EVALUATION OF PHOSPHORUS INDICES IN RECENTLY ACIDIFIED SOIL OF THE COLUMBIA BASIN, WASHINGTON STATE

By

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Coastal application of ammoniacal sources of nitrogen fertilizer can result in decreased bulk soil pH in Columbia Basin (CB) soils. This change in pH may result in altered phosphorus (P) chemistry and oppugns the validity of using certain P extraction methods. The purpose of this dissertation is: 1) identify species of P that exist in acidified CB soils, 2) determine differences in P sorption and availability extraction occur across a range of soil pH’s and 3) evaluate plant uptake and production differences in soils that have become acidified.

Several CB soils were collected representing a range of soil pH (5.2-8.4) and soil test P (3-331 mg P kg$^{-1}$, Olsen P extraction, 0.5M NaHCO$_3$, OP). Samples were analyzed by sequential extraction and $^{31}$P NMR to determine predominant P species. Phosphorus sorption isotherms compared P sorption and binding energies. Further, fertilizer additions were made and incubated soils were extracted to determine the effectiveness of several P availability indices. Lastly, a greenhouse study with various fertilizer treatments sought to compare plant P uptake and biomass production for soils with varying pH values.

Soil P speciation revealed that even at very low soil pH (5.2), the form of P that predominated was as calcium (Ca)-P (60% or more). $^{31}$P NMR spectroscopy was less
useful for making this determination than the sequential P extraction method for these samples. Comparison of extraction methods following incubation with fertilizer showed that variability was least with the OP method. Differences in sorption maximum and binding energy existed, in limited instances, suggesting CB soils changed little in these two parameters due to acidification.

Similarly, results from the greenhouse suggest that while some differences in the measured greenhouse parameters occurred, generally one can expect that soils at low pH will react similarly to high pH soils following fertilizer application in terms of biomass production and P uptake.

We conclude that differences in P speciation, sorption, extraction indices, and plant growth are not large enough to merit changing extraction methods from current preference. The OP test and fertilizer recommendations presently made using OP can be utilized on acidified CB soils reliably.
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DEDICATION

To Maren, Jens, Jillian and ??: if I can do this, you can do anything.
CHAPTER ONE

GENERAL INTRODUCTION

The Columbia Basin (CB) of Washington State is renowned for the production of a variety of crops including potatoes (*Solanum tuberosum* L.), corn (*Zea mays* L.), wheat (*Triticum aestivum* L.) and onions (*Allium cepa* L.). Nutrient removal by these crops is significant and addition of fertilizers is necessary to maintain adequate nutrition for optimum crop production. For example, a high yielding potato crop (90 Mg ha\(^{-1}\)) may remove more than 250 kg N ha\(^{-1}\) (Kleinkopf et al., 1981).

The most commonly deficient nutrient in plant production is nitrogen (N). Nitrogen is usually applied in ammoniacal forms because of availability and low cost. One consequence of using ammonia fertilizers is that as one mole of NH\(_4^+\) nitrifies, 2 moles of H\(^+\) are produced (Helyar, 1976). Continual use of ammonia based fertilizers accelerates soil acidification beyond natural N-cycle processes (Mahler and Harder, 1984; Rasmussen and Rohde, 1989).

Soils of the CB, before anthropogenic influence, have a pH of 6.1 – 9.0 (Cochran et al., 2006; Soil Survey Staff, 2012). However, recently observed soil pH values less than 5.0 are not uncommon (Stevens et al., 2003). The change in soil pH raises the question of whether the OP extraction (Olsen et al., 1954) can be used reliably on low pH soils because the method was developed for neutral to alkaline soil to determine plant available P, a nutrient that varies greatly in phyto-availability depending on soil pH. The Olsen P method, using NaHCO\(_3\) to extract plant available P, is most commonly used for calcareous soils where P complexes with calcium (Ca).
PHOSPHORUS SPECIATION RESEARCH

As the soil pH decreases, the stability of the Ca-P complex decreases, favoring either iron- (Fe-) or Aluminum- (Al-) P complexes (Lindsay, 1979). Other P extraction methods (Bray and Kurtz, 1945; Mehlich, 1984; McIntosh, 1969) for acid soils exist, however, data for use in CB soils and crops is lacking for these methods. Therefore it is vital to determine which P chemistry dominates in acidified CB soils and to identify which plant available P extraction methods are most appropriate. Sharpley (2000) indicated that the chemicals used in the extraction of available P must be suited to the particular soil properties in order to be successful.

In addition to traditional chemical extraction methods, $^{31}$P nuclear magnetic resonance spectroscopy (NMR) has been used to determine dominant Ca- or Al/Fe-P complexes in soils (Lookman et al., 1997; Delgado et al., 2000; Dougherty et al., 2005), animal waste (Hunger et al., 2004), and sediments (Reitzel et al., 2006). These studies reported results from both solid-state and solution $^{31}$P NMR analysis. Conversely, the study described herein, used the solid-state analysis to avoid problems with insoluble species and aggressive extractants that might compromise the soil pH premise of this study. The benefits of solid-state analysis together with some inherent problems are further discussed by Dougherty et al. (2005).

AVAILABLE PHOSPHORUS INDICES AND SORPTION RESEARCH

The extraction procedures for P in this study are availability indices. In other words, an amount of P that is assumed to be directly related to plant availability is extracted and is dependent on soil properties and plant requirements. Phosphorus sorption isotherms not only take into account the intensity of extracted P, but also the capacity of the soil to provide P for plant production (Singh et al., 2005). Determination of plant P requirements based on data from sorption isotherms has been demonstrated to be more accurate than using only conventional P
soil tests (Hue and Fox, 2010; Klages et al., 1988). The Langmuir equation is often fitted to P sorption isotherm data in environmental applications because the maximum sorption (b), and binding energy (k), can be calculated and analyzed statistically. The Langmuir sorption maximum has been shown to be well correlated with exchangeable calcium (Ca) (Tunesi et al., 1999) as well as iron (Fe) and aluminum (Al) oxides (Zhang et al., 2005), parameters often associated with soil pH.

PLANT PHOSPHORUS UPTAKE AND BIOMASS PRODUCTION RESEARCH

The Olsen P method (OP), which uses sodium bicarbonate (NaHCO$_3$) to extract plant available P, is most commonly used for calcareous soils where P complexes with calcium (Ca). In the Olsen P extraction, the activity of Ca decreases as it precipitates out as calcium carbonate (CaCO$_3$) leaving the plant available portion of P behind in solution which is then related to plant response (e.g. yield, uptake and vigor).

Established P fertilizer recommendations in Eastern Washington State are based on the results of Olsen P. Soil testing laboratories continue to use the Olsen P extraction on recently acidified soils regardless of soil pH (R. Miller, personal communication, 2011). Sharpley (2000) indicated that the chemicals used in the extraction of available P must be suited to the particular soil properties in order to be successful. Further, Holford (1980) suggests that good correlation with plant yield or P uptake should be the criterion by which an appropriate test should be determined across a spectrum of soil chemical properties.
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CHAPTER TWO

SPECIATION OF PHOSPHORUS IN RECENTLY ACIDIFIED SOILS OF THE
COLUMBIA BASIN, WASHINGTON

(To be submitted to Soil Science Society of America Journal)

ABSTRACT

Determination of inorganic phosphorus (P) species in recently acidified soils of the Columbia Basin (CB) is vital in understanding which available P extraction methods are most appropriate so that fertilizer recommendations can be made accurately. The objective of this work was to determine dominant P species in recently acidified soils of the CB using traditional chemical fractionation and \(^{31}\)P NMR spectroscopy. Soil samples from the CB ranging in pH (5.2-8.4) together with native calcareous and acidic soils were analyzed. Chemical fractionation to determine soluble, aluminum (Al), iron (Fe), reductant soluble (RSP), and calcium (Ca) P showed that CB soils contain more than 60% of inorganic P as Ca-P. Significant differences in other fractions were most pronounced in the Fe-P form. As soil pH decreased the amount of Fe-P extracted increased suggesting a possible transition in soil P chemistry from high to low pH. Solid state \(^{31}\)P NMR spectroscopy was limited by the low soil P content and interference from paramagnetic ions. No definitive determination of inorganic soil P species could be made. Despite these limitations, some generalized inferences can be made using the spinning side band (SSB) patterns. Because the traditional sodium bicarbonate (NaHCO\(_3\)) extractable P (Olsen phosphorus, OP) method assumes a dominant Ca-P form, fertility recommendations based on OP appears to be the best option for determining plant available P for recently acidified CB soils.
INTRODUCTION

There is growing concern for excess phosphorus (P) in the environment. Several states, including Washington (effective January 2013; RCW 15.54.500; http://apps.leg.wa.gov/rcw/default.aspx?cite=15.54.500), have passed or introduced legislation that limits the use of potential P inputs such as laundry detergents and fertilizers. The agriculture industry is a large consumer of P fertilizer and, as such, must be mindful that fertilizer inputs are made in the appropriate amounts given changing soil conditions. Soil pH is one condition that has changed from years of ammonium nitrogen (N) application because as one mole of NH$_4^+$ nitrifies, 2 moles of H$^+$ are produced (Helyar, 1976). Continual use of ammonia based fertilizers accelerates soil acidification beyond natural N-cycle processes (Mahler and Harder, 1984; Rasmussen and Rohde, 1989).

Soils of the Columbia Basin (CB), before anthropogenic influence, have a pH of 6.1 – 9.0 (Cochran et al., 2006; Soil Survey Staff, 2012). However, recently observed soil pH values less than 5.0 are not uncommon (Stevens et al., 2003). The predominant method for determining plant available P in CB soils is the Olsen (Olsen et al., 1954) phosphorus (OP) extraction. This method was developed for neutral to high pH soils where P chemistry is governed by calcium (Ca). In the extraction, the activity of Ca is decreased with the addition of sodium bicarbonate (NaHCO$_3$) as calcium carbonate (CaCO$_3$) precipitates, leaving a portion of P in solution. The measure of this P is then related to plant response (e.g. yield, uptake, vigor).

As the soil pH decreases the stability of the Ca-P complex decreases, favoring either iron-(Fe-) or Aluminum- (Al-) P complexes (Lindsay, 1979). Other P extraction methods (Bray and Kurtz, 1945; Mehlich, 1984; McIntosh, 1969) for acid soils exist, however, data for use in CB soils and crops is lacking for these methods. Therefore it is vital to determine which P chemistry
dominates in acidified CB soils and to identify which plant available P extraction methods are most appropriate. Sharpley (2000) indicated that the chemicals used in the extraction of available P must be suited to the particular soil properties in order to be successful.

