P_o data, and other techniques were used to determine if the remaining variability in the P_o data still exhibited spatial significance (a nonrandom pattern across the landscape).

To address the issue of true spatial significance, regression and Mantel tests (Bruland and Richardson, 2004) were used to identify those soil properties that explained a significant portion of the P_o variability, then fixed the effect of those variables and examined the residual variability for spatial dependence. Results identified 0.5M $NaHCO_3$ and 1.0M HCl extractable P_o fractions as exhibiting a pure spatial component in their distribution (real spatial pattern not explained by changes in soil properties). Other extractable fractions did not exhibit such spatial structure.

Explaining Spatially Significant Patterns in Sequentially Extracted P Fractions

The next logical step after determining a spatially significant pattern in sequentially extracted P_o fractions across the study sites has occurred is to try and understand why. The initial assumption is the differentiation in P_o fractions is related to redistribution of P_o via the movement of water. There are numerous examples of using topographic data to model moisture, water movement, and soil properties (e.g., Moore et al., 1991; Hornberger & Boyer, 1995; Iverson et al., 1997; Famiglietti et al. 1998; Boerner et al., 2000; Gessler et al., 2000; Western et al. 2001; Mohanty and Skaggs 2001; Case et al., 2005). Thus, one can reasonably expect there to be some topographic metric that relates to water movement that will explain the variability seen in the P_o data, assuming the distribution is, in fact, related to the movement of water.

Among topographic data, the variables can be generally described as primary or secondary (sometimes called compound) attributes (Bishop and Minasny 2006). Common primary attributes are slope gradient, slope aspect, and slope curvature. One of the most common secondary attributes is the topographic wetness index (TWI) (Bishop and Minasny 2006). In general, secondary attributes tend to be more useful than primary attributes for predicting soil properties (Bell et al. 1994; Gessler et al., 1995; McBratney et al., 2000; Bishop and Minasny 2006). In particular, TWI describes the likelihood of a location to accumulate water due to its surrounding topography (Gruber and Peckham, 2009). Another compound attribute related to water movement is the stream power index (SPI), which describes erosion and related landscape processes (Moore et al., 1991). These compound attributes utilize variables that can be calculated multiple ways. In this research, the common primary and compound terrain attributes and the multiple ways terrain attributes can be calculated were examined to determine if terrain attributes explained the residual variability in P_o distributions not explained by changes in soil properties.

It is reasonable to assume one would be able to explain the P_o distribution using these variables. In fact, Moore et al. (1993) successfully used to TWI to explain STP data at field scale. When this approach was applied to this data, the variability not explained by changes in soil properties was adequately explained by terrain attributes. Specifically, in the location with the longest history of manure applications, a spatial pattern in the 1.0 M HCl extractable P_o fraction in the surface samples was identified, the combination of Mehlich 1 extractable Ca, field subdivision and topographic wetness index explained the spatially autocorrelated variability at the location. In the other location with an extensive history of manure applications (but to a muck lower extent than the previous location) showed similar patterns in the 0.5 M NaHCO3 extractable P_o fraction from the surface samples. Similarly, when soil and topographic properties were used to model variability, the spatially autocorrelated variability was full explained. This further reinforces the concept of P_o movement by water and indicates the potential utility of topographic variables for modeling purposes.

Modeling Sequentially Extracted P Fractions Across Complex Landforms

Upon determining the topographic relationship to the observed spatial variability seen in P_o fractions cross complex landforms, the goal was to develop predictive models for sequentially extracted P fractions. Knowing P mobility depends on hydrological and chemical processes (Galeone, 1996; Easton et al., 2009) there was a reasonable expectation that these P distributions could be modeled. Given that P movement follows the direction of surface and subsurface water movement (Smeck and Runge, 1971) and that the movement of water and other materials are controlled by slope configuration (Huggett, 1975), one would expect topographic variables to become significant model terms. To this end, the spatially explicit sequentially extracted P data was divided into a model building and model testing data set. The data was used to establish a series of statistical soil-landscape models that best explained the spatial distributions these P fractions across hay and grass pastures in West Virginia, and those models compared to the actual values identified in the model test data set.

Successful models were developed. The models explained between 56% and 98% of the variability in the data. Mehlich1 STP (M1) data generally had better R2 values then the Mehlich 3 STP (M3) data. Spatially explicit STP data for the most part explained a higher percentage of variability than field averages. However, all models (M1 vs. M3 and Point vs. Field) performed well enough to potentially be useful.

Implications

Existing benchtop research can be accepted at face value. The presence of vegetation does not appear to alter the transformations of P in incubation studies. Sequentially extracted P fractions appear to establish themselves across landscapes in predictable patterns. Some of the spatial variability can be explained by changes in soil properties. However, this could be in part related to possible collinearity between soil properties and patterns of water movement. When fixed for changes in soil properties, few fractions exhibit patterns of spatial significance. When the variability explained by TWI was taken in account, none of the sequentially extracted P fractions were spatially autocorrelated. However, this does not mean sequentially extracted P fractions cannot be modeled successfully. In fact, STP levels alone and in conjunction with topographic variables can be used to create adequate models.

In time, efforts will begin to further reduce P loss into the environment. When this occurs, environmental managers will have the ability to identify the fractions that pose the greatest risk of loss, model interception strategies, and plot regional responses. As the P source in the environment that needs to be sequestered is identified, environmental professionals can develop techniques to sorb, restrain, or otherwise physically stop the P loss. The modeling techniques outlined here would enable conservation professionals to better describe risk on a field by filed basis. The modeling techniques outlined here would enable conservation professionals to allocate funds to locations with the highest potential P retained per dollar spent.

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Appendix

Sequentially extracted phosphorus fractions (benchtop)

History: Hi - long history of manure application Low - little to no manure application; Treatment: C - Control , LM -Leached Manure, M - Manure, ML Manure Leachate, P_i - Inorganic P, P_o - organic P; Vegetation: B - bare/no vegetation, V - with actively growing plants/vegetation; Rep: replicate sample number; Sequential extracted fraction: H2Ot - Deionized distilled water extractable total P fraction, H2Oi - Deionized distilled water extractable inorganic P fraction, H2Oo - Deionized distilled water extractable total P fraction, NaHCO3t - NaHCO3 extractable total P fraction, NaHCO3i - NaHCO3 extractable inorganic P fraction, NaHCO3o - NaHCO3 extractable organic P fraction, NaOHt - NaOH extractable total P fraction, NaOHi - NaOH extractable inorganic P fraction, NaOHo - NaOH extractable organic P fraction, HClt - HCl extractable total P fraction, HCli - HCl extractable inorganic P fraction, HClo - HCl extractable organic P fraction

ID	History	Treatment	Vegetation	rep	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
11	HI	C	B	3	29.0	45.5	209	54.9	5.0	9.5	22.5	39.0	24.0	36.0	186	15.9
13	HI	C	B	2	29.2	47.6	226	52.6	4.0	10.1	19.0	44.0	25.2	37.4	207	8.6
31	HI	C	B	1	23.7	46.7	203	51.6	4.5	10.0	16.0	38.0	19.2	36.7	187	13.6
10	HI	C	V	3	29.6	45.0	175	66.2	5.0	9.9	20.0	50.0	24.6	35.1	155	16.2
69	HI	C	V	2	34.2	84.4	247	46.6	5.5	11.0	24.5	39.0	28.7	73.4	223	7.6
71	HI	C	V	1	35.7	80.8	257	53.6	5.0	9.0	23.0	40.0	30.7	71.8	234	13.6
40	HI	L M	B	3	63.1	42.4	216	50.0	4.5	9.0	17.5	35.0	58.6	33.4	198	15.0
59	HI	L M	B	2	34.5	71.2	312	49.2	5.0	11.5	25.5	39.0	29.5	59.7	287	10.2
66	HI	L M	B	1	34.2	68.4	292	56.6	5.0	10.0	23.0	47.0	29.2	58.4	269	9.6
41	HI	L M	V	3	60.7	46.7	234	53.2	5.0	9.0	18.5	39.0	55.7	37.8	215	14.2
55	HI	L M	V	1	36.1	71.2	283	44.1	5.5	10.5	21.0	37.0	30.6	60.7	262	7.1
68	HI	L M	V	2	35.9		257	50.4	4.5		23.5	40.0	31.4		233	10.4
5	HI	M	B	1	31.0	49.8	183	62.8	4.0	10.3	23.0	44.0	27.0	39.5	160	18.8
48	HI	M	B	2	63.6	43.2	225	53.1	3.5	9.9	18.0	35.0	60.1	33.3	207	18.1
62	HI	M	B	3	32.4	78.9	285	51.4	4.5	10.5	23.5	43.0	27.9	68.4	262	8.4
8	HI	M	V	2	35.4	49.9	215	63.8	4.5	9.3	25.0	46.0	30.9	40.6	190	17.8
22	HI	M	V	3	31.2	41.2	204	64.5	4.5	9.7	17.0	47.0	26.7	31.5	187	17.5
49	HI	M	V	1	33.3	84.4	268	45.8	4.5	13.5	23.0	38.0	28.8	70.9	245	7.8
58	HI	M L	B	1	37.8	73.9	298	50.6	5.0	11.5	23.0	41.0	32.8	62.4	275	9.6
61	HI	M L	B	3	32.8	75.1	284	39.9	4.5	9.5	21.0	33.0	28.3	65.6	263	6.9
67	HI	M L	B	2	33.5	73.3	303	56.9	5.0	10.5	26.5	42.0	28.5	62.8	276	14.9
56	HI	M L	V	1	34.0	71.8	283	51.3	5.0	11.0	23.0	42.0	29.0	60.8	260	9.3
70	HI	M L	V	2	32.7	81.7	271	59.2	4.5	11.0	24.0	44.0	28.2	70.7	247	15.2
18	HI	P_i	B	3	27.4	51.5	206	50.2	4.0	8.7	23.0	35.0	23.4	42.8	183	15.2
36	HI	P_i	B	2	15.5	46.0	223	49.7	4.0	10.7	15.5	37.0	11.5	35.3	208	12.7
57	HI	P_i	B	1	37.8	78.1	355	47.4	5.0	11.5	21.5	33.0	32.8	66.6	333	14.4
19	HI	P_i	V	3	31.0	46.2	187	46.6	5.0	10.2	19.5	35.0	26.0	36.0	168	11.6
25	HI	P_i	V	2	33.4	50.1	187	47.6	4.0	11.4	19.0	35.0	29.4	38.6	168	12.6

