

**LONG-TERM EFFECTS OF FERTILIZATION ON PHOSPHORUS BIOGEOCHEMICAL POOLS IN  
FOREST SOILS**

By

Bradley Warren Miller

Dissertation submitted to the faculty of the  
Virginia Polytechnic Institute and State University  
in partial fulfillment of the requirements for the degree of

**DOCTOR OF PHILOSOPHY  
IN  
FORESTRY**

Thomas R. Fox, Chair  
H. Lee Allen  
W. Michael Aust  
James A. Burger  
Felipe G. Sanchez  
Lucian W. Zelazny

2 February 2009  
Blacksburg, VA

Keywords: organic phosphorus, rhizosphere, ectomycorrhiza, oxalate

Copyright February 2009

# Long-term Effects of Fertilization on Phosphorus Biogeochemical Pools in Forest Soils

Bradley Warren Miller

## ABSTRACT

Southern pines are typically limited by nitrogen (N) and phosphorus (P) availability in the soil environment. While the absolute quantities of P in forests soils may be large, the concentration of inorganic P in the soil solution is typically very small ( $< 0.01 \text{ mg L}^{-1}$ ). A onetime application of just  $56 \text{ kg P ha}^{-1}$  can substantially increase growth of pine stands over a 20 year rotation (Pritchett and Comerford, 1982; Allen *et al.*, 1990). Phosphorus fertilization of *Pinus radiata* in New Zealand has also shown long-term effects on labile P pools in the soil which improved stand growth during the subsequent rotations (Ballard, 1978; Gentle *et al.*, 1986). Identifying and quantifying the biologically available P pools in the soil environment will help foresters in making site-specific P fertilizer prescriptions.

I examined soil phosphorus pools using the Hedley sequential fractionation procedure and Mehlich-3 soil tests in a long-term loblolly pine (*Pinus taeda L.*) fertilization trial from four sites in the Atlantic and Gulf Coastal Plains. After 22 years, fertilization effects were limited to the surface depths. Mehlich-3 extractable P was largest in the soil surface (0-10 cm) of the fertilized treatments plots. Hedley labile and moderately labile P pools were also largest in the soil surface and decreased with depth.

Results from the Hedley fractionation procedure suggested that the Virginia site has a large pool of organic P in the soil surface. Organic P pools can represent 20-90% of the total P present in most mineral soils increasing with the age of the soil (Condon *et al.*, 2005). This increase in organic P pool suggests that biological cycling becomes more important as the stand develops (Wells and Jorgensen 1975). I used solution  $^{31}\text{P}$  nuclear magnetic resonance (NMR) spectroscopy to characterize organic P extracted with NaOH-EDTA in the surface of a Paleaquults from coastal Virginia. Total NaOH-EDTA extractable P was significantly larger in the fertilized treatment. Concentrations ranged from  $0.1 \text{ mg P L}^{-1}$  in the control plots to  $5.1 \text{ mg P L}^{-1}$  in fertilized plots. The surface soils in both treatments were dominated by inorganic

orthophosphate. Monoester P compounds were the only organic P compounds detected and were present in very low quantities.

The significant increase of NaOH/EDTA extractable P in the soil surface of the VA site suggested there has been a beneficial long-term effect of fertilization similar to the observations from the Mehlich 3 soil test. Results from oxalate loading experiments on ligand exchangeable versus dissolvable P pools in the bulk soil suggested that the long-term effect of P fertilization increased oxalate dissolvable P pools.

Plants and microbes have evolved a variety of mechanisms to increase P uptake in low P soil environments. These mechanisms include changes in root morphology and architecture, preferential root growth into high P microsites, the secretion of low-molecular-mass organic acids (LMMOA), and uptake via symbiotic relationships (Fox and Comerford, 1992b; Raghothama, 1999; Hinsinger, 2001; Raghothama, 2005). Results from soil samples taken from the ectomycorrhizal rhizosphere found that loblolly pine mycorrhizal roots modified the soil environment, possibly making recalcitrant P more available. In addition, the long-term effect of fertilization was a 396% increase in biologically available P.