Soil inorganic P fractionation schemes have been used for years to determine relative amounts of P pools present in soil. Pools of soluble, Fe-P, Al-P, reductant soluble P (RSP), and Ca-P can be chemically extracted in sequence by several methods (Chang and Jackson, 1957; Hedley et al., 1982; Tiessen and Moir, 1993; and Kuo, 1996). In addition to traditional chemical extraction methods, $^{31}\text{P}$ nuclear magnetic resonance spectroscopy (NMR) has been used to determine dominant Ca- or Al/Fe-P complexes in soils (Lookman et al., 1997; Delgado et al., 2000; Dougherty et al., 2005), animal waste (Hunger et al., 2004), and sediments (Reitzel et al., 2006). These studies reported results from both solid-state and solution $^{31}\text{P}$ NMR analysis. The work described herein, used the solid-state analysis to avoid problems with insoluble species and aggressive extractants that might compromise the soil pH premise of this study. The benefits of solid-state analysis together with some inherent problems are further discussed by Dougherty et al. (2005).

The objective of our research was to determine dominant P species in recently acidified soils of the CB using traditional chemical fractionation and $^{31}\text{P}$ NMR spectroscopy.

**MATERIALS AND METHODS**

To evaluate soil P speciation, 11 CB soil samples were collected in bulk from 0-30 cm representing three soil pH ranges and three OP levels within each pH range (Table 2.1; additional information in Bair and Davenport, 2012). Soil pH was determined on a 1:1 soil/water slurry using a Thermo Orion EA 940 pH meter (Waltham, MA). The three soil pH ranges were: L (<6.0), M (6.2-6.8), and H (>7.0). The three initial P ranges (measured as OP) were: 1 (<10), 2
(15-25), and 3 (>30) mg P kg\(^{-1}\). OP was used as the basis for comparisons because it is the preferred test method in the region from which the soils were collected. A CB soil with low pH (<6.0) and low OP (<10 mg kg\(^{-1}\)) was not located. Soil 1 represented a native (not influenced by agricultural activities) soil. Additionally, soils 12 and 13 were obtained from the Palouse area of Washington State used in a fractionation study by Koenig (2007). Soils 14 and 15 are part of the North American Proficiency Testing (NAPT) program and represent a non-Pacific northwest calcareous soil and a native acidic soil, respectively. Soils 16 and 17 were in-house references used only for the chemical fractionation portion of this study and are described as a CB native and western Washington native soils, respectively. In addition to available P, soils were analyzed for total P (Kuo, 1996), calcium carbonate (CaCO\(_3\)) (Loeppert and Suarez, 1996), and total Al and Fe (Gavlak et al., 2003).

Chemical P Fractionation

The inorganic P fractionation was performed by the method of Kuo (1996) and determines the amounts of P in the soluble, Fe-, Al-, RSP, and Ca-P fractions. Air-dried and ground to pass a 2-mm sieve, samples 1-17 were analyzed in triplicate, and color development was performed using the ascorbic acid method (Kuo, 1996). A slight modification suggested by Weaver (1974) for the RSP was utilized because of poor color development. Final P determination was done using a Spectronic 401 (Milton Roy, Iveyland, PA) at an 880 nm wavelength. Because the method takes several hours to complete a batch, replications were in the form of three individual batches. Analysis of variance (ANOVA) was conducted for soil inorganic P fractions using PC SAS system for Windows version 9.1 (SAS Institute, Cary, NC). Means were calculated with PROC GLM. Data were considered significantly different at \( P \leq \)
0.05 according to Tukey’s method of mean separation (Ott and Longnecker, 2001) within PROC GLM.

**31P NMR Spectroscopy**

Soil samples 1-15 were air-dried and ground to pass a 2-mm screen for analysis of solid-state 31P NMR. The spectra were collected with a spectrometer (Varian NMRS 500 MHz, Walnut Creek, CA) operating at 202.311 MHz. Because of the low amount of soil total P, 7.5-mm HX MAS zirconia rotor was used. The spin rate was 6 kHz and a total of 976 to 2,176 scans were collected per sample to acquire sufficient signal-to-noise ratios. Total acquisition time for 31P NMR spectra was around 8-12 h. The P chemical shift scale was standardized to 0 ppm using an external 85% v/v phosphoric acid standard. The P compounds were identified by their chemical shifts (ppm) and compared to published data for P speciation in soil samples.

**RESULTS AND DISCUSSION**

**Chemical P Fractionation**

Results of inorganic soil P fractionation for soils 1-17 can be found in Table 2.2. Soluble P accounted for the smallest pool of inorganic P, and ranged from <0.1-5.4% of the total extracted. Al-P totals were quite low and were only greater than 12% in soil 17. Fe-P was also low for most soils, with native acidic soils 15 and 17 containing nearly two times the percentage of the other soils tested. Measure of RSP includes P associated with Fe and Al that only becomes soluble in reduced conditions. This P fraction ranged from 0-26% of inorganic P for CB soils (including soil 13) but was greater in the low pH Palouse soil and the two native acidic soils, 12, 15 and 17, respectively. With the exception of soils 12, 15 and 17, all soils had ≥60% of inorganic P in the Ca-P form. Similarly, Koenig (2007) found that Ca-P was the dominant inorganic P species in Palouse soils of Washington State where pH varied greatly. Results of
inorganic P fractions in calcareous soils are comparable to Delgado et al. (2000), Adhami et al. (2006) and Roboredo and Coutinho (2006) in acid soils.

Table 2.2 provides the $P$-value for the factors of region (Eastern Washington vs non-Eastern Washington soils), OP, pH, and the interactive factors of pH*OP, and region*pH*OP for each inorganic P fraction. This data shows that, with the exception of the soluble P fraction, regional differences occurred for all other P fractions. Also, except for Ca-P, all fractions show significant differences ($\alpha = 0.05$) in the three-way interaction of region*pH*OP. Therefore, further analysis of different Eastern Washington (EW) soils based on OP and pH is justified.

To determine if significant differences existed in EW soils based on pH, soils 1-13 and 16 were identified based on initial OP level and differences based on pH analyzed statistically. Comparing H, M and L pH values within an OP class, significant differences occurred in soluble, Fe-, RSP, and Ca-P (Fig. 2.1). As pH increased above 7.2 the amount of Fe-P extracted decreased significantly compared to M and L pH. The same was not true for Al-P which was essentially the same across soil pH, with one exception at the low OP level. The relative amount of Ca-P was expected to trend opposite to Fe-P; however, this only occurred in the high OP soil while Ca-P was the same or significantly lower in the low and medium OP classes. Combining all CB soils, an additional graphical evaluation is made in Figure 2.2 comparing the sum of Fe- and Al-P forms and Ca-P to soil pH. From this figure it is clear that Ca-P is consistent regardless of soil pH, while Fe- and Al-P forms show an expected significant negative relationship with pH.

The significance of the Fe-P fraction in relation to CB soil pH demonstrates a possible state of transition for the soils examined. While the Fe-P constitutes only a small part of the inorganic soil P, there is evidence to suggest that this fraction is increasing as the soil pH decreases.
31P NMR Spectroscopy

Solid state 31P NMR chemical shifts for soils 1-15 can be found in Table 2.3. Previous solid state 31P NMR on soils have attempted to assign chemical shifts to Ca-P (2.2-2.6 ppm) (Rothwell et al., 1980; Hinedi, et al., 1992; Lookman et al., 1996; Delgado et al., 2000). A chemical shift in this range is related to the dominant Ca-P forms including precipitates of hydroxyapatite or fluorapatite. Bleam (1991) further explains that chemical shift peaks for Al-P crystallites occurred from 0-4 ppm and adsorbed P species from -10 to 2 ppm. In each case the shift downfield was related to lower pH.

The chemical shift of the isotropic peak in these samples falls within the range of 1.69-2.89 ppm. However, assigning peak chemical shifts to chemical P species for different soil pH levels was not possible due to lack of resolution caused by the broadness of the peaks (Fig. 2.3). These samples also have a significant spinning side band (SSB) pattern caused by the Chemical Shift Anisotropy (CSA). To reduce the SSB it is common practice to use smaller diameter rotors that are able to spin faster, but in this sample series the P concentration was low, in the range of 411 to 2281 mg P kg\(^{-1}\), therefore a rotor with a large volume was used to obtain data within a reasonable amount of time. Consequently, spinning used for these experiments was 6 kHz. Although the first set of 6 kHz side bands is outside the expected isotropic chemical shift of the P species of interest, the width of the isotropic peak did not allow for distinction of multiple species. Extraction of soil P and liquid 31P NMR analysis was not possible in this study because the methods utilize either sulfuric or hydrochloric acid (H\(_2\)SO\(_4\) or HCl) or sodium hydroxide-ethylenediaminetetraacetic (NaOH-EDTA) solutions which would alter the solution pH and subsequent P chemistry, rendering the comparisons ineffectual. The presence of paramagnetic ions caused a decrease in signal for the Fe-P species of interest and further reduced our ability to
make a meaningful interpretation. Treatment with HF (hydrofluoric acid) is commonly used to remove paramagnetic species and improve solid state $^{31}$P NMR spectra (Dougherty et al., 2007). This treatment would, however, preclude our ability to make comparisons based on pH.

The outcome and challenges of our $^{31}$P NMR study were similar to those found by Dougherty (2005) where only broad classifications of inorganic P species could be inferred. The presence of paramagnetic ions together with SSBs and low soil P all contributed to the inability to distinguish inorganic P species in the soil.

While chemical shift could not be used to determine P speciation, the spectra do show changes in SSB patterns across the samples. These line shape patterns contain information about the symmetry of the electron geometry around the phosphorus. Generally, as the SSB pattern becomes wide, the electron geometry around the P is less symmetrical. Using the data from the chemical sequential extraction, we can infer that soil #8 is the best example of a predominantly Ca-P soil while the P chemistry of soil #15 is likely in the form of Fe- or Al-P (Fig. 2.4).