ID	History	Treatment	Vegetation	rep	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
65	HI	P_i	V	1	38.0	70.3	300	52.4	5.0	10.0	24.5	39.0	33.0	60.3	276	13.4
6	HI	P_o	B	1	34.2	43.6	197	59.1	4.0	10.3	52.0	45.0	30.2	33.3	145	14.1
46	HI	P_o	B	2	69.2	45.7	237	49.0	3.5	9.7	17.5	33.0	65.7	36.0	219	16.0
53	HI	P_o	B	3	37.4	85.1	278	46.0	5.5	11.5	20.0	36.0	31.9	73.6	258	10.0
15	HI	P_o	V	1	31.3	41.2	253	56.6	4.0	8.5	18.0	42.0	27.3	32.8	235	14.6
44	HI	P_o	V	3	60.2	41.9	211	56.2	4.5	9.1	18.0	37.0	55.7	32.8	193	19.2
51	HI	P_o	V	2	41.6	78.5	276	43.4	5.5	11.5	23.5	36.0	36.1	67.0	253	7.4
14	Low	C	B	3	1.15	12.2	47.8	0.00	1.00	1.39	2.00	2.00	0.15	10.8	45.8	0.00
17	Low	C	B	1	1.53	12.9	59.2	0.00	0.50	0.41	1.50	2.00	1.03	12.5	57.7	0.00
28	Low	C	B	2	1.06	12.3	53.5	0.00	1.00	0.68	1.50	1.00	0.06	11.6	52.0	0.00
16	Low	C	V	1	1.23	11.2	47.5	0.00	0.50	0.64	2.00	2.00	0.73	10.6	45.5	0.00
21	Low	C	V	2	1.62	12.0	53.7	0.00	0.50	0.62	2.00	1.00	1.12	11.4	51.7	0.00
37	Low	C	V	3	0.41	13.2	70.6	0.00	0.50	1.13	1.50	2.00	0.00	12.1	69.1	0.00
27	Low	L M	B	2	1.17	12.9	56.2	0.00	0.50	0.67	1.50	1.00	0.67	12.2	54.7	0.00
32	Low	L M	B	1	1.04	13.4	60.3	0.54	2.00	0.95	3.00	2.00	0.00	12.5	57.3	0.00
39	Low	L M	B	3	2.94	12.9	56.5	0.00	1.50	1.39	1.50	1.00	1.44	11.5	55.0	0.00
12	Low	L M	V	3	1.89	12.5	57.7	0.00	1.50	1.76	1.50	2.00	0.39	10.7	56.2	0.00
30	Low	L M	V	2	1.10	12.7	63.8	0.46	1.00	0.81	2.50	1.00	0.10	11.9	61.3	0.00
38	Low	L M	V	1	0.10	13.0	57.8	0.00	0.50	1.79	2.00	2.00	0.00	11.3	55.8	0.00
3	Low	M	B	1	1.63	13.5	49.1	0.00	0.50	1.54	1.50	3.00	1.13	12.0	47.6	0.00
42	Low	M	B	2	2.80	12.6	61.0	0.00	1.50	1.07	2.50	3.00	1.30	11.6	58.5	0.00
52	Low	M	B	3	2.44		81.5	0.75	0.50		2.00	5.00	1.94		79.5	0.00
2	Low	M	V	2	1.48	13.2	61.1	0.00	1.50	1.13	2.00	2.00	0.00	12.1	59.1	0.00
24	Low	M	V	1	1.17	12.9	55.7	0.00	1.00	0.65	2.00	1.00	0.17	12.3	53.7	0.00
43	Low	M	V	3	2.40	12.4	76.7	0.00	1.00	0.86	3.00	2.00	1.40	11.6	73.7	0.00
9	Low	M L	B	2	1.95	12.3	49.1	0.46	1.00	0.80	2.00	2.00	0.95	11.5	47.1	0.00
20	Low	M L	B	3	1.39	12.4	55.3	0.00	0.50	2.44	2.00	2.00	0.89	10.0	53.3	0.00
64	Low	M L	B	1	1.97	18.8	76.9	0.02	0.50	0.50	1.50	2.00	1.47	18.3	75.4	0.00
23	Low	M L	V	3	1.19	11.0	57.7	0.00	1.50	0.69	2.00	2.00	0.00	10.3	55.7	0.00
29	Low	M L	V	1	0.50	12.5	53.9	0.00	1.00	1.07	1.50	2.00	0.00	11.4	52.4	0.00
50	Low	M L	V	2	2.33	21.6	71.7	0.61	0.50	1.00	2.00	2.00	1.83	20.6	69.7	0.00
35	Low	Pi	B	2	0.04	13.1	56.2	0.00	1.50	2.99	2.00	1.00	0.00	10.1	54.2	0.00
47	Low	Pi	B	1	2.51	15.2	56.4	0.00	1.50	1.01	2.50	3.00	1.01	14.2	53.9	0.00
54	Low	Pi	B	3	2.03	23.0	82.7	1.55	1.50	1.50	3.00	2.00	0.53	21.5	79.7	0.00
45	Low	Pi	V	2	2.78	14.7	55.2	0.00	0.50	1.87	3.00	3.00	2.28	12.8	52.2	0.00
63	Low	Pi	V	1	2.20	19.2	86.5	1.45	0.50	2.00	3.00	3.00	1.70	17.2	83.5	0.00
72	Low	Pi	V	3	2.11	25.8	72.5	2.27	1.50	2.00	2.50	3.00	0.61	23.8	70.0	0.00
4	Low	Po	B	1	1.87	11.8	44.7	0.00	0.50	0.59	2.00	2.00	1.37	11.2	42.7	0.00
26	Low	Po	B	2	2.10	13.2	53.4	0.28	1.00	1.41	2.00	2.00	1.10	11.8	51.4	0.00

ID	History	Treatment	Vegetation	rep	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
34	Low	Po	B	3	0.17	12.9	61.1	0.00	0.50	1.36	2.00	2.00	0.00	11.5	59.1	0.00
1	Low	Po	V	3	2.14	13.3	68.8	0.00	0.50	1.10	2.00	1.00	1.65	12.2	66.9	0.00
7	Low	Po	V	1	1.84	12.9	63.5	0.34	0.50	2.08	2.50	2.00	1.34	10.9	61.0	0.00

Sequentially extracted phosphorus fractions (field data)

Horizon: S - surface, SS ; Thickness: horizon thickness in cm; Site: H1 - high one (location 1 with long history of manure application), H2 - high two (location 2 with long history of manure application), L1 - low 1 (location 1 with little to no history of manure application), L2 - low 2 (location 2 with little to no history of manure application); ID: h1-10 (sample locations 1-10 in the high stratification) m1--10 (sample locations 1-10 in the medium stratification), l1-10 (sample location 1-10 in the low stratification); Sequential extracted fraction: H2Ot - Deionized distilled water extractable total P fraction, H2Oi - Deionized distilled water extractable inorganic P fraction, H2Oo - Deionized distilled water extractable total P fraction, NaHCO3t - NaHCO3 extractable total P fraction, NaHCO3i - NaHCO3 extractable inorganic P fraction, NaHCO3o - NaHCO3 extractable organic P fraction, NaOHt - NaOH extractable total P fraction, NaOHi - NaOH extractable inorganic P fraction, NaOHo - NaOH extractable organic P fraction, HClt - HCl extractable total P fraction, HCli - HCl extractable inorganic P fraction, HClo - HCl extractable organic P fraction

Horizon	Thickness	Site	ID	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
S	7	H1	h1	371	728	1803	2468	126	372	641	1326	245	356	1162	1142
S	5	H1	h10	292	582	1464	2050	93.0	340	522	1052	199	242	942	999
S	5	H1	h2	246	493	1857	1667	89.3	208	562	918	156	285	1295	749
S	8	H1	h3	254	646	2093	2632	78.3	358	745	1508	176	288	1349	1124
S	5	H1	h4	243	613	1373	2919	100	303	443	1604	143	310	930	1315
S	5	H1	h5	242	623	1622	2990	100	331	575	1628	141	292	1047	1362
S	6	H1	h6	320	692	1751	2387	138	376	610	1293	182	316	1141	1094
S	5	H1	h7	429	668	1919	2390	191	390	538	1250	237	278	1381	1140
S	4	H1	h8	259	600	2119	3431	108	336	785	1644	152	265	1333	1787
S	5	H1	h9	353	670	1939	3709	136	355	643	1663	218	315	1296	2045
S	9	H1	l1	320	748	3268	4606	115	332	832	1855	205	416	2436	2751
S	4	H1	l10	545	1072	2857	5602	185	418	912	2194	360	654	1945	3408
S	7	H1	l2	454	639	3043	3802	183	229	752	1728	271	410	2291	2074
S	9	H1	l3	284	613	3012	3505	113	304	742	1651	171	310	2270	1855
S	5	H1	l4	293	668	2803	3874	121	305	731	1661	171	362	2072	2212
S	8	H1	l5	200	583	2572	3035	71.3	247	628	1442	129	336	1944	1593
S	8	H1	l6	233	750	2640	4998	89.0	334	748	1903	144	416	1892	3094
S	7.5	H1	l7	425	959	2583	5945	164	462	834	2249	261	497	1749	3697
S	5	H1	l8	409	808	2624	4433	138	307	804	1954	271	501	1820	2480
S	5	H1	l9	484	964	2929	5122	167	377	896	2065	317	587	2033	3057
S	5	H1	m1	278	592	1557	2225	77.2	218	595	1204	201	374	961	1021
S	3	H1	m10	432	700	2638	5238	166	378	857	2111	267	322	1780	3127

Horizon	Thickness	Site	ID	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
S	10	H1	m2	242	991	2008	4407	66.1	381	771	1884	176	609	1237	2523
S	4	H1	m3	312	688	2021	2257	96.6	224	664	1244	216	464	1357	1013
S	7	H1	m4	244	771	1817	2714	67.4	240	655	1051	176	532	1162	1663
S	7	H1	m5	302	779	2080	2189	83.6	241	729	1193	218	538	1351	997
S	5	H1	m6	377	909	2121	3166	140	305	746	1436	237	604	1376	1730
S	6	H1	m7	382	800	2423	3529	141	327	859	1691	241	473	1564	1838
S	3	H1	m8	282	750	2430	3134	81.3	291	817	1509	201	459	1613	1626
S	3	H1	m9	311	512	2406	1750	136	216	688	930	175	295	1719	820
S	6	H1	m1	28.8	96.2	540	47.8	0.00	2.71	16.4	43.1	28.8	93.5	524	12.9
S	6.5	H2	h1	114	421	2415	524	0.00	160	725	219	114	261	1690	304
S	5	H2	h10	154	527	2491	1064	55.0	233	709	551	100	294	1782	513
S	9	H2	h2	160	538	2082	1009	35.7	235	593	510	124	303	1489	499
S	9	H2	h3	122	344	1519	725	37.7	141	441	357	84.5	203	1078	369
S	9	H2	h4	186	406	1469	749	56.8	171	390	359	129	235	1079	391
S	9	H2	h5	111	403	1870	981	35.8	98.9	565	612	75.3	304	1305	370
S	7	H2	h6	165	561	2026	944	30.7	246	529	446	134	315	1498	498
S	6	H2	h7	177	591	2342	912	59.0	226	640	434	118	365	1701	478
S	7	H2	h8	188	559	2597	955	68.0	235	744	444	120	324	1853	510
S	6	H2	h9	163	562	2193	752	31.2	234	573	438	132	328	1619	314
S	10	H2	l1	174	406	1271	962	44.1	212	414	511	130	194	857	451
S	8	H2	l10	198	400	1697	676	84.7	162	486	311	113	238	1211	365
S	10	H2	l2	137	404	1417	741	29.6	192	447	358	108	212	970	383
S	10	H2	l3	153	444	873	1261	68.8	159	536	624	83.9	285	594	636
S	19	H2	l4	80.3	316	1301	621	38.0	109	413	295	42.3	207	888	326
S	8	H2	l5	208	498	2056	823	61.4	189	525	220	146	310	1531	603
S	11	H2	l6	128	455	2064	1104	17.0	167	597	583	111	289	1467	522
S	13	H2	l7	171	484	1844	677	17.4	173	480	321	154	311	1364	356
S	9	H2	l8	149	350	1250	727	46.7	159	321	336	102	192	929	391
S	5	H2	l9	187	434	2171	793	84.9	186	553	404	102	248	1618	388
S	27	H2	m1	89.9	245	894	493	36.6	137	322	250	53.2	107	573	243
S	2	H2	m10	243	498	1376	932	90.4	217	475	455	152	280	901	477
S	9	H2	m2	172	400	1356	1334	55.4	209	483	720	117	192	873	614
S	10	H2	m3	122	369	1387	989	52.4	180	449	608	69.4	189	939	381
S	12	H2	m4	158	398	1361	1188	22.7	146	438	640	136	252	924	548
S	9	H2	m5	205	442	1104	927	76.4	177	391	521	129	265	713	406
S	10	H2	m6	130	476	1503	1277	9.19	209	549	708	121	267	954	569
S	13	H2	m7	128	426	1262	849	16.5	137	437	457	111	289	825	392
S	6	H2	m8	156	409	1202	934	12.0	159	430	455	144	250	772	479
S	13	H2	m9	163	460	1352	904	19.5	157	469	470	143	304	883	434
S	6	L1	h1	42.3	121	751	93.6	0.00	4.37	10.0	35.9	42.3	117	741	57.6