Fertilization increased loblolly pine volume growth by 57 m<sup>3</sup> ha and increased the P content in the litter layer by 118%. After the stand was harvested and replanted, mineralization of the litter layer may also increase soil P pools. Results from this long-term fertilization experiment in the Coastal Plain province of Virginia have demonstrated that there has been a significant increase in soil (33.6 kg P ha<sup>-1</sup>) and biologically available P pools (3.0 kg P ha<sup>-1</sup>).

## **DEDICATION**

The work that was poured into this study and rewards from its completion are dedicated to my beloved beautiful bride Michelle Schwarz Miller, and my children Christian Ktaadn and Terra Elizabeth. I give glory and thanks to God for allowing me to study the physical sciences in order to understand how, through mechanisms like evolution, God created this world.

## **ACKNOWLEDGEMENT**

I would like to extend my deepest gratitude to Dr. Thomas R. Fox, chair of my dissertation committee. Dr. Fox serves as an exemplary scientist and professor in a department and university of outstanding scholars. His work ethic, depth of knowledge, and wisdom has earned the respect of colleagues in academia and industry as expressed to me time and time again. As a mentor Fox's wise guidance and demand for excellence has improved my skills as hypothesis driven research scientist. As a result of his effort and success, I have been able to advance my career by making professional contacts and presenting our research at scientific conferences across the United States. While he demands extraordinary effort from his students, I was still afforded many opportunities to pursue my own scientific and academic interests. I will work to build a career as successful as he has been for my own benefit and in hopes that he will one day proudly call me a fellow scientist and former student.

I would like to thank Dr. Lee Allen, Dr. Michael Aust, Dr. James Burger, Dr. Feliepe Sanchez, and Dr. Lucian Zelazny for serving on my committee and for their efforts towards developing my scientific skills and writing. I want to thank the staff and members of the Forest Nutrition Cooperative for support including Tim Albaugh, Leandra Blevins, and Ruth Lanni. I'd like to thank members of the Virginia Tech Forestry Department including Colleen Carlson, David Mitchem, John Peterson, and Tal Roberts for technical and logistical. I'd like to thank Kathie Hollandsworth, Tracey Sherman, and Sue Snow for their help and support making many a variety of tasks easier. I also acknowledge the decades of work, as well as advice I have received from faculty of the Forestry Department including Dr. Harold E. Burkhart, Dr. John Hosner, and Dr. J. Michael Kelly. I also benefitted from support from Dr. Mark Alley, Dr. Hugo Azurmendi, Dr. Duane Berry, Dr. W. L. (Lee) Daniels, Dr. Matt Eick, and Dr. Shepard Zedaker. My thanks go out to fellow students who's discussions helped in the lab or classes including Claudia Cotton, Tripp Dowling, Salli Dymond, Patricia (Pipa) Elias, Rob Elliot, James Freeman, Charlene Kelly, Chris Kiser, Wen Lin, Aaron Pratt, Christina Siegel, Robert (Bud) Syper, Daniel Waiswa, and Jose Zerpa. A special thanks to my fellow Fox lab forest soil scientists Dr. Eduardo Arellano and Dr. Eric Sucre for their help and expertise. My time spent sharing ideas and learning from them has undoubtedly improved my training as a scientist.

Finally I'd like to acknowledge the work of Dr. Karen DePauw Vice Provost for Graduate Studies and Dean of the Graduate School and her Transformative Graduate Education (TGE)

initiative at Virginia Tech. Dr. DePauw's efforts with help from Dr. Shelli Fowler, Dr. Jeshua Pacifici, and Monika Gibson to promote a holistic approach to graduate education I feel has made me a better scientist, teacher, and citizen. Dr. DePauw has challenged the traditional notion that graduate education should be limited to passing down disciplinary knowledge and that graduate students should narrowly focus on their research. Her personal support via time in the classroom, attending graduate student functions, as well as financial support for the TGE programs demonstrate her commitment to preparing the next generation of scientists, educators, scholars, engineers, artists, and career professionals in a "flat world" with global markets and diverse cultures. I promise to continue her work throughout my career as a Citizen Scholar.