CONCLUSIONS

Chemical P fractionation of CB soils demonstrated that Ca-P forms account for 60% or more of the inorganic P. When comparing soil initial P levels, it appears that certain fractions of P are affected by soil pH. Specifically, the Fe-P fraction increased as pH decreased below 6.8. While the Fe-P fraction makes up a relatively small portion of the total inorganic P compared to Ca-P, this increase may be a sign of potential transition from a predominantly Ca-P chemistry. The Ca-P fraction appeared to be similar across all soil pH and OP levels. Solid state $^{31}$P NMR was unable to distinguish between inorganic P fractions. Low soil P together with the presence of paramagnetic ions inhibited our ability to assign an inorganic P fraction based on the peak center chemical shift of each sample. Methods to overcome these problems rely on strongly
acidic or basic treatments that were not suitable to meet the soil pH related objectives of this project. Some general inferences can be made involving the shape of the NMR spectra that relate to the geometric symmetry of electrons around the P, however, more research is needed to make this analysis definitive with respect to P speciation. Because the P chemistry of these soils is largely related to Ca, even in cases of acidification, the use of the OP extraction method appears to be the best approach to continue to test for plant available P in soils of the CB.
REFERENCES


Table 2.1. Characteristics of 17 soil samples used in this study. Included are the soil site history, pH, total P, and extractable P levels (OP, MMP, BP1, M3P)† and several other chemical parameters. Also given are rankings for pH (H = >7.0, M = 6.2-6.8, and L = <6.0) and initial P (measured as OP) (1 = <10, 2 = 15-25, and 3 = >30 mg P kg⁻¹).

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† OP = Olsen P (NaHOC₃), BP1 = Bray P-1 (HCl + NH₄F), MMP = Morgan P (NH₄OAc), M3P = Mehlich III P (HOAc + NH₄NO₃ + NH₄F + HNO₃ + EDTA)
Table 2.2. Average (n = 3) inorganic P fractionations shown in mg P kg\(^{-1}\) and as a percentage of the total inorganic P for soils 1-17. Also shown are the levels of significant difference (P-value) of the factors determined by ANOVA of region (Eastern Washington vs non-Eastern Washington soils), OP, pH, and the interactive factors of OP*pH, and region*OP*pH, for each inorganic P fraction.

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<th>Al-P mg kg(^{-1})</th>
<th>Fe-P mg kg(^{-1})</th>
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OP 0.0001 | 0.0004 | <0.0001 | <0.0001 | 0.0435
pH 0.0001 | <0.0001 | <0.0001 | 0.0420 | 0.3951
OP*pH 0.0460 | 0.0043 | 0.2857 | 0.2092 | 0.0133
Region*OP*pH 0.0363 | <0.0001 | <0.0001 | 0.0063 | 0.2758
Table 2.3. Mean chemical shift peak centers for soils 1-15 analyzed by solid state $^{31}$P NMR spectroscopy. Also given are a site history, pH and rankings for pH (H = >7.0, M = 6.2-6.8, and L = <6.0) and initial P (measured as OP) (1 = <10, 2 = 15-25, and 3 = >30 mg P kg$^{-1}$).

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Figure 2.1. Inorganic P fractions of soluble P, Al-P, Fe-P, RSP, and Ca-P for EW soils 1-13, 16.

The data represents mean values and are grouped by soil pH within an initial P (as OP) level. Means with different letters within an initial P group are significant ($\alpha = 0.05$).
Figure 2.2. Mean Ca-P and Fe+Al-P forms versus soil pH for EW soils 1-13 and 16. Best-fit lines and equations are shown for the inorganic P forms. Coefficients of determination and their significance are also given for each line.
Figure 2.3. $^{31}\text{P}$ NMR analysis of soil #4 showing the chemical shift of the center peak and the spinning side band pattern typical of Columbia Basin (CB) soils.
Figure 2.4. $^{31}$P NMR spectra of a) soil #8 (dominated by Ca-P) and b) soil #15 (dominated by Fe/Al-P. The chemical shift of each center peak is also shown.
CHAPTER THREE

INFLUENCE OF RECENT ACIDIFICATION ON AVAILABLE PHOSPHORUS INDICES
AND SORPTION IN WASHINGTON STATE SOILS

(Accepted and published by Soil Science Society of America Journal, 2012, 76:515-521)

ABSTRACT

Gradual soil acidification in the Columbia Basin (CB), Washington State, brings into question the validity of the traditional sodium bicarbonate (NaHCO₃) extractable phosphorus (Olsen P, OP) method for making fertility recommendations. Our objectives were to determine how soil pH influences the soil P indices after fertilization and sorption capacity of soils with similar levels of available P at varying soil pH levels. Collected soils represented a range of pH (5.2-8.4) and OP (3-40 mg P kg⁻¹). Soils were treated with 0, 44, 88, or 132 kg P ha⁻¹ and incubated at 20° C for 6 weeks. Subsamples were collected and analyzed for available P with OP, Bray P1 (BP1), Morgan (MMP), Mehlich-III (M3P) extractions. Additionally, P sorption isotherms were performed on untreated soils. Analysis of variance indicated that the slope of the change in extractable P was generally not significant (α = 0.05) for OP, BP1, MMP, and M3P given a P addition for several soils at different pH but of similar initial available P levels. Grouping soils by pH showed differences in slope with OP (0.27-0.34) yielding the least difference and MMP (0.12-0.33) the greatest related to soil pH. Langmuir parameters of b, sorption capacity, and k, binding energy, showed variability for soils grouped by initial available P for several pH levels. Grouped by pH there was little difference in P sorption in relation to soil pH. Continued use of OP on acidified soils of the CB appears to be a viable soil testing strategy.
INTRODUCTION

The Columbia Basin (CB) of Washington State is renowned for the production of a variety of crops including potatoes \((\text{Solanum tuberosum } \text{L.})\), corn \((\text{Zea mays } \text{L.})\), wheat \((\text{Triticum aestivum } \text{L.})\) and onions \((\text{Allium cepa } \text{L.})\). Nutrient removal by these crops is significant and addition of fertilizers is necessary to maintain adequate nutrition for optimum crop production. For example, a high yielding potato crop \((90 \text{ Mg ha}^{-1})\) may remove more than \(250 \text{ kg N ha}^{-1}\) (Kleinkopf et al., 1981).

The most commonly deficient nutrient in plant production is nitrogen (N). Nitrogen is usually applied in ammoniacal forms because of availability and low cost. One consequence of using ammonia fertilizers is that as one mole of \(\text{NH}_4^+\) nitrifies, 2 moles of \(\text{H}^+\) are produced (Helyar, 1976). Continual use of ammonia based fertilizers accelerates soil acidification beyond natural N-cycle processes (Mahler and Harder, 1984; Rasmussen and Rohde, 1989).

Soils of the CB, before anthropogenic influence, have a pH of 6.1 – 9.0 (Cochran et al., 2006; Soil Survey Staff, 2012). All soil pH data in this work refer to a 1:1 soil/water measurement. However, recently observed soil pH values less than 5.0 are not uncommon (Stevens et al., 2003). The change in soil pH raises the question of whether the OP extraction (Olsen et al., 1954) can be used reliably on low pH soils because the method was developed for neutral to alkaline soil to determine plant available P, a nutrient that varies greatly in phyto-availability depending on soil pH. The OP method, using \(\text{NaHCO}_3\) to extract plant available P, is most commonly used for calcareous soils where P complexes with calcium (Ca).

Eastern Washington State has established P fertilizer recommendations based on the results of the OP method. Soil testing laboratories continue to use the OP extraction on recently
acidified soils regardless of soil pH (R. Miller, personal communication, 2011). Sharpley (2000) indicated that the reagents used in the extraction of available P must be suited to the particular soil properties in order to be successful. Further, Holford (1980) suggests that the P test utilized should have good correlation with plant yield or P uptake across varying soil chemical conditions. Alternative extraction methods of plant available P for acidic soils are: BP1 (Bray and Kurtz, 1945), M3P (Mehlich, 1984), MMP (McIntosh, 1969), or ion exchange resins. Data for use in CB soils and crops are lacking for these methods; however, OP has shown promise (Kuo, 1990) in extracting plant available P from low pH soils. For this reason the OP method continues to be the preferred method for CB soils regardless of soil pH.

The extraction procedures for P in this study are availability indices. In other words, an amount of P that is assumed to be directly related to plant availability is extracted and is dependent on soil properties and plant requirements. Phosphorus sorption isotherms not only take into account the intensity of extracted P, but also the capacity of the soil to provide P for plant production (Singh et al., 2005). Determination of plant P requirements based on data from sorption isotherms has been demonstrated to be more accurate than using only conventional P soil tests (Hue and Fox, 2010; Klages et al., 1988). The Langmuir equation is often fitted to P sorption isotherm data in environmental applications because the maximum sorption (b), and binding energy (k), can be calculated and analyzed statistically. The Langmuir sorption maximum has been shown to be well correlated with exchangeable calcium (Ca) (Tunesi et al., 1999) as well as iron (Fe) and aluminum (Al) oxides (Zhang et al., 2005), parameters often associated with soil pH.
The objectives of this research are 1) to determine how soil pH influences the results of the soil P indices after fertilization for soils with similar levels of plant available P and 2) to evaluate the P sorption capacity of soils with similar levels of available P at varying soil pH levels.

MATERIALS AND METHODS

To evaluate plant available P extractions, 10 CB soil samples were collected in bulk from 0-30 cm representing three soil pH ranges and three OP levels within each pH range (Table 3.1). Soil pH was determined on a 1:1 soil/water slurry using a Thermo Orion EA 940 pH meter (Waltham, MA). The three soil pH ranges were: L (<6.0), M (6.2-6.8), and H (>7.0). The three initial P ranges (measured as OP) were: 1 (<10), 2 (15-25), and 3 (>30) mg P kg\(^{-1}\). OP was used as the basis for comparisons because it is the preferred test method in the region from which the soils were collected. A CB soil with low pH (<6.0) and low OP (<10 mg kg\(^{-1}\)) was not located. Soil 1 represented a native (not influenced by agricultural activities) soil. In addition to available P, soils were analyzed for total P (Kuo, 1996), calcium carbonate (CaCO\(_3\)) (Loeppert and Suarez, 1996), organic matter (OM), exchangeable Al, Ca, potassium (K), magnesium (Mg) and sodium (Na), and cation exchange capacity (CEC) by ammonium replacement (Gavlak et al., 2003). Total bases and percent base saturation (sum of Ca, Mg, K, and Na divided by CEC) were calculated.