Horizon	Thickness	Site	ID	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
S	8	L1	h10	50.3	119	666	49.5	12.1	36.5	85.4	48.8	38.2	82.2	581	1.46
S	6	L1	h2	40.4	130	750	88.6	0.00	15.2	9.78	7.68	40.4	115	741	80.9
S	5	L1	h3	46.3	127	679	130	1.40	0.00	0.00	16.1	44.9	127	679	114
S	6	L1	h4	54.4	98.9	831	155	0.03	24.4	35.0	47.1	54.4	74.5	796	108
S	7	L1	h5	57.9	117	689	68.4	0.00	30.3	72.5	73.2	57.9	86.4	616	21.4
S	7	L1	h6	83.4	133	645	86.5	0.00	48.3	72.4	157	83.4	84.8	573	8.17
S	9	L1	h7	20.1	95.6	539	29.9	0.00	12.0	59.8	23.9	20.1	83.6	479	6.00
S	7	L1	h8	37.1	125	655	42.0	37.4	37.4	62.2	24.9	0.00	87.3	593	17.2
S	8	L1	h9	42.8	141	681	44.5	0.00	47.9	72.1	48.1	42.8	92.8	609	0.00
S	8	L1	l1	19.5	79.5	547	44.4	0.45	18.5	22.2	58.8	19.1	61.0	525	0.00
S	9	L1	l10	19.2	104	756	58.1	0.00	0.54	0.00	3.26	19.2	103	756	54.8
S	7	L1	l2	40.8	126	433	24.5	2.73	24.2	27.7	77.1	38.1	102	405	0.00
S	9	L1	l3	25.8	103	730	64.9	0.00	11.2	0.00	28.3	25.8	91.7	730	36.6
S	9	L1	l4	53.0	120	573	199	8.66	13.6	9.11	40.8	44.4	107	564	158
S	6	L1	l5	43.8	87.6	537	48.5	23.1	15.9	7.77	8.27	20.7	71.8	529	40.3
S	8	L1	l6	29.4	88.0	522	58.3	0.00	11.2	0.76	15.2	29.4	76.8	522	43.2
S	5	L1	l7	90.2	152	731	98.2	39.3	29.4	30.2	30.7	50.9	122	700	67.4
S	7	L1	l8	39.1	111	719	103	0.00	12.4	4.96	19.7	39.1	98.4	715	83.1
S	9	L1	l9	27.3	92.3	564	115	0.00	5.26	2.55	12.1	27.3	87.1	561	103
S	5	L1	m10	73.3	192	746	68.2	19.4	50.0	8.83	95.7	53.8	142	737	0.00
S	8	L1	m2	18.9	116	590	28.6	0.00	7.17	34.3	58.7	18.9	109	555	0.00
S	5	L1	m3	35.9	85.1	604	67.4	0.00	4.58	4.69	59.9	35.9	80.5	600	9.17
S	6	L1	m4	40.9	97.3	538	83.5	2.93	21.3	28.3	75.9	37.9	76.0	510	7.54
S	7	L1	m5	40.2	125	602	52.8	4.07	21.0	30.5	71.7	36.1	104	571	7.95
S	9	L1	m6	32.2	106	507	44.7	0.00	15.8	9.39	75.8	32.2	90.0	497	0.00
S	8	L1	m7	39.1	122	571	35.4	2.37	26.4	33.0	80.7	36.8	96.0	538	0.00
S	9	L1	m8	26.0	86.5	471	35.6	0.00	0.00	7.47	64.1	26.0	86.5	463	0.00
S	6	L1	m9	46.0	126	663	73.3	2.48	16.2	10.1	69.8	43.5	110	653	15.4
S	4.5	L2	h1	39.8	93.9	566	25.0	0.00	0.00	30.1	0.00	39.8	93.9	536	25.0
S	5	L2	h10	21.7	97.5	453	6.98	0.00	5.31	9.49	14.3	21.7	92.1	443	2.94
S	3.5	L2	h2	61.5	133	737	60.2	0.00	42.5	69.0	19.2	61.5	90.5	668	41.0
S	5	L2	h3	19.5	134	568	18.9	0.00	5.29	32.6	17.9	19.5	129	535	3.98
S	5	L2	h4	19.8	74.4	485	11.2	0.00	9.18	4.06	0.00	19.8	65.2	481	11.2
S	4	L2	h5	11.6	121	735	54.9	0.00	10.6	61.0	18.9	11.6	110	674	36.1
S	3	L2	h6	19.2	41.2	429	15.1	0.00	0.00	6.54	30.3	19.2	41.2	423	0.00
S	5.5	L2	h7	23.7	49.0	417	19.5	0.00	17.4	21.1	22.4	23.7	31.7	396	5.22
S	5	L2	h8	15.9	45.3	392	6.06	4.24	3.48	15.5	0.00	11.7	41.8	377	6.06
S	5	L2	h9	16.4	75.1	436	4.70	0.00	1.91	14.2	4.03	16.4	73.2	421	3.95
S	4	L2	l2	28.8	167	605	79.0	0.00	18.3	52.8	0.00	28.8	149	552	79.0
S	6	L2	l3	18.5	97.7	387	7.48	0.00	59.6	49.2	0.00	18.5	38.2	338	7.48

Horizon	Thickness	Site	ID	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
S	5	L2	l4	20.1	158	614	53.0	0.00	22.2	40.3	29.8	20.1	136	574	23.2
S	4.5	L2	l5	20.5	143	540	28.7	0.00	21.9	20.9	35.6	20.5	121	520	0.73
S	4	L2	l6	23.3	123	533	46.3	0.00	25.7	51.7	15.1	23.3	96.9	481	31.2
S	4	L2	l7	21.4	152	536	43.8	0.00	18.3	18.0	31.0	21.4	134	518	12.8
S	5	L2	l8	35.4	255	546	22.7	6.93	21.2	38.1	0.00	28.4	234	508	22.7
S	4	L2	l9	47.3	159	733	61.2	0.00	13.7	51.0	1.13	47.3	145	682	60.1
S	8	L2	m1	30.6	159	781	98.8	0.00	48.6	109	48.6	30.6	110	671	50.3
S	4	L2	m10	24.0	94.5	363	19.4	0.00	31.1	16.8	14.5	24.0	63.4	346	8.49
S	4	L2	m2	30.8	153	759	68.3	0.00	61.0	98	36.5	30.8	92.0	662	31.7
S	4	L2	m3	25.9	127	661	44.4	0.00	52.4	82.0	46.8	25.9	74.9	579	0.00
S	5	L2	m4	23.5	99.0	497	21.7	18.0	24.2	72.5	24.2	5.47	74.8	424	0.00
S	5	L2	m5	37.1	174	911	101	0.00	61.6	142	73.9	37.1	112	769	26.8
S	7.5	L2	m6	23.6	128	653	62.0	12.3	61.3	116	73.5	11.4	66.6	537	0.00
S	6	L2	m7	26.5	233	507	49.0	0.00	46.3	118	0.00	26.5	186	389	49.0
S	4	L2	m8	16.5	152	467	31.5	0.00	15.7	64.1	7.93	16.5	137	403	23.5
S	3	L2	m9	61.6	186	505	26.7	0.00	25.4	0.00	2.46	61.6	160	505	24.3
SS	10	H1	h1	229	678	1322	2179	71.8	344	519	1191	158	334	803	988
SS	10	H1	h10	230	487	1005	1208	86.9	285	341	648	143	202	664	560
SS	10	H1	h2	250	612	1934	1450	89.4	278	670	782	160	334	1264	668
SS	10	H1	h3	206	644	1778	2522	76.4	345	644	1337	130	300	1134	1186
SS	10	H1	h4	221	674	1084	2126	86.6	350	422	1178	135	324	662	948
SS	10	H1	h5	237	606	1413	2113	94.2	301	563	1183	142	305	850	929
SS	10	H1	h6	231	646	1274	2174	84.0	357	570	1180	147	288	704	994
SS	10	H1	h7	276	611	1533	2160	97.6	344	506	1146	179	267	1027	1014
SS	10	H1	h8	253	590	1842	2138	98.7	319	660	1107	154	270	1182	1031
SS	10	H1	h9	270	609	1505	2662	89.2	346	615	1343	180	263	890	1319
SS	10	H1	l1	292	765	2514	3881	95.2	352	713	1765	197	413	1802	2116
SS	10	H1	l10	392	999	2417	4517	113	422	900	2004	280	577	1518	2513
SS	10	H1	l2	326	613	2603	2776	127	277	724	1355	198	336	1879	1420
SS	10	H1	l3	219	502	1897	1016	71.2	239	575	511	148	263	1322	505
SS	10	H1	l4	229	652	2265	3818	90.0	286	720	1735	139	366	1544	2083
SS	10	H1	l5	216	656	2297	2402	86.1	289	791	1118	130	366	1506	1283
SS	10	H1	l6	173	513	1199	2308	53.2	212	366	1113	119	301	834	1195
SS	10	H1	l7	319	975	2123	4523	103	438	862	2005	216	537	1260	2518
SS	10	H1	l8	320	771	2490	3035	118	230	859	1668	202	541	1632	1367
SS	10	H1	l9	361	968	2524	3810	115	389	875	1860	246	580	1649	1950
SS	10	H1	m1	251	574	1278	1578	75.2	216	537	865	176	358	741	713
SS	10	H1	m10	310	837	2250	5071	110	391	849	2159	200	446	1402	2912
SS	10	H1	m2	164	683	937	2625	39.2	287	438	1418	125	397	499	1208
SS	10	H1	m3	234	612	1455	1068	56.4	208	592	616	178	404	863	452

Horizon	Thickness	Site	ID	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
SS	10	H1	m4	220	791	1512	2511	56.4	289	631	1285	163	502	881	1227
SS	10	H1	m5	253	779	1988	1615	52.9	281	798	878	200	499	1191	737
SS	10	H1	m6	286	661	1585	1880	91.1	252	656	1011	195	409	928	869
SS	10	H1	m7	295	822	2404	2650	103	309	726	1318	192	513	1678	1332
SS	10	H1	m8	281	812	2131	1993	79.8	261	811	1074	202	551	1320	918
SS	10	H1	m9	314	570	2689	1870	113	194	817	1006	200	376	1872	865
SS	10	H1	m1	13.0	56.8	368	24.7	0.00	1.02	7.41	55.5	13.0	55.7	360	0.00
SS	10	H2	h1	89.4	368	2170	445	12.1	149	690	206	77.3	219	1480	238
SS	10	H2	h10	102	407	1995	502	20.4	173	541	261	81.4	234	1454	241
SS	10	H2	h2	91.0	308	1311	579	5.61	134	351	257	85.4	174	960	321
SS	10	H2	h3	108	347	1231	418	9.10	134	361	168	99.2	214	870	250
SS	10	H2	h4	105	307	1007	452	20.7	138	309	190	84.3	169	698	262
SS	10	H2	h5	80.0	310	1491	538	4.51	112	420	290	75.5	198	1071	248
SS	10	H2	h6	79.5	372	1798	524	12.1	170	501	298	67.5	201	1297	226
SS	10	H2	h7	92.3	302	1509	609	0.00	113	412	276	92.3	189	1097	334
SS	10	H2	h8	98.0	380	1914	627	24.4	154	572	301	73.6	226	1343	326
SS	10	H2	h9	95.2	411	1739	717	15.0	194	445	351	80.2	217	1294	366
SS	10	H2	l1	94.7	292	956	369	11.7	165	359	197	83.0	127	597	172
SS	10	H2	l10	101	321	1403	580	19.4	148	393	263	81.6	173	1009	317
SS	10	H2	l2	119	368	1074	367	8.28	188	384	160	111	180	690	207
SS	10	H2	l3	106	359	1256	713	54.1	123	433	311	52.1	236	823	402
SS	10	H2	l4	46.9	100	465	290	0.00	31.2	117	302	46.9	68.6	349	124
SS	10	H2	l5	188	499	1929	702	71.8	188	580	329	116	311	1349	374
SS	10	H2	l6	100	337	1164	547	0.85	149	321	295	100	188	843	252
SS	10	H2	l7	212	536	1831	419	32.1	222	533	197	180	313	1298	222
SS	10	H2	l8	128	347	1111	610	86.9	161	343	290	41.1	186	768	319
SS	10	H2	l9	136	414	1923	512	17.0	173	534	280	119	241	1389	231
SS	10	H2	m1	52.1	90.1	418	82.7	0.00	49.3	134	39.4	52.1	40.9	284	43.4
SS	10	H2	m10	147	353	1230	1185	33.1	185	400	607	114	168	830	578
SS	10	H2	m2	135	342	1075	877	8.40	185	310	485	127	157	765	391
SS	10	H2	m3	105	320	1139	797	2.13	152	431	457	103	168	708	340
SS	10	H2	m4	127	396	1141	616	15.8	192	447	309	112	204	694	307
SS	10	H2	m5	148	401	953	630	4.67	115	369	339	144	286	584	290
SS	10	H2	m6	87.0	282	763	419	0.00	129	288	218	87.0	152	475	201
SS	10	H2	m7	82.3	257	914	515	0.00	66.2	318	260	82.3	190	596	255
SS	10	H2	m8	145	402	1205	717	21.6	144	363	422	124	258	841	295
SS	10	H2	m9	150	458	1230	715	35.9	218	475	373	114	240	754	342
SS	10	L1	h1	17.8	32.9	441	48.7	0.00	0.00	0.00	3.72	17.8	32.9	441	45.0
SS	10	L1	h10	16.1	49.0	378	15.6	0.00	37.1	61.9	24.8	16.1	11.9	316	0.00
SS	10	L1	h2	13.4	40.1	524	99.3	0.00	1.37	1.35	27.2	13.4	38.7	522	72.1