## Table of Contents

<b>ABSTRACT .....</b>	<b>iii</b>
<b>DEDICATION.....</b>	<b>v</b>
<b>ACKNOWLEDGEMENT.....</b>	<b>vi</b>
<b>LIST OF FIGURES.....</b>	<b>x</b>
<b>LIST OF TABLES.....</b>	<b>xii</b>
<b>CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW</b>	
Justification and Objectives.....	<b>1</b>
Soil Phosphorus Pools and Silvicultural Treatments.....	<b>3</b>
Phosphorus Reactions in Soils.....	<b>6</b>
Hedley Sequential Fractionation.....	<b>8</b>
Organic P and <sup>31</sup> P-NMR Spectroscopy.....	<b>9</b>
Low-Molecular-Mass-Organic Acids and P Sorption.....	<b>12</b>
Literature Cited.....	<b>17</b>
<b>CHAPTER 2: HEDLEY PHOSPHORUS POOLS IN SOILS FROM FOUR LOBLOLLY PINE (<i>PINUS TAEDA</i>) PLANTATIONS IN THE SOUTHEASTERN UNITED STATES.</b>	
Abstract.....	<b>23</b>
Introduction.....	<b>23</b>
Material and Methods .....	<b>26</b>
Results .....	<b>36</b>
Discussion.....	<b>44</b>
Conclusion.....	<b>51</b>
Literature Cited.....	<b>52</b>
<b>CHAPTER 3: LONG-TERM EFFECTS OF FERTILIZATION ON ORGANIC PHOSPHORUS POOLS IN A LOBLOLLY PINE (<i>Pinus taeda</i>) PLANTATION DETERMINED BY <sup>31</sup>P NMR SPECTROSCOPY</b>	
Abstract.....	<b>56</b>
Introduction.....	<b>56</b>
Material and Methods.....	<b>60</b>
Results .....	<b>64</b>

Discussion.....	74
Conclusion.....	76
Literature Cited.....	78
<b>CHAPTER 4: COMPARISON OF OXALATE EXCHANGEABLE PHOSPHORUS IN THE BULK SOIL AND BIOLOGICALLY AVAILABLE PHOSPHORUS POOLS IN THE ECTOMYCORRHIZAL RHIZOPHERE OF A MATURE LOBLOLLY PINE (<i>Pinus taeda</i>) PLANTATION.</b>	
Abstract.....	83
Introduction.....	84
Material and Methods.....	88
Results and Discussion.....	99
Conclusion.....	115
Literature Cited.....	116
<b>CHAPTER 5: SUMMARY AND CONCLUSIONS.....</b>	<b>120</b>
Literature Cited.....	124
<b>DISSERTATION REFERENCES.....</b>	<b>125</b>
<b>APPENDICES.....</b>	<b>137</b>

## LIST OF FIGURES

Figure 2-1. Schematic representation of Hedley sequential fractionation procedure modified by Tiessen and Moir (1993)..... 33

Figure 2-2. Hedley extractable phosphorus pools (mg/kg) from Coastal Plain soils supporting loblolly pine plantations in the southeastern US. Labile P (DiH<sub>2</sub>O-Pi, NaHCO<sub>3</sub> – Pi, and NaHCO<sub>3</sub> – Po). Moderately labile P (NaOH – Po). Recalcitrant P (NaOH – Pi, 1M HCl – Pi, hot concentrated HCl – Pi and –Po; H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> - Pi). Error bars represent the standard error of the mean for total Hedley extractable P. Cn= control, Fert = fertilized, Trt = treatment..... 38

Figure 2-3. Mehlich 3 extractable phosphorus (mg/kg) from Coastal Plains soils supporting loblolly pine plantations in the southeastern US. Values represent least square means and the bars are the standard error of the least square means. Bars with different letters are significantly different at the  $p < 0.1$ ..... 39

Figure 2-4 Total Hedley extractable phosphorus (kg/ha) from Coastal Plains soils supporting loblolly pine plantations in the southeastern US. Labile P (DiH<sub>2</sub>O-Pi, NaHCO<sub>3</sub> – Pi, and NaHCO<sub>3</sub> – Po). Moderately labile P (NaOH – Po). Recalcitrant P (NaOH – Pi, 1M HCl – Pi, hot concentrated HCl – Pi and –Po; H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> - Pi). Values represent least square means. Error bars represent the standard error of the least square means for Hedley total extractable P. Cn= control, Fert = fertilized, Trt = treatment..... 50