Soil Incubation

To determine how extraction method results differ across soil pH ranges following fertilization, 250 g of each soil was weighed into 450 ml polystyrene cups, fertilized with 0, 21, 42, 63 mg P kg\(^{-1}\) (which represent field application levels of 0, 44, 88 or 132 kg P ha\(^{-1}\)) added as
monoammonium phosphate, MAP (11-52-0) in four replicates. Because no plants were to be grown, the N was not adjusted to reach the same N application rate across treatments. Soils were mixed thoroughly, moistened to slightly less than saturation, and maintained with de-ionized water at 20°C for six weeks. At 2 week intervals, 50 g subsamples were taken from each cup, air dried, and ground to pass a 2 mm sieve.

Subsamples from 2, 4, and 6 week time intervals were extracted by OP, BP1, MMP, and M3P (Gavlak et al., 2003). Phosphorus determination was performed colorimetrically (Milton Roy Spectronic 401, Ivyland, PA; Watanabe and Olsen, 1965) for OP, BP1, and MMP. M3P was not analyzed colorimetrically because the color development is prohibitively rapid. M3P analysis was performed with a Perkin Elmer DV3300 Optima Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES, Perkin Elmer, Waltham, MA).

Analysis of variance (ANOVA) was conducted for available P by incubation time, and available P by soil pH interactions using PC SAS system for Windows version 9.1 (SAS Institute, Cary, NC). Means were calculated with PROC GLM. Data were considered significantly different at $P \leq 0.05$ according to Tukey’s method of mean separation within PROC GLM. Multiple contrasts were made using Bonferroni’s method.

P Sorption Isotherms

Phosphorus sorption isotherms were determined on soils 1-10 by adding P solutions of 0, 6.45, 16.13, 32.26, 161.3, and 323 µmol P L$^{-1}$ as KH$_2$PO$_4$ in 0.01 M CaCl$_2$ at a 1:25 soil/solution ratio (Nair et al., 1984) into 50 ml plastic centrifuge tubes. After end-over-end shaking for 24 hours at 25°C the samples were filtered (0.45 µm) and analyzed colorimetrically for P concentration by the same method used for the soil incubation samples. This method has been
used by other researchers studying P sorption (Sharpley, 2003; Fang et al., 2002; Young and Ross, 2001; Pau1ter and Sims, 2000). The difference between P added and the P remaining in solution was calculated as the amount of P sorbed. This analysis was conducted on three replicates of untreated soil sample and means presented. Pooled sorbed P \( (Y = \text{mg P kg}^{-1}) \) data separated only by soil pH and equilibrium P concentration \( (X = \text{mg P L}^{-1}) \) were fitted using the Langmuir isotherm equation:

\[
Y = \frac{bX}{1 + kX}
\]

where \( Y \) is the sorbed concentration (mg kg\(^{-1}\)), \( b \) is the maximum sorption capacity of the soil (mg kg\(^{-1}\)), \( k \) is the binding constant of the Langmuir equation (L mg\(^{-1}\)) and \( X \) is the equilibrium concentration (mg L\(^{-1}\)). Best fit lines for the Langmuir equation were obtained by minimizing the sum of errors squared of the actual versus modeled isotherm data using the solver tool in Microsoft Excel (Redmond, WA) to adjust the values of \( b \) and \( k \). Means of \( b \) and \( k \) were compared using Bonferroni’s a priori method using the PROC GLM procedure in SAS. Correlation statistics were obtained using PROC REG for the relationship between observed and the modeled Langmuir isotherm.

RESULTS AND DISCUSSION

Soil Incubation

To test the hypothesis that soil pH influences the available P index following fertilizer application, change in tested available P over the 0 kg P ha\(^{-1}\) treatment was plotted against P rate for each soil at 2, 4, and 6 weeks of incubation time. The slope of the line from the plotted data heretofore references the expected change in soil test available P at a given P fertilizer rate. The
change in tested P following fertilizer application was comparable to the findings of other researchers (Yang and Jacobsen, 1990; Rubio et al., 2008). ANOVA revealed that line slopes were similar for individual soils within an extraction method and showed no change in available P from week 2 to week 6 of incubation (data not shown). Therefore, comparisons were made pooling data from weeks 2, 4, and 6.

Slopes created from the relationship between the changes in extractable P following fertilizer application were analyzed using Bonferroni *a priori* contrasts for similar initial P level soils across the pH range represented (Table 3.2) and suggest that, on average, within an extractable P class (low, medium, or high available P), soil P extraction is not affected by pH with any of the tested methods. The single exception was with the MMP extraction of a low P soil where the slope for the higher pH soil was significantly (*P* = 0.03) higher than the medium pH soil.

If the P response slopes are pooled only by soil pH, a trend of higher slopes with higher pH can be observed for OP and MMP, while the opposite (lower soil pH, higher slope) occurs for M3P and BP1 extractions (Figure 3.1). The higher slopes with higher pH for OP are the result of the NaHCO₃ extractant. As the pH of the soil increases, there is greater tendency to form CaCO₃ precipitates, leaving greater amounts of formerly Ca bound P in solution. For M3P and BP1 the likely reason for lower slopes occurring with high pH soils is because Ca (Blanchar and Caldwell, 1964) and CaCO₃ (Randall and Grava, 1971) have a neutralizing effect on acid extractants resulting in an incomplete P extraction. Previous research (Nesse, et al., 1988) comparing non- and violently-effervescent (>7% calcium carbonate equivalent) soils
demonstrated that the reliability of acid extractions (M3P and BP1) decreases significantly with increasing effervescence.

Of the four extraction methods tests, OP shows the least amount of slope variability (0.27—0.34) across soil pH while MMP (0.12—0.33) exhibited the greatest differences. Similar consistency in soil test P increases in relation to P fertilization with the OP extraction has been demonstrated on low pH soils (Davenport et al., 2009).

P Sorption Isotherms

Phosphorus sorption isotherms were conducted to test whether differences in sorption capacity could be attributed to soil pH. Measured isotherm data was plotted with the best fit lines for the Langmuir isotherm equation for soils 1-10 and the results appear in Fig. 3.2. The observed data for all soils were well correlated ($P < 0.001$) with the modeled Langmuir isotherm (Table 3.3). Other researchers (Olsen and Wanatabe, 1957; Del Bubba et al., 2003; Whalen and Chang, 2002) have also found the Langmuir model appropriate for sandy and calcareous soils.

The general shape of the curve was similar for most soils with soils 6 and 7 being outliers (Figure 3.2a, b). These two soils draw attention to the fact that despite similarities between many of the soil P sorption capacities, a generalization for all soils in the region is not realistic. Previous research has shown that several factors other than soil pH can account for differences in P sorption capacity of soil: soil surface area (Olsen and Wanatabe, 1957; Del Bubba et al., 2003; Casson et al., 2006), Ca and Mg content of the soil as well as porosity, bulk density, hydraulic conductivity (Del Bubba et al., 2003), and soil organic matter content (Kang et al., 2009). These soils belong to the Outlook series and are distinguished from the other soils in this project by
being fine-textured alluvial soils with a high water table. The fine fraction of the soil texture likely contributes to the large increase in sorption capacity compared to the other soils.

Table 3.3 gives the mean separation for the binding energy, $k$, and the sorption maximum, $b$, for the soils separated by initial available P level. With exception of soil 5, the lowest values of $k$ were observed in the highest initial P soils. Overall these high P soils also showed the greatest sorption maximum (excluding soils 6 and 7). This suggests that the soils with low and moderate amounts of initial P can sorb less P and the energy required for P removal is greater than other soils with high initial P.

Separating the data only by soil pH and removing soils 6 and 7, Fig. 3.3, soil pH was not a factor in P sorption capacity for the 10 CB soils tested. Further analysis (Table 3.4), shows some separation in $k$ for the soils tested, however, sorption maximum for low, medium and high pH soils is not significant ($\alpha = 0.05$).

CONCLUSION

The results from this study showed that the increase in several extractable P forms following incubation with fertilizer for soils that vary in initial P status and pH did not significantly differ with soil pH. When the slopes for extractable P concentration with increasing P rate (fertilizer P addition) are compared based solely on soil pH, different slopes were found for each extraction method and were likely related to the chemistry of the extraction reagents. Of the extraction methods tested, OP demonstrated the least variability in slope across the pH ranges tested and MMP the most. This means that if a P fertilizer addition is made to low or high pH soils, the amount of P extracted by OP will be very similar for both. Further, assessment of plant need for fertilizer P at a given level of OP appears to be valid for several pH levels in the
CB and the fertilizer calibrations made on high pH soils are suitable under low pH conditions. There are two possible explanations for OP working well with low pH soil: 1) the soils of the CB, although low in pH in some cases, still exhibit a dominant Ca-P chemistry or 2) if Fe/Al-P complexes exist, the amount of P extracted is similar to the traditionally assumed Ca-P state.

Isotherm data for soils 1-10 were well correlated with modeled Langmuir values. Analysis of sorption maxima calculated from the Langmuir isotherm show some variability based on soil pH for given initial P ranges. Pooled by soil pH, differences in sorption maximum for low, medium and high pH soils were not statistically significant.
REFERENCES


Table 3.1. Soil characteristics of 10 Columbia Basin bulk samples. Included are the soil site and taxonomic descriptions, pH, total P, and extractable P levels (OP, MMP, BP1, M3P)† and several other chemical parameters. Also given are rankings for pH (H = >7.0, M = 6.2-6.8, and L = <6.0) and initial P (measured as OP) (1 = <10, 2 = 15-25, and 3 = >30 mg P kg\(^{-1}\)).