Horizon	Thickness	Site	ID	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
SS	10	L1	h3	20.6	102	501	112	0.00	3.53	4.67	1.73	20.6	98.7	496	110
SS	10	L1	h4	19.8	74.3	555	96.7	0.00	0.00	0.00	15.7	19.8	74.3	555	81.1
SS	10	L1	h5	25.1	59.5	472	31.0	0.00	12.0	60.1	24.0	25.1	47.5	412	6.96
SS	10	L1	h6	28.6	67.4	475	36.7	0.00	18.4	61.6	61.5	28.6	48.9	414	0.00
SS	10	L1	h7	12.2	38.1	288	6.68	5.84	0.00	35.9	23.9	6.35	38.1	252	0.00
SS	10	L1	h8	18.0	60.7	445	48.8	46.2	63.5	46.3	34.8	0.00	8.25	399	14.0
SS	10	L1	h9	14.7	48.6	303	18.0	0.00	42.1	72.1	24.0	14.7	6.61	231	0.00
SS	10	L1	l1	13.5	36.1	198	6.17	1.55	12.8	3.33	41.2	12.0	23.3	195	0.00
SS	10	L1	l10	18.5	35.7	426	37.4	0.00	1.96	0.00	0.00	18.5	33.8	426	37.4
SS	10	L1	l2	14.6	61.5	278	3.69	0.00	9.29	0.00	43.3	14.6	52.2	278	0.00
SS	10	L1	l3	15.1	41.0	421	36.5	0.00	0.98	0.00	4.44	15.1	40.0	421	32.1
SS	10	L1	l4	14.6	21.1	220	17.2	0.00	0.00	0.00	0.00	14.6	21.1	220	17.2
SS	10	L1	l5	16.4	59.8	392	49.8	0.00	5.65	0.00	2.76	16.4	54.2	392	47.0
SS	10	L1	l6	16.1	15.9	199	28.5	0.00	0.00	1.68	0.00	16.1	15.9	197	28.5
SS	10	L1	l7	29.0	118	627	91.8	0.00	30.8	7.07	11.8	29.0	87.5	620	80.0
SS	10	L1	l8	18.0	46.4	427	70.4	0.00	11.4	0.25	0.00	18.0	35.0	427	70.4
SS	10	L1	l9	20.4	21.5	220	31.6	0.00	0.00	0.00	15.3	20.4	21.5	220	18.7
SS	10	L1	m10	28.2	94.9	556	40.3	0.00	18.9	19.8	214	28.2	75.9	536	0.00
SS	10	L1	m2	10.9	51.7	383	22.9	0.00	2.43	0.00	38.7	10.9	49.2	383	4.12
SS	10	L1	m3	16.1	72.4	408	31.6	2.82	0.00	6.52	19.8	13.2	72.4	402	11.8
SS	10	L1	m4	12.0	39.7	310	25.3	0.00	7.48	0.00	36.4	12.0	32.2	310	0.75
SS	10	L1	m5	14.8	61.9	394	29.6	0.00	0.00	0.00	63.3	14.8	61.9	394	0.00
SS	10	L1	m6	12.4	34.4	218	2.93	0.00	0.00	0.00	12.1	12.4	34.4	218	0.00
SS	10	L1	m7	12.3	34.1	275	10.3	0.00	3.10	0.00	36.8	12.3	31.0	275	0.00
SS	10	L1	m8	14.4	23.5	194	10.2	0.00	0.00	0.00	31.3	14.4	23.5	194	0.00
SS	10	L1	m9	18.9	84.3	552	55.3	0.00	4.23	31.6	90.3	18.9	80.0	521	0.00
SS	10	L2	h1	14.4	35.3	237	0.00	0.00	2.77	5.26	0.00	14.4	32.5	232	0.00
SS	10	L2	h10	7.95	30.4	213	0.00	0.00	0.00	1.24	0.30	7.95	30.4	212	0.00
SS	10	L2	h2	10.5	43.6	331	4.28	0.00	5.73	25.9	0.00	10.5	37.9	305	4.28
SS	10	L2	h3	7.22	14.7	128	0.00	0.00	0.00	0.00	0.40	7.22	14.7	128	0.00
SS	10	L2	h4	9.35	10.9	150	0.00	0.94	3.22	2.58	1.34	8.41	7.66	147	0.00
SS	10	L2	h5	15.0	28.4	277	9.58	0.00	0.08	19.6	7.18	15.0	28.3	257	3.16
SS	10	L2	h6	11.1	11.5	147	0.00	0.00	2.25	0.00	7.73	11.1	9.23	147	0.00
SS	10	L2	h7	25.4	48.4	423	8.13	3.83	23.4	72.3	3.13	21.6	25.0	351	5.00
SS	10	L2	h8	12.4	15.7	135	0.00	5.87	1.61	0.00	7.15	6.90	14.1	135	0.00
SS	10	L2	h9	11.8	29.7	192	0.00	0.00	12.2	0.00	14.5	11.8	17.6	192	0.00
SS	10	L2	l2	7.68	45.8	258	14.5	0.00	63.3	1.90	0.00	7.68	0.00	256	14.5
SS	10	L2	l3	9.51	18.0	119	0.00	0.00	13.5	0.00	0.00	9.51	9.12	119	0.00
SS	10	L2	l4	11.7	61.0	329	22.8	0.00	21.9	49.1	0.00	11.7	39.1	280	22.8
SS	10	L2	l5	8.05	37.0	232	4.93	0.00	8.34	6.81	6.10	8.05	28.7	225	2.50

Horizon	Thickness	Site	ID	H2Ot	NaHCO3t	NaOHt	HClt	H2Oi	NaHCO3i	NaOHi	Hcli	H2Oo	NaHCO3o	NaOHo	Hclo
SS	10	L2	l6	7.36	18.9	130	1.23	0.00	3.66	6.23	0.00	7.36	15.2	124	1.23
SS	10	L2	l7	8.17	42.2	236	18.7	0.00	15.7	17.8	10.2	8.17	26.5	218	8.52
SS	10	L2	l8	4.18	35.3	129	0.00	7.85	9.84	8.90	0.00	0.90	25.4	120	0.00
SS	10	L2	l9	10.3	53.1	300	0.00	0.00	20.2	29.5	0.00	10.3	32.8	270	0.00
SS	10	L2	m1	11.6	28.2	176	0.25	31.3	55.9	80.9	37.3	4.95	0.00	94.9	0.00
SS	10	L2	m10	4.88	15.7	123	0.00	0.00	0.68	2.95	0.00	4.88	15.0	120	0.00
SS	10	L2	m2	10.4	55.5	330	19.1	0.00	72.4	84.5	60.5	10.4	0.00	245	0.00
SS	10	L2	m3	15.0	28.2	190	0.72	0.00	542	71.8	72.0	15.0	0.00	118	0.00
SS	10	L2	m4	6.68	21.6	164	0.00	0.00	60.9	61.0	24.4	6.68	0.00	103	0.00
SS	10	L2	m5	19.2	56.5	362	25.4	5.95	66.5	139	48.3	13.3	0.00	223	0.00
SS	10	L2	m6	11.8	24.4	168	4.37	6.13	48.6	91.0	36.3	5.96	0.00	76.8	0.00
SS	10	L2	m7	4.10	82.4	276	15.2	0.00	14.6	8.52	0.00	4.10	67.8	267	15.2
SS	10	L2	m8	3.65	27.0	158	0.44	0.00	0.43	27.2	0.00	3.65	26.6	131	0.44
SS	10	L2	m9	4.52	50.2	183	0.00	0.00	1.16	92.0	0.00	4.52	49.1	91.4	0.00

Soil test, pH, carbon, and particle size data (field data)

Horizon: S - surface, SS ; Thickness: horizon thickness in cm; Site: H1 - high one (location 1 with long history of manure application), H2 - high two (location 2 with long history of manure application), L1 - low 1 (location 1 with little to no history of manure application), L2 - low 2 (location 2 with little to no history of manure application); ID: h1-10 (sample locations 1-10 in the high stratification) m1--10 (sample locations 1-10 in the medium stratification), l1-10 (sample location 1-10 in the low stratification); % Carbon - % carbon; pH - pH in water; pH in CaCl - pH in $CaCl_2$; Soil Test Results: M1 Al - Mehlich 1 extractable Al, M1 Fe - Mehlich 1 extractable Fe, M1 Ca - Mehlich 1 extractable Ca, M1 P - Mehlich 1 extractable P, M3 P - Mehlich 3 extractable P; Soil Particle size distribution: % Sand, % Silt, % Clay.

Horizon	Thickness	Site	ID	% Carbon	pH	pH in CaCl	M1 Al	M1 Fe	M1 Ca	M1 P	M3 P	% Sand	% Silt	% Clay
S	7	H1	h1	3.68	6.32	6.05	238	12.6	3536	852	55.1	27.2	52.1	20.7
S	5	H1	h10	3.30	6.43	6.05	210	16.9	2727	652	38.7	22.7	58.0	19.3
S	5	H1	h2	5.20	6.25	5.87	247	12.3	2675	582	40.4	24.9	52.7	22.4
S	8	H1	h3	4.46	6.36	5.87	284	18.0	2946	788	53.5	23.0	60.6	16.4
S	5	H1	h4	4.38	6.45	6.03	222	12.3	3594	857	48.5	28.6	50.6	20.8
S	5	H1	h5	3.89	6.36	5.91	238	15.4	3136	854	12.7	29.0	49.5	21.4
S	6	H1	h6	4.63	6.38	6.00	237	13.6	3178	697	8.38	25.4	53.2	21.4
S	5	H1	h7	4.47	6.41	6.14	256	10.8	3078	790	8.44	25.1	54.3	20.6
S	4	H1	h8	4.56	6.48	6.05	285	16.1	3762	951	11.0	24.0	55.8	20.3
S	5	H1	h9	4.74	6.41	6.05	251	12.6	3732	973	8.24	23.1	57.1	19.8
S	9	H1	l1	6.22	6.48	6.23	300	19.4	4742	1448	80.0	22.5	53.9	23.6
S	4	H1	l10	8.29	6.41	6.14	243	18.6	5757	1430	84.6	26.2	49.4	24.3
S	7	H1	l2	4.36	6.38	5.94	280	14.1	3225	1101	56.8	30.4	49.1	20.5
S	9	H1	l3	7.41	6.57	6.02	288	18.0	4038	1007	51.0	25.7	56.8	17.5
S	5	H1	l4	5.78	6.75	6.39	286	15.1	3686	1192	83.6	28.6	53.2	18.2
S	8	H1	l5	4.62	6.45	6.05	347	21.9	3977	882	63.6	23.6	56.6	19.8
S	8	H1	l6	5.08	6.70	6.29	213	21.5	3401	1263	72.7	26.9	52.1	21.0
S	7.5	H1	l7	7.73	6.63	6.23	250	16.5	7180	1805	106	26.7	47.7	25.6
S	5	H1	l8	10.85	6.50	6.05	262	13.4	5229	1061	62.6	19.6	62.8	17.6
S	5	H1	l9	5.00	6.57	6.16	369	24.0	6351	1553	87.0	25.3	54.0	20.7
S	5	H1	m1	5.47	6.38	5.85	279	14.5	3525	751	48.6	22.6	45.3	32.1
S	3	H1	m10	7.10	6.36	5.98	267	17.8	5486	1545	85.2	26.0	49.8	24.2
S	10	H1	m2	5.19	6.75	6.38	215	19.6	7146	1493	88.0	26.0	52.2	21.8
S	4	H1	m3	7.73	6.27	5.87	265	14.0	2963	711	50.5	16.9	60.7	22.4
S	7	H1	m4	5.68	6.39	6.02	331	16.0	5300	1081	67.3	20.6	57.9	21.5
S	7	H1	m5	5.69	5.98	5.67	278	16.6	2873	649	54.4	23.8	54.6	21.6
S	5	H1	m6	6.34	6.68	6.29	300	14.5	4765	987	69.0	23.3	55.0	21.7
S	6	H1	m7	5.22	6.43	6.03	369	17.7	4662	1061	66.5	23.0	53.2	23.8
S	3	H1	m8	7.81	6.41	5.96	377	15.3	4273	755	57.5	24.4	53.8	21.8
S	3	H1	m9	6.45	6.27	5.89	310	12.9	4174	712	48.6	27.8	47.6	24.7
S	6	H1	m1	4.33	5.55	5.21	98.7	6.79	757	12.1	4.44	46.3	44.6	9.1
S	6.5	H2	h1	5.67	5.33	5.13	85.9	117	1283	76.0	9.24	17.6	51.9	30.5
S	5	H2	h10	4.29	5.60	5.42	117	98.8	1650	158	17.7	20.1	57.9	22.0