Figure 3-1 Long-term response of the litter (Oi) and fragmented (Oe) horizons of the litter layer to P fertilization (56 kg P ha<sup>-1</sup>) 27 years after application. Values are means (n=3) and error bars represent standard deviation. Bars with different letters represent significant effects of fertilization at the  $p < 0.05$ ..... 65

Figure 3-2 Mehlich 3 extractable phosphorus (mg/kg) determined colorimetrically from Grantham soils series supporting loblolly pine in the southeastern US. Values represent least square means. Error bars represent the standard error of the least square means. Bars with different letters represent significant effects of depth at the  $p < 0.05$ ..... 66

Figure 3-3 Long-term fertilization affects on total extractable P (0.25M NaOH + 0.05M EDTA) from the surface 10 cm of a Grantham soil series. Values are means (n=18) and error bars represent standard deviation. Bars with different letters represent significant effects of fertilization at the  $p < 0.05$ ..... 69

Figure 3-4 Solution <sup>31</sup>P NMR spectra from 0.25M NaOH & 0.05M EDTA extracts from fertilized and control plots..... 71

Figure 4-1 Effects of oxalate loading on metal and fulvic acid desorption in the bulk soil of a loblolly pine forest in coastal Virginia. Values represent mean and bars are std. error of the mean. CuCl<sub>2</sub> extractable Al (dashed line). CuCl<sub>2</sub> extractable Fe (solid line)..... 102

Figure 4-2 Effect of oxalate concentrations on desorbable fulvic acids in solution..... 103

Figure 4-3 Phosphorus desorption in the bulk soil of the control (top) and fertilized (bottom) treatments in response to oxalate loading in a loblolly pine forest soil of coastal Virginia. Values represent mean and S.E.M. CuCl<sub>2</sub> extractable Al (dashed line).CuCl<sub>2</sub> extractable Fe (solid line)..... 106

Figure 4-4 Phosphorus desorption in response to oxalate loading in a loblolly pine forest soil of coastal Virginia. Values represent mean and S.E.M..... 107

Figure 4-5 Fertilization x ECM rhizosphere interactions effects (p = 0.0869) on desorbable P pools extracted with 100 μmol oxalate g<sup>-1</sup> soil. Samples were taken from a loblolly pine forest soil of coastal Virginia. Values represent mean and S.E.M. Bars with different letters are significantly different at p < 0.05..... 111

Figure 4-6 Phosphorus desorption in the ECM rhizosphere and bulk soil of the control (top) and fertilized (bottom) treatments from a loblolly pine forest soil of coastal Virginia. Values represent mean and S.E.M..... 112

Figure 4-7 Total oxalate desorbable phosphorus and Mehlich-3 extractable P concentration in the surface soil (0-5 cm) of a loblolly pine plantation of coastal Virginia. Values represent mean (S.E.M)..... 113