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>Description</th>
<th>Taxonomy</th>
<th>pH</th>
<th>Initial Rank</th>
<th>OP Rank</th>
<th>OP</th>
<th>MMP</th>
<th>BP1</th>
<th>M3P</th>
<th>Total</th>
<th>CaCO(_3)</th>
<th>OM</th>
<th>Al</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>Total Bases</th>
<th>CEC</th>
<th>Saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Native Winchester Sand, Mixed, mesic Xeric Torripsamments</td>
<td></td>
<td>7.2</td>
<td>H</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>12</td>
<td>8.0</td>
<td>1023</td>
<td>0.6</td>
<td>0.68</td>
<td>1010</td>
<td>194</td>
<td>29.9</td>
<td>7.6</td>
<td>8.4</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Agricultural Timmerman Course Sandy Loam, Sandy, mixed, mesic Xeric Haplocambids</td>
<td></td>
<td>6.2</td>
<td>M</td>
<td>2</td>
<td>20</td>
<td>6</td>
<td>23</td>
<td>73.7</td>
<td>943</td>
<td>1.2</td>
<td>1.2</td>
<td>2.33</td>
<td>1270</td>
<td>249</td>
<td>50.6</td>
<td>8.9</td>
<td>12</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>Lightly Manured Agricultural Royal Loamy Fine Sand, Coarse-loamy, mixed, superactive, mesic Xeric Haplocambids</td>
<td></td>
<td>7.7</td>
<td>H</td>
<td>3</td>
<td>33</td>
<td>42</td>
<td>37</td>
<td>63.4</td>
<td>1100</td>
<td>3.5</td>
<td>1.7</td>
<td>0.68</td>
<td>3300</td>
<td>200</td>
<td>69.0</td>
<td>19.7</td>
<td>14.2</td>
<td>139</td>
</tr>
<tr>
<td>4</td>
<td>Agricultural Warden Silt Loam, Coarse-silty, mixed, superactive, mesic Xeric Haplocambids</td>
<td></td>
<td>5.9</td>
<td>L</td>
<td>2</td>
<td>25</td>
<td>7</td>
<td>40</td>
<td>45.7</td>
<td>647</td>
<td>0.7</td>
<td>1.0</td>
<td>0.82</td>
<td>1420</td>
<td>254</td>
<td>55.2</td>
<td>13.5</td>
<td>11.9</td>
<td>88</td>
</tr>
<tr>
<td>5</td>
<td>Garden Strat Stoney Loam, Loamy-skeletal, mixed, superactive, mesic Calcic Haploxerolls</td>
<td></td>
<td>7.9</td>
<td>H</td>
<td>2</td>
<td>20</td>
<td>24</td>
<td>25</td>
<td>40.1</td>
<td>482</td>
<td>1.0</td>
<td>1.7</td>
<td>0.83</td>
<td>1240</td>
<td>327</td>
<td>190.9</td>
<td>11.9</td>
<td>11.6</td>
<td>103</td>
</tr>
<tr>
<td>6</td>
<td>Pasture Outlook Very Fine Sandy Loam, Coarse-silty, mixed, superactive, calcareous, mesic Aeric Halaquepts</td>
<td></td>
<td>6.4</td>
<td>M</td>
<td>2</td>
<td>15</td>
<td>4</td>
<td>9</td>
<td>17.9</td>
<td>707</td>
<td>1.2</td>
<td>1.4</td>
<td>0.50</td>
<td>960</td>
<td>450</td>
<td>234.6</td>
<td>10.6</td>
<td>10.4</td>
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<tr>
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<td>Pasture Outlook Very Fine Sandy Loam, Coarse-silty, mixed, superactive, calcareous, mesic Aeric Halaquepts</td>
<td></td>
<td>8.4</td>
<td>H</td>
<td>1</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>11.3</td>
<td>664</td>
<td>8.1</td>
<td>2.3</td>
<td>0.18</td>
<td>3680</td>
<td>956</td>
<td>112.7</td>
<td>27.3</td>
<td>15.8</td>
<td>173</td>
</tr>
<tr>
<td>8</td>
<td>Agricultural Warden Silt Loam, Coarse-silty, mixed, superactive, mesic Xeric Haplocambids</td>
<td></td>
<td>5.2</td>
<td>L</td>
<td>3</td>
<td>40</td>
<td>8</td>
<td>49</td>
<td>100.0</td>
<td>816</td>
<td>0.3</td>
<td>0.9</td>
<td>14.33</td>
<td>920</td>
<td>206</td>
<td>34.5</td>
<td>7.2</td>
<td>10.1</td>
<td>71</td>
</tr>
<tr>
<td>9</td>
<td>Agricultural Ephraita Gravely Sandy Loam, Coarse-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Xeric Haplocambids</td>
<td></td>
<td>6.7</td>
<td>M</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>9.5</td>
<td>633</td>
<td>1.2</td>
<td>1.4</td>
<td>1.02</td>
<td>1500</td>
<td>242</td>
<td>59.8</td>
<td>10.0</td>
<td>12.4</td>
<td>81</td>
</tr>
<tr>
<td>10</td>
<td>Agricultural Warden Silt Loam, Coarse-silty, mixed, superactive, mesic Xeric Haplocambids</td>
<td></td>
<td>6.5</td>
<td>M</td>
<td>3</td>
<td>32</td>
<td>21</td>
<td>56</td>
<td>70.2</td>
<td>617</td>
<td>1.8</td>
<td>1.1</td>
<td>1.38</td>
<td>2000</td>
<td>370</td>
<td>20.7</td>
<td>14.1</td>
<td>16.4</td>
<td>86</td>
</tr>
</tbody>
</table>

† OP = Olsen P (NaHOC\(_3\)), BP1 = Bray P-1 (HCl + NH\(_4\)F), MMP = Morgan P (NH\(_4\)OAc), M3P = Mehlich III P (HOAc + NH\(_4\)NO\(_3\) + NH\(_4\)F + HNO\(_3\) + EDTA)
Table 3.2. Average slope values (n = 12) of the lines for change in soil extractable P versus P addition rate using 4 different P extraction techniques (OP, BP1, MMP and M3P). Levels of significance for slope comparison using the Bonferroni \textit{a priori} contrasts for soils of similar initial P level (<10 = 1, 15-25 = 2, >30 mg P kg\(^{-1}\) = 3) across high (>7.0, H), medium (6.2-6.8, M) and low (<6.0, L) pH.

<table>
<thead>
<tr>
<th>Initial P Rank</th>
<th>Soil pH Rank</th>
<th>OP</th>
<th>BP1</th>
<th>MMP</th>
<th>M3P</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,7 H</td>
<td>0.34</td>
<td>0.14</td>
<td>0.29</td>
<td>0.46</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>9 M</td>
<td>0.22</td>
<td>0.28</td>
<td>0.10</td>
<td>0.31</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.25</td>
<td>0.87</td>
<td>0.03</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5 H</td>
<td>0.45</td>
<td>0.65</td>
<td>0.46</td>
<td>0.64</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>2,6 M</td>
<td>0.25</td>
<td>0.31</td>
<td>0.14</td>
<td>0.34</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>4 L</td>
<td>0.33</td>
<td>0.50</td>
<td>0.12</td>
<td>0.49</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.19</td>
<td>0.44</td>
<td>0.52</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3 H</td>
<td>0.43</td>
<td>0.40</td>
<td>0.37</td>
<td>0.48</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>10 M</td>
<td>0.37</td>
<td>0.63</td>
<td>0.27</td>
<td>0.57</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>8 L</td>
<td>0.36</td>
<td>0.52</td>
<td>0.11</td>
<td>0.53</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.17</td>
<td>0.60</td>
<td>0.75</td>
<td>0.92</td>
<td></td>
</tr>
</tbody>
</table>

† OP = Olsen P (NaHOC\(_3\)), BP1 = Bray P-1 (HCl + NH\(_4\)F), MMP = Morgan P (NH\(_4\)OAc), M3P = Mehlich III P (HOAc + NH\(_4\)NO\(_3\) + NH\(_4\)F + HNO\(_3\) + EDTA)
Table 3.3. Mean separation for sorption maximum, \( b \) and binding energy, \( k \) for the Langmuir isotherm constants performed on soils 1-10 (\( n = 3 \)). Soils are grouped by H (>7.0), M (6.2-6.8) and L (<6.0) pH within an initial P (as OP) level (<10 = 1, 15-25 = 2, >30 mg P kg\(^{-1}\) = 3). Means with different letters for constants within an initial P group are significant (\( \alpha = 0.05 \)). Correlation and significance of observed vs. the modeled Langmuir isotherm are also given.

<table>
<thead>
<tr>
<th>Initial P Rank</th>
<th>Soil pH</th>
<th>Langmuir Constants</th>
<th>( k ) ( \text{mg P L}^{-1} )</th>
<th>( b ) ( \text{mg P kg}^{-1} )</th>
<th>( R^2 )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>1.17 a</td>
<td>64.5 c</td>
<td>0.97</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>H</td>
<td>0.42 b</td>
<td>282 a</td>
<td>0.99</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>0.58 b</td>
<td>112 b</td>
<td>0.98</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LSD(_{0.05})</td>
<td>0.28</td>
<td>19.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>H</td>
<td>0.20 c</td>
<td>101 c</td>
<td>0.96</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>0.76 a</td>
<td>107 c</td>
<td>0.98</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>0.69 ab</td>
<td>255 a</td>
<td>0.95</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>L</td>
<td>0.32 bc</td>
<td>150 b</td>
<td>0.98</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LSD(_{0.05})</td>
<td>0.37</td>
<td>27.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>0.11 b</td>
<td>251 a</td>
<td>0.98</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>0.23 a</td>
<td>200 ab</td>
<td>0.96</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>L</td>
<td>0.20 ab</td>
<td>173 b</td>
<td>0.98</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LSD(_{0.05})</td>
<td>0.11</td>
<td>59.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.4. Mean separation for sorption maximum, $b$ and binding energy, $k$ for the Langmuir isotherm constants performed on soils 1-10 grouped only by pH ($H = >7.0$ (n = 12), $M = 6.2-6.8$ (n = 12), $L = <6.0$ (n = 6)). Means with different letters for constants within a pH group are significant ($\alpha = 0.05$). Correlation and significance of observed vs. the modeled Langmuir isotherm are also given.