Horizon	Thickness	Site	ID	% Carbon	pH	pH in CaCl	M1 Al	M1 Fe	M1 Ca	M1 P	M3 P	% Sand	% Silt	% Clay
S	9	H2	h2	4.96	6.18	5.94	99.2	23.2	2747	219	15.6	20.6	47.0	32.4
S	9	H2	h3	2.39	6.03	5.44	171	13.4	1621	194	20.1	23.4	52.0	24.7
S	9	H2	h4	4.59	6.20	5.89	160	14.8	1942	285	25.2	20.8	54.8	24.5
S	9	H2	h5	3.85	6.21	5.87	116	25.7	2170	154	12.9	20.6	53.3	26.0
S	7	H2	h6	3.83	6.45	6.27	129	39.0	2454	237	22.6	25.4	57.3	17.4
S	6	H2	h7	5.16	6.11	5.82	120	56.1	1793	188	20.1	17.1	53.9	28.9
S	7	H2	h8	4.08	5.64	5.48	104	77.6	1472	186	16.0	18.3	61.5	20.3
S	6	H2	h9	4.36	6.07	5.78	119	49.5	1787	173	18.5	20.2	57.9	22.0
S	10	H2	l1	3.18	5.93	5.51	181	15.4	1919	244	19.5	24.8	55.7	19.5
S	8	H2	l10	3.40	6.09	5.71	184	15.2	1929	267	23.6	25.0	52.6	22.4
S	10	H2	l2	0.30	5.75	5.31	198	17.1	1682	256	26.0	24.8	57.1	18.1
S	10	H2	l3	3.22	5.76	5.46	167	16.9	2242	375	25.4	37.8	38.4	23.9
S	19	H2	l4	2.24	6.72	6.20	117	33.2	2255	99.3	7.27	35.5	39.6	24.9
S	8	H2	l5	4.67	6.03	5.76	206	9.71	2057	305	32.0	24.6	50.5	25.0
S	11	H2	l6	3.62	5.55	5.15	126	49.7	1560	157	18.0	30.0	46.3	23.7
S	13	H2	l7	2.43	6.05	5.67	214	10.6	1676	253	30.4	30.7	51.0	18.4
S	9	H2	l8	3.42	6.23	5.89	156	9.74	2011	250	23.7	27.6	51.7	20.6
S	5	H2	l9	4.20	5.71	5.31	179	12.0	1658	198	26.5	24.3	47.0	28.6
S	27	H2	m1	2.36	6.48	5.87	141	13.7	2081	137	9.71	24.5	48.0	27.5
S	2	H2	m10	4.44	6.52	6.03	178	18.1	2013	320	27.6	28.9	52.2	18.9
S	9	H2	m2	3.69	6.14	5.76	161	18.4	2441	363	26.4	26.8	50.5	22.7
S	10	H2	m3	3.62	5.80	5.53	143	23.1	2422	246	16.4	27.4	51.1	21.5
S	12	H2	m4	2.69	5.78	5.39	179	12.8	1745	262	25.8	31.3	53.8	14.9
S	9	H2	m5	3.52	6.29	5.91	170	9.67	2433	357	30.9	26.2	52.6	21.2
S	10	H2	m6	3.32	6.14	5.78	135	32.8	2386	212	16.7	28.8	49.4	21.7
S	13	H2	m7	3.33	6.38	5.98	146	18.2	2491	187	16.0	30.9	44.0	25.1
S	6	H2	m8	2.81	6.14	5.58	162	15.8	1569	215	24.3	30.8	50.3	18.9
S	13	H2	m9	2.98	6.00	5.57	198	15.3	1806	293	32.0	28.2	54.0	17.8
S	6	L1	h1	3.76	5.31	4.92	116	14.0	786	20.1	6.81	34.2	53.9	12.0
S	8	L1	h10	3.81	5.39	4.97	89.7	23.0	574	12.8	4.96	48.0	42.2	9.8
S	6	L1	h2	3.52	5.39	4.94	164	14.7	852	14.8	5.48	34.7	52.9	12.4
S	5	L1	h3	3.57	5.31	4.95	121	20.2	747	17.8	7.22	65.9	28.6	5.5
S	6	L1	h4	5.69	5.44	5.21	124	6.66	699	16.7	4.94	42.9	47.7	9.4
S	7	L1	h5	4.51	5.53	5.22	109	6.54	876	15.5	4.78	48.4	42.8	8.8
S	7	L1	h6	4.76	5.76	5.58	106	6.55	1167	34.4	6.34	46.4	44.3	9.4
S	9	L1	h7	2.72	4.91	4.52	115	25.3	633	3.86	1.94	39.9	45.5	14.5
S	7	L1	h8	3.15	5.24	4.94	126	16.1	696	12.6	6.61	50.6	41.8	7.7
S	8	L1	h9	4.91	5.26	4.94	117	21.0	1207	11.0	4.43	31.8	53.8	14.4
S	8	L1	l1	3.89	5.10	4.61	107	23.8	880	5.18	2.16	31.6	50.4	17.9
S	9	L1	l10	3.91	4.97	4.61	127	17.4	528	3.31	2.20	22.1	57.6	20.3

Horizon	Thickness	Site	ID	% Carbon	pH	pH in CaCl	M1 Al	M1 Fe	M1 Ca	M1 P	M3 P	% Sand	% Silt	% Clay
S	7	L1	l2	3.47	5.73	4.86	132	12.1	503	15.2	4.96	54.7	39.1	6.2
S	9	L1	l3	4.45	5.24	4.76	132	7.38	584	4.47	2.24	21.9	56.2	21.9
S	9	L1	l4	5.26	5.35	4.92	113	26.7	1214	51.7	4.08	30.1	54.0	15.9
S	6	L1	l5	3.50	5.60	5.35	128	5.55	790	10.6	6.19	69.6	26.4	4.0
S	8	L1	l6	4.84	5.46	5.12	93.7	20.1	1102	7.40	2.24	26.1	54.7	19.2
S	5	L1	l7	6.16	5.57	5.22	122	10.7	1210	37.1	9.77	51.5	41.6	7.0
S	7	L1	l8	3.23	5.46	4.88	196	12.5	710	22.0	7.46	42.4	51.0	6.6
S	9	L1	l9	4.77	5.60	5.55	114	34.6	1252	9.47	1.70	3.5	75.8	20.7
S	5	L1	m10	7.69	5.35	5.03	88.9	22.3	1131	25.8	7.02	31.4	52.5	16.2
S	8	L1	m2	3.11	4.97	5.61	177	14.8	441	10.6	5.06	49.4	42.4	8.2
S	5	L1	m3	4.08	5.44	4.85	106	24.2	449	9.17	3.59	57.4	32.2	10.5
S	6	L1	m4	5.74	5.58	5.33	85.9	6.09	1119	15.9	4.50	43.0	45.1	11.9
S	7	L1	m5	4.64	5.31	4.88	117	9.88	615	12.3	4.76	46.5	44.0	9.5
S	9	L1	m6	4.10	5.15	4.72	126	36.7	953	12.8	4.04	38.6	48.4	13.1
S	8	L1	m7	4.10	5.46	5.01	132	13.0	684	16.0	6.59	53.8	40.2	5.9
S	9	L1	m8	4.59	5.49	5.12	89.5	16.6	1068	5.85	1.63	24.1	54.2	21.7
S	6	L1	m9	4.36	5.42	5.03	138	12.2	887	10.5	8.41	48.5	43.6	7.9
S	4.5	L2	h1	5.62	5.19	4.68	169	59.6	842	9.04	5.52	21.4	66.4	12.2
S	5	L2	h10	5.13	4.88	4.43	170	118	679	6.99	5.42	22.2	66.8	11.0
S	3.5	L2	h2	8.59	4.90	4.68	186	38.8	808	21.2	4.95	16.9	63.1	20.0
S	5	L2	h3	7.34	4.81	4.27	258	133	664	7.53	2.14	21.1	65.3	13.6
S	5	L2	h4	4.83	5.01	4.38	252	100	513	7.97	2.28	19.3	68.8	11.9
S	4	L2	h5	4.52	4.40	3.95	289	177	411	11.2	3.91	19.1	69.6	11.3
S	3	L2	h6	3.77	5.40	4.86	140	43.7	974	11.9	2.28	18.0	70.1	11.9
S	5.5	L2	h7	3.26	5.58	5.06	125	39.4	747	12.3	2.78	18.8	69.6	11.6
S	5	L2	h8	3.09	5.08	4.56	169	84.7	509	8.59	2.86	20.0	68.7	11.3
S	5	L2	h9	4.16	5.03	4.56	183	70.3	648	8.85	1.55	23.9	64.9	11.2
S	4	L2	l2	4.36	4.90	4.56	162	45.9	768	17.3	5.40	19.6	67.1	13.2
S	6	L2	l3	3.83	5.12	4.68	163	32.0	846	10.7	3.17	20.1	67.5	12.4
S	5	L2	l4	3.79	5.08	4.77	137	27.0	756	14.6	6.00	17.0	69.6	13.5
S	4.5	L2	l5	4.28	5.24	4.85	139	19.6	928	13.4	5.36	17.3	69.7	12.9
S	4	L2	l6	4.08	5.22	4.99	123	27.5	759	10.4	3.85	16.1	70.4	13.5
S	4	L2	l7	4.40	5.08	4.79	125	25.4	847	12.3	4.77	19.7	67.0	13.4
S	5	L2	l8	6.78	4.94	4.36	219	110	494	13.3	3.67	21.9	66.2	11.9
S	4	L2	l9	7.42	5.01	4.63	191	8.06	466	7.21	4.12	14.4	72.2	13.4
S	8	L2	m1	6.93	4.99	4.70	189	57.3	1278	17.2	4.64	16.1	73.1	10.7
S	4	L2	m10	3.89	5.30	4.94	129	33.1	684	12.6	3.49	23.9	64.7	11.4
S	4	L2	m2	5.15	5.01	4.50	170	58.1	1038	14.4	4.39	17.0	72.4	10.6
S	4	L2	m3	4.21	5.21	4.67	156	46.5	654	10.1	3.95	23.6	65.5	11.0
S	5	L2	m4	3.39	5.26	4.59	174	57.2	662	6.96	2.53	25.5	63.6	10.8

Horizon	Thickness	Site	ID	% Carbon	pH	pH in CaCl	M1 Al	M1 Fe	M1 Ca	M1 P	M3 P	% Sand	% Silt	% Clay
S	5	L2	m5	5.51	5.13	4.76	141	44.7	974	15.5	5.32	12.5	73.1	14.4
S	7.5	L2	m6	4.59	5.10	4.68	123	31.9	854	10.8	4.23	14.1	72.5	13.3
S	6	L2	m7	4.95	5.06	4.81	140	52.1	585	15.9	5.88	23.1	66.8	10.1
S	4	L2	m8	4.48	4.95	4.72	145	49.9	559	9.13	3.63	21.5	68.3	10.3
S	3	L2	m9	5.96	5.15	4.61	174	58.9	673	24.8	5.45	20.7	67.3	12.0
SS	10	H1	h1	2.22	6.68	6.45	243	9.31	3544	736		27.9	53.3	18.8
SS	10	H1	h10	0.79	6.59	6.14	233	12.1	2591	612		27.6	53.0	19.4
SS	10	H1	h2	2.98	6.36	5.91	315	17.4	2445	661		26.1	51.9	22.0
SS	10	H1	h3	2.28	6.63	6.12	300	21.9	3087	877		27.9	51.9	20.1
SS	10	H1	h4	1.39	6.65	6.11	237	16.5	2881	864		31.6	47.7	20.7
SS	10	H1	h5	2.11	6.32	5.85	266	17.5	2641	824		28.3	52.3	19.4
SS	10	H1	h6	1.85	6.50	6.11	261	11.1	2861	757		36.9	43.3	19.7
SS	10	H1	h7	2.74	6.29	6.00	268	10.6	2565	645		27.5	52.8	19.7
SS	10	H1	h8	3.01	6.02	5.51	345	22.3	2869	881		24.5	54.6	20.9
SS	10	H1	h9	2.11	6.14	5.89	299	14.3	2937	903		27.1	54.4	18.5
SS	10	H1	l1	2.45	6.81	6.32	334	18.0	4916	1471		27.9	51.0	21.1
SS	10	H1	l10	3.60	6.50	6.05	348	28.7	5785	1540		30.1	48.0	21.9
SS	10	H1	l2	3.99	6.18	5.89	340	17.1	3763	1162		33.2	48.1	18.7
SS	10	H1	l3	2.24	6.47	6.05	333	16.5	2222	520		28.6	53.2	18.2
SS	10	H1	l4	2.58	6.88	6.38	331	18.5	5233	1287		34.3	48.7	17.1
SS	10	H1	l5	2.62	6.57	6.12	440	19.2	3780	1102		41.2	41.8	17.0
SS	10	H1	l6	1.83	6.68	6.12	239	25.7	3233	822		25.2	53.9	20.9
SS	10	H1	l7	3.04	6.84	6.39	225	15.1	7686	1838		30.0	50.5	19.5
SS	10	H1	l8	3.81	6.07	5.73	394	28.9	4668	1167		28.4	49.9	21.7
SS	10	H1	l9	4.62	6.47	6.16	404	23.3	5462	1434		28.0	50.3	21.7
SS	10	H1	m1	2.50	6.38	5.89	326	13.2	2962	730		26.4	53.9	19.7
SS	10	H1	m10	3.10	6.47	6.03	333	24.4	6280	1648		27.7	48.5	23.9
SS	10	H1	m2	1.85	7.04	6.45	276	16.6	5307	1148		24.2	54.8	20.9
SS	10	H1	m3	2.68	5.98	5.55	314	16.1	2005	556		19.7	59.1	21.2
SS	10	H1	m4	2.77	6.43	6.00	383	18.0	4497	1107		20.1	59.7	20.2
SS	10	H1	m5	2.28	5.73	5.35	350	25.2	2250	620		21.4	58.1	20.5
SS	10	H1	m6	2.84	6.29	5.75	386	18.1	2962	844		21.9	56.9	21.2
SS	10	H1	m7	2.14	6.56	6.11	496	22.9	4052	1188		24.9	52.4	22.7
SS	10	H1	m8	3.17	5.96	5.37	473	21.3	2583	801		24.6	52.7	22.7
SS	10	H1	m9	2.76	5.58	5.15	379	22.0	2451	713		29.9	47.1	23.0
SS	10	H1	m1	1.66	5.26	4.85	147	8.49	343	6.48		50.2	41.5	8.3
SS	10	H2	h1	2.76	5.19	4.86	96.6	131	1038	40.5		17.9	59.2	23.0
SS	10	H2	h10	2.22	5.46	5.21	108	84.1	1403	114		23.6	58.1	18.3
SS	10	H2	h2	2.38	6.72	6.27	118	16.3	2211	104		22.3	47.3	30.4
SS	10	H2	h3	1.16	6.05	5.53	165	8.73	1521	179		24.4	51.9	23.7