<table>
<thead>
<tr>
<th>pH</th>
<th>Langmuir Constants</th>
<th>$R^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k$ [mg P L$^{-1}$]</td>
<td>$b$ [mg P kg$^{-1}$]</td>
<td></td>
</tr>
<tr>
<td>$H$</td>
<td>0.16 b</td>
<td>176 a</td>
<td>0.97</td>
</tr>
<tr>
<td>$M$</td>
<td>0.51 a</td>
<td>131 a</td>
<td>0.95</td>
</tr>
<tr>
<td>$L$</td>
<td>0.27 ab</td>
<td>175 a</td>
<td>0.98</td>
</tr>
<tr>
<td>LSD$_{0.05}$</td>
<td>0.25</td>
<td>61.5</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.1. Slope analysis of data pooled for all initial P (as OP) levels within soil pH: >7.0 (n = 36), 6.2-6.8 (n = 36), <6.0 (n = 18)) for OP, MMP, BP1, and M3P† following 6-week incubation with fertilizer for soils 1-10. Slopes with different letters are statistically significant at (α = 0.05)

† OP = Olsen P (NaHOC₃), BP1 = Bray P-1 (HCl + NH₄F), MMP = Morgan P (NH₄OAc), M3P = Mehlich III P (HOAc + NH₄NO₃ + NH₄F + HNO₃ + EDTA)
Figure 3.2. Best fit lines for Langmuir P sorption isotherms conducted for soils 1-10 (n = 3) grouped by initial P (as OP) level. High (>7.0), medium (6.2-6.8) and low (<6.0) pH soils are shown where applicable. Sorbed P is calculated as the difference between the P added and the P remaining in solution following incubation and 24 hour shaking. The points are the collected data.
Figure 3.3. Results of P sorption isotherms conducted for soils 1-5 and 8-10 grouped by High (>7.0, n = 9), Medium (6.2-6.8, n = 9) and Low (<6.0, n = 6) soil pH. Sorbed P is calculated as the difference between the P added and the P remaining in solution following incubation and 24 hour shaking. Best fit lines and equations for the Langmuir isotherm are shown for the data sets.
CHAPTER FOUR

PLANT AVAILABLE PHOSPHORUS ANALYSIS FOR RECENTLY ACIDIFIED SOILS OF THE COLUMBIA BASIN WASHINGTON STATE

ABSTRACT

Soil acidification in the Columbia Basin (CB) of Washington State, brings into question the validity of the sodium bicarbonate (NaHCO$_3$) extractable phosphorus (Olsen P, OP) method for making fertilizer recommendations. The objectives of this research are to 1) identify the soil test method (Olsen, OP; Bray P, BP1; Mehlich-III, M3P; Morgan, MMP; and plant root simulator, PRS$^{TM}$ probes) that best correlates with plant available P at pH < 7.0 in CB soils and 2) evaluate relationships between OP and biomass production, plant tissue P and plant P uptake in acidified CB soils.

Corn and wheat plants were grown in soils representing a range of pH (5.2-8.4) and OP (3-40 mg P kg$^{-1}$) and were treated with 0, 44, 88, or 132 kg P ha$^{-1}$. Half of the replicates contained PRS$^{TM}$ probes and were analyzed for P bi-weekly. Plants were measured for height, biomass, and tissue P concentration following a 6-week growth period. Coefficients of determination for plant biomass as a function of soil test phosphorus were: M3P (0.81), PRS$^{TM}$-P (0.70), MMP (0.69), OP (0.68), and BP1 (0.49) for the corn crop and OP (0.76), M3P (0.73), Bray (0.53), MMP (0.29), and PRS$^{TM}$-P (0.25) for wheat. Analysis of variance indicated that plant biomass, plant P content, and PRS$^{TM}$-P were not significantly influenced by soil pH at a given OP level for corn and wheat crops. Therefore, one can expect similar plant response to a given OP
measurement regardless of soil pH. Continued use of OP on acidified CB soils appears to be the more suitable alternative than to switching extraction methods.

INTRODUCTION

The Columbia Basin (CB) of Washington State is renowned for the production of a variety of crops including potatoes (Solanum tuberosum L.), corn (Zea mays L.), wheat (Triticum aestivum L.) and onions (Allium cepa L.). Nutrient removal by these crops is significant and addition of fertilizers is necessary to maintain soil fertility levels for optimum crop production. For example, a high yielding potato crop (90 Mg ha\(^{-1}\)) may remove more than 250 kg N ha\(^{-1}\) (Kleinkopf et al., 1981).

The most commonly deficient nutrient in plant production is nitrogen (N). Nitrogen is usually applied in ammoniacal forms because of their availability and low cost. One consequence of using ammoniacal fertilizers is that as one mole of NH\(_4^+\) nitrifies, 2 moles of H\(^+\) are produced (Helyar, 1976). Continual use of ammonia based fertilizers accelerates soil acidification beyond natural N-cycle processes (Mahler and Harder, 1984; Rasmussen and Rohde, 1989).

Soils of the CB, before anthropogenic influence, have a pH of 6.1 – 9.0 (Cochran et al., 2006; Soil Survey Staff, 2012). However, recently, observed soil pH values less than 5.0 are common (Stevens et al., 2003). The change in soil pH raises the question of the validity of using the Olsen (Olsen et al., 1954) phosphorus (P) extraction which was developed for neutral to alkaline soil to determine plant available phosphorus, a nutrient that varies greatly in phyto-availability depending on soil pH. The Olsen P method (OP), which uses sodium bicarbonate (NaHCO\(_3\)) to extract plant available P, is most commonly used for calcareous soils where P complexes with calcium (Ca). In the Olsen P
extraction, the activity of Ca decreases as it precipitates out as calcium carbonate 
(CaCO$_3$) leaving the plant available portion of P behind in solution which is then related 
to plant response (e.g. yield, uptake and vigor).

Established P fertilizer recommendations in Eastern Washington State are based 
on the results of Olsen P. Soil testing laboratories continue to use the Olsen P extraction 
on recently acidified soils regardless of soil pH (R. Miller, personal communication, 
2011). Sharpley (2000) indicated that the chemicals used in the extraction of available P 
must be suited to the particular soil properties in order to be successful. Further, Holford 
(1980) suggests that good correlation with plant yield or P uptake should be the criterion 
by which an appropriate test should be determined across a spectrum of soil chemical 
properties. Alternative P extraction methods for acidic soils are: Bray-P1 (BP1) (Bray 
and Kurtz, 1945), Mehlich I and III (M3P) (Mehlich, 1984), Morgan (MMP) (Morgan, 
1941), modified Morgan (McIntosh, 1969), or ion exchange resins. Data for use in CB 
soils and crops is lacking for these methods; however, OP has shown promise (Kuo, 
1990) in extracting plant available P in low pH soils. For this reason the Olsen P method 
continues to be the preferred method for CB soils regardless of soil pH.

The objectives of this research are to 1) identify the soil test method (Olsen, OP; 
Bray P, BP1; Mehlich-III, M3P; Morgan, MMP; and plant root simulator, PRS$^\text{TM}$ probes) 
that correlates with plant available P at pH < 7.0 in CB soils and 2) evaluate relationships 
between OP and biomass production, plant tissue P and plant P uptake in acidified CB 
soils.
MATERIALS AND METHODS

To determine if the Olsen P extraction correlates with plant available P in acidic conditions, 9 CB soil samples were collected in bulk from 0-30 cm representing three soil pH ranges and three OP levels within each pH range (Table 4.1). The three soil pH ranges were: L (<6.0), M (6.2-6.8), and H (>7.0). Soil pH was determined on a 1:1 soil/water slurry using a Thermo Orion EA 940 pH meter (Waltham, MA). The three initial P ranges (measured as OP) were: 1 (<10), 2 (15-25), and 3 (>30) mg P kg\(^{-1}\). A CB soil with low pH (<6.0) and low OP (<10 mg kg\(^{-1}\)) was not located. In addition, soil 1 represented a native Eastern Washington soil and was used only for biomass correlation analysis with the available P extraction indices. In addition to available P, soils were analyzed for total P (Kuo, 1996), calcium carbonate (CaCO\(_3\)) (Loeppert and Suarez, 1996), organic matter (OM), exchangeable Al, Ca, magnesium (Mg) and sodium (Na), and cation exchange capacity (CEC) by ammonium replacement (Gavlak et al., 2003). Total bases and percent base saturation (sum of Ca, Mg, potassium (K), and Na divided by CEC) were calculated.

Potted Plant Study

Bulk soil samples collected were thoroughly mixed and approximately 2.5 kg of soil for each treatment was placed in plastic pots and planted with either ‘Expresso’ wheat (eight seeds per pot thinned to six) or ‘Dekalb 52-62’ corn (three seeds per pot, thinned to two plants). The corn experiment was conducted on soils 1-9 beginning on day 363 of 2009 and wheat on soils 2-10 beginning on day 15 of 2011. Each soil was treated with 0, 44, 88 or 132 kg P ha\(^{-1}\) added as monoammonium phosphate, MAP (11-52-0) in four replicates. Additional N was applied as calcium nitrate (Ca(NO\(_3\))\(_2\)) so each
pot had a uniform N application of 112 kg N ha\(^{-1}\). The pots were completely randomized and plants were allowed to grow for six weeks.

One replicate per treatment for each soil contained an EC-5 moisture probe connected to an EM-50 data logger (Decagon Devices, Pullman, WA). All pots were irrigated near field capacity (FC) without allowing water to drain from the pot. Field capacity was determined using the Soil-Plant-Atmosphere-Water (SPAW) Characteristics model with soil texture as the input (Saxton and Willey, 2006). Corn pots were irrigated with de-ionized water to 85% of FC on average (61% of FC minimum) while the wheat pot moisture averaged 88% of FC (71% of FC minimum)(data not reported). Greenhouse conditions included 12 hour photoperiods provided by fluorescent light and an average air temperature of 20\(^{\circ}\)C.

Two replicates per treatment for each soil had an anion plant root simulator (PRS\(^{TM}\)) probe (Western Ag Innovations, Saskatoon, Canada) placed in the soil for a two week period then exchanged with a recharged probe to estimate the continuous quantity of available P to which the plant root was exposed. Probes were eluted with 20 ml of 0.5 M hydrochloric acid (Quian et al., 1992) and phosphorus was determined with a Perkin Elmer DV3300 Optima Inductively Coupled Plasma Atomic Emission Spectrophotometer (ICP-AES, Perkin Elmer, Waltham, MA).

Following the 6-week growth period, plants were measured for height and biomass and harvested at the soil surface. Harvested plant tissue was dried at 65\(^{\circ}\)C for two days, weighed, and then ground to 40 mesh. The concentration of P in dry matter was determined by ICP-AES following nitric/perchloric acid digestion (Gavlak et al., 2003).
Analysis of variance (ANOVA) was conducted and correlations between selected soils were determined using PC SAS system for Windows version 9.1 (SAS Institute, Cary, N.C.). Means were calculated with PROC GLM. Data were considered significantly different at $P \leq 0.05$ according to Tukey’s method of mean separation within PROC GLM.