Horizon	Thickness	Site	ID	% Carbon	pH	pH in CaCl	M1 Al	M1 Fe	M1 Ca	M1 P	M3 P	% Sand	% Silt	% Clay
SS	10	H2	h4	2.04	6.30	5.85	141	11.9	1613	177		22.7	54.0	23.3
SS	10	H2	h5	2.89	6.30	5.96	109	17.8	2182	118		22.1	52.7	25.1
SS	10	H2	h6	2.21	6.41	6.07	128	68.6	1549	138		27.5	56.1	16.4
SS	10	H2	h7	1.64	6.21	5.73	128	46.3	1235	85.0		16.0	62.1	22.0
SS	10	H2	h8	2.00	5.75	5.48	114	68.2	1028	108		19.8	62.5	17.7
SS	10	H2	h9	2.42	6.05	5.69	147	53.4	1704	119		20.7	58.5	20.8
SS	10	H2	l1	1.38	6.18	5.64	176	11.1	1380	139		26.7	55.2	18.2
SS	10	H2	l10	2.04	6.14	5.75	178	13.4	1658	211		29.7	50.3	20.0
SS	10	H2	l2	3.96	6.14	5.49	211	12.5	1392	201		23.9	59.6	16.4
SS	10	H2	l3	1.62	6.11	5.57	173	13.8	1591	239		26.8	48.8	24.4
SS	10	H2	l4	1.09	7.08	6.34	115	19.8	1639	29.2		34.9	39.2	25.9
SS	10	H2	l5	2.74	6.00	5.51	243	16.3	1679	281		30.7	47.6	21.7
SS	10	H2	l6	1.63	6.21	5.60	148	11.4	1290	156		31.7	49.3	19.1
SS	10	H2	l7	1.47	5.70	5.15	248	12.9	928	214		31.7	52.4	15.9
SS	10	H2	l8	2.11	6.30	5.73	142	8.51	1451	178		29.5	50.9	19.6
SS	10	H2	l9	2.35	5.87	5.44	189	13.0	1480	181		27.7	47.7	24.6
SS	10	H2	m1	1.18	6.70	5.98	131	12.0	1653	35.0		9.5	55.3	35.2
SS	10	H2	m10	2.64	6.00	5.53	214	12.5	1901	344		29.6	53.6	16.8
SS	10	H2	m2	1.82	6.40	5.85	176	22.5	2114	309		34.3	46.4	19.3
SS	10	H2	m3	2.24	6.03	5.73	148	16.7	2119	229		31.6	49.2	19.2
SS	10	H2	m4	1.48	6.32	5.64	247	16.0	1544	284		31.5	54.8	13.7
SS	10	H2	m5	1.87	6.52	6.00	194	8.85	2204	314		27.3	50.7	21.9
SS	10	H2	m6	1.72	6.54	6.16	124	21.2	1873	94.6		27.4	49.4	23.3
SS	10	H2	m7	2.43	6.36	6.00	143	14.2	1917	128		20.4	55.0	24.6
SS	10	H2	m8	2.42	6.09	5.53	185	16.1	1667	236		33.1	50.8	16.1
SS	10	H2	m9	1.94	6.23	5.71	225	12.1	1789	287		22.7	63.3	14.0
SS	10	L1	h1	1.23	5.39	4.92	132	7.95	527	4.20		33.4	55.1	11.5
SS	10	L1	h10	1.63	5.37	4.95	108	9.59	474	2.32		47.7	42.7	9.6
SS	10	L1	h2	1.42	5.17	4.88	176	11.0	593	4.97		34.9	56.0	9.1
SS	10	L1	h3	1.90	5.62	4.99	174	18.1	623	11.0		58.2	36.8	5.0
SS	10	L1	h4	2.68	5.44	5.01	119	4.79	432	4.80		51.1	41.9	7.0
SS	10	L1	h5	2.24	5.69	5.15	126	6.32	483	5.48		50.6	42.0	7.4
SS	10	L1	h6	2.36	6.12	5.71	123	4.76	698	9.62		48.0	44.4	7.7
SS	10	L1	h7	1.66	5.58	5.12	98.8	11.2	814	1.28		27.3	55.2	17.5
SS	10	L1	h8	1.80	5.40	4.99	150	9.37	490	7.26		55.5	39.4	5.2
SS	10	L1	h9	1.79	5.51	5.04	130	17.3	878	4.15		35.5	50.5	14.0
SS	10	L1	l1	1.28	5.75	4.88	99.3	10.9	633	1.63		38.4	46.9	14.7
SS	10	L1	l10	1.62	5.40	4.85	100	7.56	391	2.10		26.1	54.6	19.3
SS	10	L1	l2	1.23	5.51	4.61	147	9.15	250	3.56		59.6	35.4	4.9
SS	10	L1	l3	2.30	5.67	5.03	109	4.13	390	1.08		22.7	57.3	20.0

Horizon	Thickness	Site	ID	% Carbon	pH	pH in CaCl	M1 Al	M1 Fe	M1 Ca	M1 P	M3 P	% Sand	% Silt	% Clay
SS	10	L1	l4	1.46	5.53	5.06	105	10.8	480	1.58		31.3	55.7	13.0
SS	10	L1	l5	1.74	5.42	4.97	146	12.3	381	3.42		58.0	36.8	5.2
SS	10	L1	l6	1.12	5.82	5.01	81.8	8.57	455	0.56		28.9	54.2	16.8
SS	10	L1	l7	2.02	5.10	4.68	188	19.4	501	18.5		45.0	49.1	5.9
SS	10	L1	l8	1.63	5.73	4.98	218	18.4	266	4.74		51.4	43.0	5.6
SS	10	L1	l9	1.62	5.89	5.46	171	31.1	783	2.69		26.7	49.8	23.5
SS	10	L1	m10	4.58	5.19	4.56	126	31.5	1032	9.18		36.8	48.5	14.6
SS	10	L1	m2	1.74	5.35	4.85	176	9.00	401	4.58		51.0	41.1	7.8
SS	10	L1	m3	2.40	5.37	4.74	113	20.0	324	4.59		48.6	41.7	9.7
SS	10	L1	m4	1.54	5.82	5.17	118	5.15	382	2.78		47.4	42.3	10.4
SS	10	L1	m5	1.88	5.35	4.72	119	7.53	244	2.80		50.2	40.6	9.2
SS	10	L1	m6	1.54	5.48	4.79	123	19.2	648	4.12		39.2	49.3	11.6
SS	10	L1	m7	1.33	5.98	5.21	200	6.35	435	4.12		55.7	39.0	5.3
SS	10	L1	m8	1.53	5.67	5.08	76.3	7.13	757	1.27		23.7	61.1	15.2
SS	10	L1	m9	2.05	5.26	4.81	184	12.6	575	11.6		52.4	41.7	5.9
SS	10	L2	h1	1.80	5.06	4.32	273	109	252	3.21		25.2	64.0	10.8
SS	10	L2	h10	1.88	5.04	4.29	215	94.9	322	3.11		17.5	73.6	8.9
SS	10	L2	h2	3.24	4.83	4.32	559	62.8	220	5.24		18.7	59.9	21.5
SS	10	L2	h3	1.20	4.83	4.00	365	104	134	2.19		24.2	64.4	11.4
SS	10	L2	h4	0.93	4.95	4.04	264	47.6	78.1	2.15		21.5	66.0	12.5
SS	10	L2	h5	1.35	4.86	4.11	254	94.8	161	8.24		19.6	70.0	10.3
SS	10	L2	h6	1.37	5.66	5.01	119	44.9	589	1.99		17.4	71.9	10.6
SS	10	L2	h7	1.27	5.60	4.85	165	56.7	557	21.7		21.9	64.6	13.5
SS	10	L2	h8	0.91	5.15	4.25	214	34.7	152	1.83		19.4	67.1	13.5
SS	10	L2	h9	1.42	5.08	4.32	236	105	219	4.18		28.0	62.0	10.0
SS	10	L2	l2	1.63	5.51	4.61	170	36.3	512	6.57		20.3	66.1	13.6
SS	10	L2	l3	0.98	5.44	4.47	263	18.3	206	2.30		20.1	66.7	13.2
SS	10	L2	l4	1.57	5.57	4.88	142	32.6	434	8.36		21.1	67.9	11.0
SS	10	L2	l5	1.38	5.44	4.72	178	18.3	407	3.72		22.1	66.4	11.5
SS	10	L2	l6	1.02	5.21	4.63	136	26.0	303	2.72		15.1	75.2	9.7
SS	10	L2	l7	1.47	5.67	4.88	151	30.3	511	4.65		24.3	64.6	11.1
SS	10	L2	l8	1.68	4.89	3.96	210	8.32	494	9.01		24.4	66.9	8.6
SS	10	L2	l9	2.89	5.08	4.38	100	7.02	1253	1.37		14.9	72.0	13.1
SS	10	L2	m1	0.84	5.21	4.41	287	43.9	299	6.06		22.2	64.0	13.8
SS	10	L2	m10	1.09	5.64	4.63	157	35.3	282	2.61		25.4	63.2	11.3
SS	10	L2	m2	1.61	5.10	4.43	194	54.1	446	7.38		17.3	72.3	10.4
SS	10	L2	m3	1.11	5.62	4.58	235	38.3	304	4.14		23.9	63.7	12.4
SS	10	L2	m4	1.25	5.44	4.77	168	36.3	326	1.68		26.7	64.7	8.6
SS	10	L2	m5	1.26	5.67	4.77	150	29.0	607	11.9		13.3	72.6	14.0
SS	10	L2	m6	1.08	5.64	4.92	126	23.8	443	2.57		13.3	72.5	14.1

Horizon	Thickness	Site	ID	% Carbon	pH	pH in CaCl	M1 Al	M1 Fe	M1 Ca	M1 P	M3 P	% Sand	% Silt	% Clay
SS	10	L2	m7	2.09	5.08	4.56	172	54.4	313	7.32		23.9	66.8	9.3
SS	10	L2	m8	1.14	5.22	4.41	176	34.8	230	3.20		21.9	68.9	9.2
SS	10	L2	m9	1.99	5.31	4.29	480	39.6	130	4.92		19.7	68.1	12.2

Primary spatial data

Site: H1 - high one (location 1 with long history of manure application), H2 - high two (location 2 with long history of manure application), L1 - low 1 (location 1 with little to no history of manure application), L2 - low 2 (location 2 with little to no history of manure application); ID: h1-10 (sample locations 1-10 in the high stratification) m1--10 (sample locations 1-10 in the medium stratification), l1-10 (sample location 1-10 in the low stratification); Z Elevation - Z score of in site elevation; Z Aspect - Z score of slope aspect; Z Slope - Z score of slope gradient; PRC - Profile curvature; PLC - Plan curvature; TANC - Tangential curvature; CI - Convergence index; MFD - flow accumulation (multiple directions method); D8 - flow accumulation (deterministic 8 method); INF - flow accumulation (deterministic infinity method)