RESULTS AND DISCUSSION

Soil Available P Extractions

To determine which soil P extraction method was most suitable for use in recently acidified soils, dry plant biomass of the control pots (no P fertilization) was plotted against each soil P index (Figure 4.1). A second order polynomial fitted line was used to compute $R^2$ values for each extraction method and crop. The fitted lines show that, in most instances, biomass for both corn and wheat increases at low extraction values, peaks, and then declines again at the highest levels of extracted P. Therefore, addition of P was not beneficial for biomass production when extractable P was high, even in soils that have become acidified over time.

Cate-Nelson splits (Cate and Nelson, 1965) were also made for each crop and extraction method. This technique attempts to homogenize data by separating the points into responsive (lower left) and non-responsive (upper right) quadrants. This is done by transposing a horizontal and vertical line on the graph so the number of points in the responsive and non-responsive quadrants is maximized. The position of the vertical line on the x-axis then gives the critical level for that test. The reliability of the Cate-Nelson analysis in this case is somewhat limited by the number of soils used in the study ($n = 9$ for corn and $n = 10$ for wheat). Relatively small changes in the point location can effect
large variation in the line and therefore critical level reading. The critical level for wheat is lower than corn for the OP and MMP tests and the opposite for BP1, M3P and PRSTM-P.

For the corn, M3P extraction had the best correlation ($R^2 = 0.81$) between dry biomass and extractable P followed by cumulative PRSTM-P ($R^2 = 0.70$), MMP ($R^2 = 0.69$), OP ($R^2 = 0.68$), and BP1 ($R^2 = 0.49$) P. For wheat, OP ($R^2 = 0.76$) showed the highest correlation and was followed by M3P ($R^2 = 0.73$), BP1 ($R^2 = 0.53$), MMP ($R^2 = 0.29$) and cumulative PRSTM-P ($R^2 = 0.25$). Kuo (1990) found that OP and MMP extractions were highly correlated with yield, tissue P and P content in 7 acid soils.

Cumulative PRSTM-P for the wheat trial showed the lowest correlation. Plotting plant tissue P concentration against PRSTM-P resulted in an improved correlation, $R^2 = 0.81$ and $R^2 = 0.20$, for corn and wheat, respectively (Figure 4.2). This showed that the PRSTM system in this case was better at determining plant P concentration and not necessarily biomass production.

The same correlation analysis was performed separately for soils grouped by low (pH >7.0) and high pH (<7.0) (Table 4.2). For soils with pH <7.0, OP had the highest coefficient of determination ($R^2 = 0.97$) for corn, versus PRSTM-P ($R^2 = 0.34$) for wheat at low soil pH. At pH >7.0, MMP, M3P, and PRSTM-P were highest ($R^2 = 0.99$) for corn and MMP was the highest ($R^2 = 0.79$) for wheat.

Plant Tissue P and Uptake

Plant P concentration was significantly different by crop ($P<0.0001$) and treatment ($P<0.0001$) and the interactive factors of crop*pH ($P<0.0001$), however, no significant differences were found in tissue P in relation to soil OP level. Therefore,
individual crop data were separated by soil pH for each treatment to determine any
differences due to soil pH. Figure 4.3 shows plant tissue P concentration as a function of
fertilizer P added for wheat and corn. Tissue P concentration increased with added P for
both corn and wheat plants, however, comparison of tissue P across soil pH levels within
a treatment showed substantial variation in many cases and imparted few differences.
Corn tissue P concentration varied from 1.2-10.7 g P kg\(^{-1}\) with the mean at 2.8 g P kg\(^{-1}\),
just below the sufficiency range of 3-5 g P kg\(^{-1}\) dry tissue (Jones et al., 1991) while wheat
tissue P ranged from 1.8-8.1 g P kg\(^{-1}\) and a mean of 5.5 g P kg\(^{-1}\), slightly greater than the
sufficiency range of 2-5 g P kg\(^{-1}\).

The plant dry biomass did show statistical differences relating to crop, treatment,
OP, and pH levels (Table 4.3). Therefore, contrasts relate to plant biomass (biomass per
plant, biomass as a percent of the 0 kg P ha\(^{-1}\) treatment and plant P content) were made
for similar OP level soils within each pH range. Considering both biomass per plant and
biomass as a percentage of the control treatment, there were very few instances of
statistically significant differences related to P rate treatments and soil OP levels (Table
4.4). Where significant differences were observed, no pH level could be attributed to the
increase in biomass. Significant differences in plant P content occurred more frequently
than in biomass characteristics, and soils with medium and high pH dominated instances
of increase. This is likely an effect of a combination of somewhat higher tissue P
concentrations with higher pH soils (Fig. 4.3) and modestly higher plant production at
higher soil pH (Table 4.3). Wheat and corn P content was greater for high and medium
soil pH levels and was most pronounced in the high OP soils.
Cumulative PRS\textsuperscript{TM}-P

Cumulative PRS\textsuperscript{TM}-P was generally not significantly different by treatment at a given OP level and when it was, no specific pattern dominated with either corn or wheat pots (Table 4.4). The lack of statistical difference was due to large variations in measured PRS\textsuperscript{TM}-P between replicates in the same treatment. One possible explanation for the variation is that root competition with the PRS\textsuperscript{TM} membrane in the pots was neither consistent nor predictable. Also, Quian and Schoenau (2002) note that potential microscale variations can cause large differences in individual membrane supply rates. The PRS\textsuperscript{TM} probes did, however, distinguish between P treatments very effectively.

CONCLUSION

Indicators of plant response to a fertilizer application given a level of OP including plant biomass, plant biomass as a percentage of the control treatment, and P content indicate that there is generally little difference given soil pH. When a difference was observed, the higher pH soil exhibited higher mean values with a few exceptions. Correlation analysis of plant biomass with extractable P indices was more favorable for the corn crop than for wheat. Ranking extractions by $R^2$ values yielded M3P, cumulative PRS\textsuperscript{TM}-P, MMP, OP, and BP1 for corn and OP, M3P, BP1, MMP and cumulative PRS\textsuperscript{TM}-P for wheat. This data shows the versatility of using the OP extraction on varying pH soils. At similar levels of OP, plant biomass production on acidified soil responds similarly to fertilizer application on soil with a higher pH. This suggests OP can be reliably used on recently acidified soils and that plant response is analogous to the soils on which the recommendation calibration was originally performed. Future research should focus on explaining why the OP test works well in recently acidified soils in the
CB. Possible explanations include a still dominant Ca-P chemistry in the soils or if Al/Fe-P complexes exist, the amount of P extracted by OP is similar to the traditionally assumed Ca-P chemistry. Further, in-field testing should be done to further substantiate these claims.
REFERENCES


Table 4.1. Soil characteristics of 10 Columbia Basin bulk samples. Included are the soil site and taxonomic descriptions, pH, total P, and extractable P levels (OP, MMP, BP1, M3P)† and several other chemical parameters. Also given are rankings for pH (H = >7.0, M = 6.2-6.8, and L = <6.0) and initial P (measured as OP) (1 = <10, 2 = 15-25, and 3 = >30 mg P kg⁻¹).

<table>
<thead>
<tr>
<th>Soil ID</th>
<th>Description</th>
<th>Taxonomy</th>
<th>pH</th>
<th>Initial P Rank</th>
<th>pH Rank</th>
<th>OP</th>
<th>MMP</th>
<th>BP1</th>
<th>M3P</th>
<th>Total CaCO₃</th>
<th>OM</th>
<th>Al</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>Total Bases</th>
<th>CEC</th>
<th>Saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Native</td>
<td>Winchester Sand, Mixed, mesic Xeric Torripsamments</td>
<td>7.2</td>
<td>H</td>
<td>1</td>
<td>7</td>
<td>4</td>
<td>12</td>
<td>8.0</td>
<td>1023</td>
<td>1.2</td>
<td>0.6</td>
<td>0.68</td>
<td>1010</td>
<td>194</td>
<td>29.9</td>
<td>7.6</td>
<td>8.4</td>
</tr>
<tr>
<td>2</td>
<td>Agricultural</td>
<td>Tammerman Course Sandy Loam, Sandy, mixed, mesic Xeric Haplocambids</td>
<td>6.2</td>
<td>M</td>
<td>2</td>
<td>20</td>
<td>6</td>
<td>23</td>
<td>73.7</td>
<td>943</td>
<td>1.2</td>
<td>1.2</td>
<td>2.33</td>
<td>1270</td>
<td>249</td>
<td>50.6</td>
<td>8.9</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Lightly Manured Agricultural</td>
<td>Royal Loamy Fine Sand, Coarse-loamy, mixed, superactive, mesic Xeric Haplocambids</td>
<td>7.7</td>
<td>H</td>
<td>3</td>
<td>33</td>
<td>42</td>
<td>37</td>
<td>63.4</td>
<td>1100</td>
<td>3.5</td>
<td>1.7</td>
<td>0.68</td>
<td>3300</td>
<td>200</td>
<td>69.0</td>
<td>19.7</td>
<td>14.2</td>
</tr>
<tr>
<td>4</td>
<td>Agricultural</td>
<td>Warden Silt Loam, Coarse-silty, mixed, superactive, mesic Xeric Haplocambids</td>
<td>5.9</td>
<td>L</td>
<td>2</td>
<td>25</td>
<td>7</td>
<td>40</td>
<td>45.7</td>
<td>647</td>
<td>0.7</td>
<td>1.0</td>
<td>0.82</td>
<td>1420</td>
<td>254</td>
<td>55.2</td>
<td>13.5</td>
<td>11.9</td>
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<td>5</td>
<td>Garden</td>
<td>Strat Stoney Loam, Loamy-skeletal, mixed, superactive, mesic Calclic Haploxerolls</td>
<td>7.9</td>
<td>H</td>
<td>2</td>
<td>20</td>
<td>24</td>
<td>25</td>
<td>40.1</td>
<td>482</td>
<td>1.0</td>
<td>1.7</td>
<td>0.83</td>
<td>1240</td>
<td>327</td>
<td>190.9</td>
<td>11.9</td>
<td>11.6</td>
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<tr>
<td>6</td>
<td>Pasture</td>
<td>Outlook Very Fine Sandy Loam, Coarse-silty, mixed, superactive, calcareous, mesic Aeric Haplaquepts</td>
<td>6.4</td>
<td>M</td>
<td>2</td>
<td>15</td>
<td>4</td>
<td>9</td>
<td>17.9</td>
<td>707</td>
<td>1.2</td>
<td>1.4</td>
<td>0.50</td>
<td>960</td>
<td>450</td>
<td>234.6</td>
<td>10.6</td>
<td>10.4</td>
</tr>
<tr>
<td>7</td>
<td>Pasture</td>
<td>Outlook Very Fine Sandy Loam, Coarse-silty, mixed, superactive, calcareous, mesic Aeric Haplaquepts</td>
<td>8.4</td>
<td>H</td>
<td>1</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>11.3</td>
<td>661</td>
<td>8.1</td>
<td>2.3</td>
<td>0.18</td>
<td>3680</td>
<td>956</td>
<td>112.7</td>
<td>27.3</td>
<td>15.8</td>
</tr>
<tr>
<td>8</td>
<td>Agricultural</td>
<td>Warden Silt Loam, Coarse-silty, mixed, superactive, mesic Xeric Haplocambids</td>
<td>5.2</td>
<td>L</td>
<td>3</td>
<td>40</td>
<td>8</td>
<td>49</td>
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<td>816</td>
<td>0.3</td>
<td>0.9</td>
<td>14.33</td>
<td>920</td>
<td>206</td>
<td>34.5</td>
<td>7.2</td>
<td>10.1</td>
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<tr>
<td>9</td>
<td>Agricultural</td>
<td>Ephrata Gravelly Sandy Loam, Coarse-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Xeric Haplocambids</td>
<td>6.7</td>
<td>M</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>8</td>
<td>9.5</td>
<td>633</td>
<td>1.2</td>
<td>1.4</td>
<td>1.02</td>
<td>1500</td>
<td>242</td>
<td>59.8</td>
<td>10.0</td>
<td>12.4</td>
</tr>
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<td>10</td>
<td>Agricultural</td>
<td>Warden Silt Loam, Coarse-silty, mixed, superactive, mesic Xeric Haplocambids</td>
<td>6.5</td>
<td>M</td>
<td>3</td>
<td>32</td>
<td>21</td>
<td>56</td>
<td>70.2</td>
<td>617</td>
<td>1.8</td>
<td>1.1</td>
<td>1.38</td>
<td>2000</td>
<td>370</td>
<td>20.7</td>
<td>14.1</td>
<td>16.4</td>
</tr>
</tbody>
</table>