Site	Location	Z Elevation	Z Aspect	Z Slope	PRC	PLC	TANC	CI	MFD	D8	INF
L2	h2	0.54	-0.024	-0.171	0.001	-0.005	-0.001	-3.20	147	108	121
L2	h3	0.01	-0.002	-0.317	-0.006	-0.083	-0.009	2.57	244	144	196
L2	h4	0.01	0.010	-0.361	0.006	0.008	0.001	-7.49	267	207	269
L2	h5	-2.36	-0.047	0.133	0.009	0.025	0.005	-34.66	452	9	548
L2	h6	-1.18	-0.012	0.049	0.009	0.034	0.006	1.89	185	72	183
L2	h7	0.75	-0.023	0.002	-0.009	0.009	0.001	1.09	125	90	99
L2	h8	0.50	-0.037	-0.207	0.006	0.015	0.002	-2.66	161	108	195
L2	h9	0.66	-0.034	-0.038	-0.008	-0.049	-0.007	4.81	128	171	103
L2	l2	0.22	-0.030	0.246	0.015	0.041	0.008	3.47	78	54	65
L2	l3	1.95	0.019	0.088	0.010	0.060	0.010	14.93	42	36	44
L2	l4	0.28	0.039	0.094	-0.005	-0.011	-0.002	3.74	81	81	55
L2	l5	0.20	0.016	0.466	-0.003	0.006	0.001	10.54	53	36	46
L2	l6	-0.19	-0.025	0.175	0.005	0.047	0.009	4.49	113	18	112
L2	l7	0.54	0.016	-0.031	0.002	0.031	0.005	4.46	80	54	68
L2	l8	0.80	0.113	-0.067	-0.008	0.131	0.019	4.45	37	9	24
L2	l9	1.38	0.001	-0.034	0.008	0.093	0.014	16.94	35	45	42

Site	Location	Z Elevation	Z Aspect	Z Slope	PRC	PLC	TANC	CI	MFD	D8	INF
L2	m1	-1.75	-0.031	0.081	-0.001	0.013	0.002	-8.24	199	36	137
L2	m2	-1.65	-0.008	0.150	0.002	-0.007	-0.001	-3.58	224	126	188
L2	m3	-0.25	0.030	0.029	-0.013	-0.012	-0.002	-0.55	108	72	80
L2	m4	-0.90	0.030	-0.075	0.000	0.000	0.000	5.14	144	126	109
L2	m5	-0.52	-0.004	-0.011	0.011	0.060	0.009	-2.56	131	72	126
L2	m6	-0.97	-0.018	0.108	0.003	0.021	0.004	0.43	169	99	150
L2	m8	-0.06	-0.023	0.012	0.004	0.018	0.003	-2.61	115	54	112
L2	m9	1.36	0.066	0.145	0.000	0.000	0.000	5.95	35	27	36
H1	h1	0.27	-0.012	0.350	-0.002	-0.005	-0.001	-11.56	295	343	252
H1	h2	1.22	0.893	-0.248	0.000	0.014	0.002	10.66	161	135	163
H1	h3	-2.33	-0.559	-0.208	0.000	0.004	0.001	0.91	200	99	202
H1	h4	0.62	0.099	0.065	0.002	-0.003	-0.001	-8.19	160	126	153
H1	h5	0.60	0.043	0.081	0.006	0.023	0.004	4.43	171	190	160
H1	h6	0.33	0.002	0.345	-0.001	0.002	0.001	-13.74	226	262	164
H1	h7	0.26	0.055	0.390	-0.001	-0.018	-0.004	-9.40	332	388	364
H1	h8	1.02	1.197	0.171	0.006	-0.015	-0.003	-8.42	166	181	148
H1	h9	0.17	-0.010	0.318	0.001	0.003	0.001	-15.81	242	325	204
H1	l1	-0.28	0.033	-0.118	-0.002	0.097	0.015	31.17	47	27	30
H1	l10	-0.94	-0.421	-0.435	0.003	0.097	0.010	36.64	26	18	16
H1	l2	-1.80	-0.602	0.289	0.002	0.010	0.002	9.10	97	54	61
H1	l3	0.22	0.088	-0.148	-0.002	-0.015	-0.002	24.67	100	45	103
H1	l4	-0.01	-0.056	-0.055	0.003	0.057	0.009	30.95	75	18	59
H1	l5	-0.16	-0.720	0.459	-0.003	-0.012	-0.003	13.67	86	81	57
H1	l6	-0.35	0.220	0.096	0.003	0.074	0.014	16.17	49	27	33
H1	l7	-0.62	-0.786	0.213	0.010	0.054	0.011	13.46	60	9	54
H1	l8	-1.07	-0.520	-0.376	0.001	0.019	0.002	26.39	49	36	36
H1	l9	-0.55	-0.965	-0.108	0.016	0.108	0.017	22.92	38	36	46
H1	m1	0.32	-0.114	0.235	-0.001	0.012	0.003	11.56	150	36	117

Site	Location	Z Elevation	Z Aspect	Z Slope	PRC	PLC	TANC	CI	MFD	D8	INF
H1	m10	-1.04	0.259	-0.292	0.003	0.003	0.000	11.85	74	90	77
H1	m2	-0.31	0.700	0.300	0.010	0.012	0.003	8.99	70	27	42
H1	m3	1.15	0.536	-0.114	0.000	-0.037	-0.006	8.27	112	126	108
H1	m4	0.09	-0.021	-0.192	0.003	0.046	0.006	28.56	92	72	83
H1	m5	-2.19	-0.392	-0.137	0.000	0.015	0.002	12.34	105	63	79
H1	m6	0.91	-0.085	0.004	-0.001	0.005	0.001	16.24	124	54	120
H1	m7	0.74	-0.466	-0.835	-0.002	1.091	0.031	18.68	19	9	42
H1	m8	1.37	0.812	-0.010	-0.001	0.005	0.001	8.50	136	108	116
H1	m9	1.65	0.738	-0.105	0.005	0.028	0.004	16.34	63	63	52
H2	h1	-1.10	-0.701	-0.504	-0.001	-0.023	-0.001	3.38	201	304	243
H2	h10	-0.95	-0.311	-0.352	-0.005	-0.076	-0.003	2.07	109	403	101
H2	h2	-1.11	-0.687	-0.433	-0.001	0.048	0.002	-1.48	90	18	64
H2	h3	1.02	-0.638	1.062	-0.004	-0.020	-0.003	2.63	70	72	101
H2	h4	0.69	-0.003	0.090	0.003	-0.096	-0.007	2.11	152	27	164
H2	h5	-1.37	-0.669	-0.498	-0.001	0.002	0.000	-18.30	361	90	177
H2	h6	-0.94	2.689	-0.725	0.000	-0.424	-0.008	-16.47	1336	528	1309
H2	h7	-1.33	2.525	-0.398	-0.001	-0.018	-0.001	-19.86	158	107	113
H2	h8	-0.93	-0.470	-0.430	-0.003	0.000	0.000	-13.12	241	18	117
H2	h9	-1.34	2.496	-0.434	0.000	-0.008	0.000	-19.18	180	179	136
H2	l1	-0.68	-0.342	0.007	0.001	0.037	0.002	11.20	66	233	69
H2	l10	1.26	-0.452	0.619	0.001	0.024	0.003	0.49	79	63	72
H2	l2	0.57	-0.612	-0.035	0.000	0.020	0.001	29.20	66	54	48
H2	l3	1.52	-0.561	0.679	0.003	-0.007	-0.001	17.65	25	18	28
H2	l4	-0.48	0.172	0.341	-0.009	-0.060	-0.005	-33.68	276	107	90
H2	l5	1.04	-0.639	0.546	0.003	0.008	0.001	24.46	75	18	69
H2	l6	1.56	-0.462	0.224	0.002	0.009	0.001	13.60	58	45	49
H2	l7	-0.09	-0.903	0.115	0.000	0.051	0.004	20.62	65	107	66
H2	l8	1.34	-0.440	0.629	0.001	-0.002	0.000	9.77	82	54	67

Site	Location	Z Elevation	Z Aspect	Z Slope	PRC	PLC	TANC	CI	MFD	D8	INF
H2	l9	1.38	2.351	0.215	0.019	0.216	0.017	22.88	27	9	13
H2	m1	-1.24	-0.863	-0.462	0.001	0.044	0.002	7.81	143	90	51
H2	m10	-0.07	-0.635	-0.524	-0.003	0.042	0.001	20.67	101	134	87
H2	m2	0.91	-0.446	0.759	-0.003	-0.010	-0.001	-3.62	129	90	103
H2	m3	-0.14	-0.221	-0.305	0.001	0.042	0.002	17.75	112	18	130
H2	m4	0.14	-0.689	-0.304	0.000	0.007	0.000	18.73	122	107	102
H2	m5	0.82	-0.473	0.564	-0.001	-0.005	-0.001	-3.17	132	90	100
H2	m6	-0.05	-0.717	0.363	-0.007	-0.034	-0.003	-2.03	90	54	54
H2	m7	-1.19	2.705	-0.514	0.000	0.059	0.002	13.46	73	18	47
H2	m8	0.41	-0.347	-0.192	-0.001	0.016	0.001	9.27	111	295	109
H2	m9	0.37	-0.659	-0.102	0.000	0.011	0.001	25.08	85	63	67
L1	h1	1.28	-0.155	-0.127	0.003	-0.006	-0.001	-4.22	239	621	249
L1	h10	-0.36	0.157	-0.180	0.001	-0.011	-0.001	-17.29	698	1133	539
L1	h2	-0.20	-0.065	-0.106	0.002	-0.006	-0.001	-16.03	362	441	384
L1	h3	1.28	-0.138	-0.077	0.001	-0.011	-0.002	-1.38	132	99	146
L1	h4	0.42	0.096	-0.141	-0.001	-0.015	-0.002	-6.42	302	162	212
L1	h5	0.01	0.118	-0.110	-0.003	-0.006	-0.001	-10.29	712	243	607
L1	h6	0.13	-0.025	-0.255	0.002	-0.008	-0.001	-19.86	587	117	409
L1	h7	-0.53	0.207	-0.024	0.009	-0.011	-0.002	-15.63	668	216	1074
L1	h8	0.13	0.109	-0.152	0.002	0.006	0.001	-8.99	601	234	456
L1	h9	-0.53	-0.108	-0.060	0.001	-0.019	-0.003	-10.74	432	288	418
L1	l1	-1.55	0.047	0.422	-0.002	0.005	0.001	7.28	120	63	94
L1	l10	-0.57	-0.048	0.171	-0.002	-0.007	-0.001	6.43	103	108	91
L1	l2	1.07	-0.076	0.095	-0.003	0.004	0.001	13.90	105	171	92
L1	l3	-0.73	-0.018	0.273	0.001	0.011	0.002	7.37	96	99	84
L1	l4	-1.31	0.053	0.468	0.000	0.011	0.002	9.05	110	72	84
L1	l5	1.15	-0.068	0.104	0.001	0.026	0.004	16.90	80	63	65
L1	l6	-1.18	-0.020	0.245	0.000	0.007	0.001	3.92	148	153	128

Site	Location	Z Elevation	Z Aspect	Z Slope	PRC	PLC	TANC	CI	MFD	D8	INF
L1	l7	1.77	-0.146	-0.155	-0.003	0.006	0.001	14.83	109	36	84
L1	l8	1.48	-0.033	-0.204	0.003	0.038	0.004	22.21	64	99	63
L1	l9	-1.31	0.190	0.257	-0.001	0.193	0.035	3.58	27	9	17
L1	m1	0.46	0.009	-0.359	-0.004	0.039	0.004	18.02	96	9	71
L1	m10	-1.68	0.045	0.338	-0.004	0.003	0.001	3.05	133	72	103
L1	m2	0.17	-0.089	-0.038	0.000	0.002	0.000	-0.62	197	225	174
L1	m3	0.13	0.109	-0.213	0.001	0.004	0.000	-7.98	413	207	285
L1	m4	0.25	0.101	-0.208	0.000	-0.007	-0.001	-8.77	545	216	380
L1	m5	0.58	-0.089	-0.062	0.000	0.002	0.000	5.72	165	369	152
L1	m6	-1.06	-0.063	0.123	0.000	0.009	0.001	-11.90	244	189	157
L1	m7	0.05	-0.108	-0.149	-0.002	-0.010	-0.001	1.80	190	99	171
L1	m8	-1.10	-0.051	0.161	0.001	0.010	0.002	8.55	136	126	161
L1	m9	1.77	0.058	-0.038	-0.002	0.000	0.000	8.99	111	117	86