† OP = Olsen P (NaHOC₃), BP1 = Bray P-1 (HCl + NH₄F), MMP = Morgan P (NH₄OAc), M3P = Mehlich III P (HOAc + NH₄NO₃ + NH₄F + HNO₃ + EDTA)
Table 4.2. Coefficients of determination for corn and wheat dry biomass production as a function of five available P extraction methods (OP, MMP, BP1, M3P and PRS<sup>TM</sup>-P<sup>†</sup>) for soils with pH > 7.0 and <7.0.

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Soil pH</th>
<th>Crop</th>
<th>&lt;7.0</th>
<th>&gt;7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP</td>
<td>Corn</td>
<td>0.97</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>0.89</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>MMP</td>
<td>Corn</td>
<td>0.85</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>0.64</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>BP1</td>
<td>Corn</td>
<td>0.75</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>0.36</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>M3P</td>
<td>Corn</td>
<td>0.84</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>0.78</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>PRS&lt;sup&gt;TM·P&lt;/sup&gt;</td>
<td>Corn</td>
<td>0.71</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat</td>
<td>0.34</td>
<td>0.93</td>
<td></td>
</tr>
</tbody>
</table>

<sup>†</sup> OP = Olsen P (NaHOC<sub>3</sub>), BP1 = Bray P-1 (HCl + NH<sub>4</sub>F), MMP = Morgan P (NH<sub>4</sub>OA<sub>c</sub>), M3P = Mehlich III P (HOAc + NH<sub>4</sub>NO<sub>3</sub> + NH<sub>4</sub>F + HNO<sub>3</sub> + EDTA), PRS<sup>TM·P</sup> = Plant root simulator (ion exchange resin).
Table 4.3. Average plant biomass, plant biomass as a percentage of the control (0 kg P ha$^{-1}$) treatment, plant P content, and cumulative PRS$^{TM}$-P and level of significance (LOS) for each crop, initial P (measured as OP) (1 = <10, 2 = 15-25, and 3 = >30 mg P kg$^{-1}$), pH (H = >7.0, M = 6.2-6.8, and L = <6.0), and treatment. The significance of interactive factors is shown.

<table>
<thead>
<tr>
<th>Crop (C)</th>
<th>Corn</th>
<th>Wheat</th>
<th>Biomass (g plant$^{-1}$)</th>
<th>LOS ($P$)</th>
<th>% of Control</th>
<th>LOS ($P$)</th>
<th>Plant P (g P pot$^{-1}$)</th>
<th>LOS ($P$)</th>
<th>PRS$^{TM}$ P†</th>
<th>LOS ($P$)</th>
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<td>1.20</td>
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<td>109</td>
<td>115</td>
<td>0.035</td>
<td>1.27</td>
<td>1.10</td>
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<td>110</td>
<td>107</td>
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<td>pH (s.u.)</td>
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<td>118</td>
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<td>107</td>
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<td>0.0008</td>
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† $\mu$g P ml$^{-1}$ 10 cm$^{-2}$ 6 weeks$^{-1}$
Table 4.4. Tables show that given an initial P (Olsen P, NaHCO$_3$) (OP) (1 = <10, 2 = 15-25, and 3 = >30 mg P kg$^{-1}$) level and a treatment within that level, which soil pH (H = >7.0 (n = 8), M = 6.2-6.8 (n = 8), and L = <6.0 (n = 4)) resulted in the highest mean values. An “O” indicates that at a certain level of OP and treatment, there is no significant difference ($\alpha = 0.05$) in the indicated characteristic across the soil pH levels.

<table>
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<th>Corn Biomass per Plant</th>
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<td>(kg ha$^{-1}$)</td>
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<th>Corn Cumulative PRS$^\text{TM}$-P</th>
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<td>(kg ha$^{-1}$)</td>
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<tr>
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Figure 4.1. Average dry biomass vs. OP (Olsen P, NaHCO$_3$), MMP (Morgan P, NH$_4$OAc), BP1 (Bray P1, HCl + NH$_4$F), M3P (Mehlich-III P, HOAc + NH$_4$NO$_3$ + NH$_4$F + HNO$_3$ + EDTA), and cumulative PRS$^{TM}$-P for corn and wheat pots. Fitted curves with corresponding $R^2$ values are given for both corn and wheat crops. Coefficients of determination with different letters show significance across extraction methods but within a crop designation ($\alpha = 0.05$). Cate-Nelson quadrants are represented with solid lines corresponding to the corn and dashed to the wheat crop.
Figure 4.2. Correlation of plant tissue P (%) with cumulative PRSTM-P for corn and wheat crops. The coefficients of determination are also given for each crop.
Figure 4.3. Plant tissue P concentration as a function of P fertilizer application for corn and wheat plants. For each fertilizer treatment, tissue P is separated by pH: High = >7.0 (n = 36), Medium = 6.2-6.8 (n = 36), and Low = <6.0 (n = 24). Error bars represent tissue P standard deviation.
CHAPTER FIVE

GENERAL CONCLUSIONS

Chemical P fractionation revealed that soils in the CB with a pH as low as 5.2 still contained nearly 60% of inorganic P as Ca-P. While other P fractions were quite low, as pH dropped below 6.8, the amount of Fe-P increased significantly. This increase may be a sign of potential transition from a Ca-P predominated chemistry. Solid state $^{31}$P NMR was unable to distinguish between inorganic P fractions. Some general inferences can be made involving the shape of the NMR spectra that relates to the geometric symmetry of electrons around the P, however, more research is needed to make this analysis definitive with respect to P speciation.

Comparison of slopes for extractable P concentration demonstrated OP variability was the least across the pH ranges tested and MMP the most. This means that if a P fertilizer addition is made to low or high pH soils, the amount of P extracted by OP will be very similar for both. Further, assessment of plant need for fertilizer P at a given level of OP appears to be valid for several pH levels in the CB and the fertilizer calibrations made on high pH soils are suitable under low pH conditions. This is true because soils of the CB, although low in pH in some cases, still exhibit a dominant Ca-P chemistry. Isotherm data for soils 1-10 were well correlated with modeled Langmuir values. Pooled by soil pH, differences in sorption maximum for low, medium and high pH soils were not statistically significant.

Finally, analysis of plant P uptake and biomass production in a greenhouse setting showed that, in most cases, differences in several measured parameters cannot be attributed to changes in soil pH. Ranking extractions by $R^2$ values yielded M3P.
cumulative PRS\textsuperscript{TM}-P, MMP, OP, and BP1 for corn and OP, M3P, BP1, MMP and cumulative PRS\textsuperscript{TM}-P for wheat. These data show the versatility of using the OP extraction on varying pH soils. At similar levels of OP, plant biomass production on acidified soil responds similarly to fertilizer application on soil with a higher pH. Ca-P forms still dominate in low soil pH conditions and extractable P levels are similar for a range of soil pH values. Therefore, plant response was similar for many pH levels, further validating the claim that OP can be reliably used on recently acidified soils and that plant response is analogous to the soils on which the recommendation calibration was originally performed.

While several methods exist to test for plant available P, even beyond those in this study, OP appears to be the best method in determining fertilizer recommendations for high and low pH soils of the CB. The reasons for this conclusion are based on how speciation, extraction, sorption and plant response differences in relation to soil pH in CB soils are currently not large enough to warrant changing extraction methodology.

Opportunities for additional research on this topic are abundant. The most obvious next-step would be to take the principles of the greenhouse study in this project and apply them to field conditions. This would make the information more meaningful to a production crop system. The limitations inherent to greenhouse studies encumber extrapolation of the data to real world situations. Also, more work and experimentation are needed to determine how to best utilize solid state $^{31}$P NMR for determination of inorganic P for soil samples. Different treatments to remove paramagnetic elements or liquid extractions that will not interfere with \textit{in-situ} P speciation could be explored. Lastly, in-field calibration of alternative extraction indices could be the project for an
ambitious researcher. The benefit of doing so is that a universal extractant, such as M3P, could be used to extract multiple elements simultaneously, reducing the workload for weary soil analysis professionals.