Compound topographic data

Site: H1 - high one (location 1 with long history of manure application), H2 - high two (location 2 with long history of manure application), L1 - low 1 (location 1 with little to no history of manure application), L2 - low 2 (location 2 with little to no history of manure application); ID: h1-10 (sample locations 1-10 in the high stratification) m1--10 (sample locations 1-10 in the medium stratification), l1-10 (sample location 1-10 in the low stratification); TWI_s - topographic wetness index (saga method); D8 TWI - topographic wetness index (deterministic 8 method); MFD TWI- topographic wetness index (multiple flow direction method); D∞ TWI - topographic wetness index (deterministic infinity method); MC SPI - stream power index (calculated with saga modified catchment method); D8 SPI - stream power index (deterministic 8 method); MFD SPI- stream power index (multiple flow direction method); D∞ SPI - stream power index (deterministic infinity method)

Site	Location	TWI_s	D8 TWI	MFD TWI	D∞ TWI	MFD SPI	MC SPI	D∞ SPI	D8 SPI
L2	h2	2.47	5.12	5.43	5.23	95.0	109.4	78.0	69.8
L2	h3	2.81	3.21	3.74	3.52	1413.3	1499.7	1135.4	834.8
L2	h4	2.87	5.52	5.78	5.78	221.7	265.8	222.9	171.7
L2	h5	3.12	9.11	13.02	13.21	-568.0	-639.6	-689.1	-11.3
L2	h6	2.64	4.02	4.97	4.96	238.9	251.7	236.5	92.8
L2	h7	2.37	6.33	6.66	6.42	20.1	22.0	15.9	14.5
L2	h8	2.51	9.98	10.38	10.57	0.8	0.9	1.0	0.5

Site	Location	TWI$_s$	D8 TWI	MFD TWI	D∞ TWI	MFD SPI	MC SPI	D∞ SPI	D8 SPI
L2	h9	2.51	12.05	11.76	11.54	-63.7	-69.9	-51.4	-85.3
L2	l2	2.29	3.78	4.14	3.96	95.7	106.1	80.1	66.6
L2	l3	1.98	0.43	0.59	0.63	981.6	1240.4	1016.0	839.5
L2	l4	2.36	11.30	11.30	10.92	-1554.5	-1816.8	-1063.2	-1554.0
L2	l5	1.91	2.51	2.89	2.75	154.6	191.3	134.5	105.5
L2	l6	2.45	9.80	11.64	11.62	-27.2	-29.8	-26.8	-4.3
L2	l7	2.37	10.90	11.29	11.13	-30.1	-34.4	-25.6	-20.3
L2	l8	1.92	9.11	10.51	10.07	-48.9	-50.7	-31.5	-11.9
L2	l9	1.93	10.71	10.46	10.65	-15.2	-16.9	-18.2	-19.5
L2	m1	2.67	1.69	3.40	3.03	1319.2	1386.7	911.1	238.7
L2	m2	2.72	11.74	12.32	12.15	-165.1	-172.3	-138.8	-92.9
L2	m3	2.32	4.66	5.07	4.77	73.4	82.2	54.7	49.0
L2	m4	2.53	11.74	11.87	11.60	-248.5	-264.8	-189.4	-218.1
L2	m5	2.49	11.18	11.79	11.74	-4.9	-5.2	-4.7	-2.7
L2	m6	2.59	11.50	12.04	11.92	-597.4	-629.8	-530.3	-350.0
L2	m8	2.43	5.08	5.83	5.80	38.6	41.8	37.6	18.2
L2	m9	1.99	10.20	10.46	10.48	-30.0	-41.4	-30.6	-23.3
H1	h1	2.73	1.76	1.61	1.45	17441.5	17641.0	14933.9	20286.8
H1	h2	2.69	5.38	5.56	5.57	100.2	103.5	101.3	84.1
H1	h3	2.63	3.50	4.20	4.21	600.4	630.8	608.1	298.6
H1	h4	2.52	11.75	11.98	11.94	-45.3	-45.4	-43.2	-35.8
H1	h5	2.57	9.55	9.44	9.38	2.3	2.3	2.2	2.6
H1	h6	2.68	3.35	3.21	2.89	2072.1	2352.7	1505.2	2397.3
H1	h7	2.79	12.87	12.71	12.81	-400.8	-402.2	-439.1	-468.0
H1	h8	2.57	12.10	12.02	11.91	-15934.4	-16281.7	-14197.2	-17296.9
H1	h9	3.09	5.33	5.03	4.86	382.9	832.6	322.8	513.7
H1	l1	1.92	10.21	10.76	10.30	-15.8	-15.8	-10.0	-9.1
H1	l10	2.20	3.66	4.01	3.53	11.9	11.9	7.4	8.4
H1	l2	2.09	4.62	5.20	4.73	51.8	51.9	32.4	29.0
H1	l3	2.30	10.72	11.52	11.54	-112.4	-112.5	-115.5	-50.5
H1	l4	2.20	2.91	4.33	4.10	74.1	74.2	58.4	17.8
H1	l5	1.99	5.53	5.58	5.17	27.8	27.8	18.4	26.3
H1	l6	1.90	4.57	5.16	4.77	13.6	13.7	9.2	7.6
H1	l7	2.10	9.11	11.00	10.90	-65.5	-65.6	-58.7	-9.8
H1	l8	2.44	1.38	1.69	1.39	444.4	444.5	330.5	327.2
H1	l9	2.06	10.49	10.54	10.73	-5.5	-5.5	-6.7	-5.2
H1	m1	2.43	10.49	11.92	11.67	-72.2	-72.4	-56.2	-17.3

Site	Location	TWI$_s$	D8 TWI	MFD TWI	D∞ TWI	MFD SPI	MC SPI	D∞ SPI	D8 SPI
H1	m10	2.68	11.41	11.21	11.26	-16.7	-16.6	-17.5	-20.5
H1	m2	2.05	3.49	4.45	3.93	58.2	58.4	34.7	22.4
H1	m3	2.48	11.75	11.62	11.59	-27.7	-28.1	-26.8	-31.3
H1	m4	2.31	1.27	1.52	1.42	1860.7	1862.7	1682.2	1457.1
H1	m5	2.33	11.05	11.57	11.28	-81.0	-93.6	-60.9	-48.5
H1	m6	2.41	10.90	11.73	11.70	-505.5	-509.1	-487.3	-219.9
H1	m7	2.93	9.11	9.83	10.65	-5.0	-25.0	-11.3	-2.4
H1	m8	2.57	-0.49	-0.25	-0.42	23989.6	24976.6	20434.9	19053.1
H1	m9	2.33	11.05	11.05	10.86	-6.4	-7.1	-5.3	-6.5
H2	h1	4.08	8.30	7.88	8.07	15.3	34.5	18.5	23.1
H2	h10	4.40	5.41	4.10	4.02	197.2	1519.9	183.2	728.9
H2	h2	3.97	3.39	5.00	4.67	54.1	210.1	38.9	10.8
H2	h3	2.78	4.20	4.17	4.54	74.8	102.8	108.3	76.8
H2	h4	3.20	3.27	5.01	5.08	155.3	192.7	167.6	27.4
H2	h5	4.82	6.66	8.05	7.34	41.5	214.9	20.3	10.3
H2	h6	4.32	13.18	14.11	14.08	-6105.0	-5479.4	-5980.3	-2413.1
H2	h7	4.11	4.71	5.09	4.76	152.7	498.3	109.7	104.0
H2	h8	4.30	3.35	5.95	5.22	151.9	593.1	73.5	11.3
H2	h9	4.23	5.72	5.72	5.44	106.4	313.1	80.2	105.7
H2	l1	3.49	6.79	5.53	5.57	17.3	38.1	18.1	61.0
H2	l10	2.83	3.50	3.74	3.64	149.7	167.3	135.6	118.1
H2	l2	3.03	10.89	11.09	10.79	-1.2	-1.2	-0.9	-1.0
H2	l3	2.31	0.58	0.89	1.04	245.7	376.7	284.8	179.6
H2	l4	4.80	11.58	12.53	11.40	-241.5	-1291.2	-78.3	-93.9
H2	l5	3.07	3.24	4.67	4.58	52.7	53.6	48.6	12.6
H2	l6	2.87	10.71	10.96	10.79	-623.3	-728.8	-525.2	-484.3
H2	l7	2.95	4.34	3.84	3.85	92.0	92.0	92.6	151.0
H2	l8	3.12	3.18	3.60	3.39	183.1	194.1	149.2	120.4
H2	l9	2.92	9.10	10.21	9.48	-847.7	-1493.9	-406.9	-278.3
H2	m1	4.12	5.49	5.96	4.93	52.5	156.0	18.8	33.0
H2	m10	3.46	11.81	11.52	11.37	-5.4	-5.5	-4.6	-7.2
H2	m2	3.07	11.40	11.77	11.55	-288.1	-309.8	-230.7	-199.7
H2	m3	3.64	1.30	3.13	3.28	547.7	718.1	637.9	87.7
H2	m4	3.43	3.06	3.18	3.00	615.8	616.1	513.9	543.2
H2	m5	3.16	4.61	5.00	4.72	117.4	122.3	88.6	79.5
H2	m6	2.93	10.89	11.40	10.90	-58.8	-79.5	-35.5	-35.2
H2	m7	4.20	7.44	8.85	8.40	0.8	4.0	0.5	0.2

Site	Location	TWI$_s$	D8 TWI	MFD TWI	D∞ TWI	MFD SPI	MC SPI	D∞ SPI	D8 SPI
H2	m8	3.37	12.60	11.61	11.60	-187.9	-220.4	-185.4	-501.7
H2	m9	3.19	11.05	11.35	11.11	-41.7	-41.7	-32.7	-30.7
L1	h1	3.17	8.55	7.60	7.64	28.7	31.6	29.8	74.3
L1	h10	3.51	13.94	13.46	13.20	-534.0	-536.2	-412.3	-867.7
L1	h2	3.19	6.92	6.72	6.78	158.2	163.1	167.7	192.6
L1	h3	2.86	4.48	4.76	4.86	148.1	166.4	163.8	111.3
L1	h4	3.01	11.99	12.62	12.26	-28.6	-28.7	-20.1	-15.4
L1	h5	3.43	6.45	7.53	7.37	272.7	276.2	232.3	93.0
L1	h6	3.61	3.03	4.65	4.29	3311.1	4116.3	2307.6	659.1
L1	h7	3.60	12.28	13.41	13.89	-17152.1	-17161.2	-27553.4	-5540.0
L1	h8	3.35	12.36	13.31	13.03	-155.1	-156.3	-117.6	-60.4
L1	h9	3.28	5.02	5.43	5.39	823.1	823.3	796.7	548.1
L1	l1	2.28	11.05	11.70	11.45	-502.6	-503.1	-393.5	-262.8
L1	l10	2.29	3.41	3.36	3.24	368.3	368.3	326.3	385.9
L1	l2	2.54	6.76	6.27	6.13	20.8	20.8	18.2	34.0
L1	l3	2.28	11.50	11.47	11.34	-35.9	-36.0	-31.5	-37.1
L1	l4	2.25	11.18	11.61	11.34	-88.5	-88.6	-67.7	-58.0
L1	l5	2.44	5.21	5.45	5.25	27.4	27.5	22.5	21.7
L1	l6	2.45	11.94	11.91	11.76	-144.6	-144.7	-124.4	-149.2
L1	l7	2.61	10.49	11.60	11.34	-33.3	-34.1	-25.7	-11.0
L1	l8	2.49	11.50	11.07	11.04	-99.5	-99.8	-97.0	-153.6
L1	l9	2.40	9.10	10.21	9.76	-18.6	-105.6	-11.9	-6.2
L1	m1	2.57	9.10	11.47	11.17	-10.9	-10.9	-8.0	-1.0
L1	m10	2.32	4.69	5.30	5.05	87.4	87.5	68.1	47.5
L1	m2	2.85	3.62	3.49	3.37	1186.8	1188.1	1050.3	1354.3
L1	m3	3.19	12.24	12.93	12.56	-888.9	-891.9	-614.0	-445.4
L1	m4	3.30	12.28	13.21	12.85	-984.3	-990.2	-686.7	-390.1
L1	m5	2.77	5.33	4.53	4.44	292.9	293.4	269.9	656.8
L1	m6	3.20	5.63	5.89	5.45	165.3	276.5	106.5	127.8
L1	m7	2.91	11.50	12.16	12.05	-40.5	-40.5	-36.3	-21.0
L1	m8	2.45	4.04	4.11	4.28	300.9	301.0	357.4	279.7
L1	m9	2.46	2.93	2.87	2.62	693.1	708.5	536.6	732.1