## LIST OF TABLES

Table 2-1 Study site descriptions and experimental treatments applied.....	28
Table 2-2 Treatment means from four loblolly pine stands in the Coastal Plains province of the southeastern United States.....	29
Table 2-3 Georgia Type 3 table test of fertilization and depth treatment effects on Hedley Labile (A) Moderately Labile (A) Recalcitrant (C) and Total (D) P pools. ....	40
Table 2-4 South Carolina Type 3 table test of fertilization and depth treatment effects on Hedley Labile (A) Moderately Labile (A) Recalcitrant (C) and Total (D) P pools.....	41
Table 2-5 Alabama Type 3 table test of fertilization and depth treatment effects on Hedley Labile (A) Moderately Labile (A) Recalcitrant (C) and Total (D) P pools.....	42
Table 2-6 Virginia Type 3 table test of fertilization and depth treatment effects on Hedley Labile (A) Moderately Labile (A) Recalcitrant (C) and Total (D) P pools.....	43
Table 3-1 Study site description and experimental treatments.....	62
Table 3-2 Long-term effects of P fertilization on total extractable phosphorus (0.25 M NaOH + 0.05 M EDTA) determined by ICP-AES from Grantham soil series in the Coastal Plains of Virginia. Values are means (n=18) with standard deviation in parenthesis. Values with different letters represent significant effect of fertilization at the $p < 0.05$ .....	68
Table 3-3 Chemical shift and proportion of P compounds determined by NaOH/EDTA extraction and solution P NMR spectroscopy. Phosphorus was extracted from a loblolly pine fertilization field trail installed on an eastern Virginia Grantham soil series.....	72
Table 3-4 Phosphorus pools determined by $^{31}\text{P}$ NMR spectroscopy. Values are the mean (n=3) and S.E.M. Values with different letters represent significant effects of fertilization at the $p < 0.05$ .....	73
Table 4-1 Experimental treatments imposed on a loblolly pine plantation growing on a Grantham soil series in coastal VA.....	89
Table 4-2. Type three table for fertilization and oxalate concentration treatment effects on oxalate exchangeable P pools.....	95

Table 4-3. Type 3 table test of fertilization and oxalate concentration treatment effects on oxalate dissolvable P pools.....	96
Table 4-4 Type three table for fertilization and ectomycorrhizal rhizosphere (ECMR) treatment effects on oxalate dissolvable P pools.....	97
Table 4-5. Type three table for fertilization and ectomycorrhizal rhizosphere treatment effects on total oxalate dissolvable P pools.....	98
Table 4-6 Surface soil (0-5 cm) properties from a loblolly pine plantation on Grantham soil series in coastal VA.....	101
Table 4-7. Biologically available phosphorus pools in the ectomycorrhizal rhizosphere from a loblolly pine plantation of coastal VA.....	114

## **Chapter 1 Introduction and Literature Review**

### **Justification and Objectives**

Forest fertilization in the southeastern United States is a common silvicultural tool for intensively managed pine forests. Generally forested lands are located on less fertile soils. The pressures to develop land for suburban development in addition to the demand for fertile agricultural lands has reduced forest cover in the southeastern United States to 87 million ha<sup>-1</sup> (Conner and Hartsell, 2002). Twelve million hectares are intensively managed pine plantations. Despite common knowledge of P deficiencies in southern pine forests and the use of P fertilizers to address these deficiencies, important questions remain unanswered in relation to the effects of management practices on P pools. We know that fertilization mitigates nutrient deficiencies but for how long? Do the responses vary by soil and site? How does the soil biota influence P pools in southern pine forests? Do common soil tests detect long-term changes in plant available P pools?

Depending upon the physical and chemical properties of the soils examined, there are a variety of soil tests used to estimate the concentration of plant available or “labile” P (Pierzynski, 2000). Routine soil tests such as Mehlich-1, Mehlich-3, Olsen-P, Bray and Kurtz P-1 use a variety of ligands to break P covalent bonds from colloidal surfaces, and hold the orthophosphate anion and organic P in solution. Phosphorus concentration were quantified using colorimetric analysis or inductively coupled plasma (ICP) spectroscopy (Pierzynski, 2000). The logic behind these soil tests are that the chemicals used to cleave P bonds from soil colloids approximate the ability of soil organisms to break these same P bonds and absorb the phosphate ions. Among the challenges for accurately predicting P uptake is that soil microbes have a variety of mechanisms for accessing different P pools.

The results of these soil tests are correlated with plant growth experiments and used to determine soil test P critical levels. These critical levels are established based upon the demand of the agronomic crop in the observed soil series. The empirically derived equations used to estimate soil P critical levels typically account for 50-60% of the observed variability in crop growth (Tiessen and Moir, 1993). These critical values have worked well for agronomic crops in surface soils that have been homogenized because of years of tillage and limited influence of weed species.

Soils from forested ecosystems are very complex. In order to determine if soil P pools can meet the demand of the crop tree species, forest soil scientists are challenged with heterogeneous soils, decadal crop rotations, and a plethora of above- and belowground biota influencing P pools and cycles. In order to make accurate predictions of labile P pools in forest soils, scientists must account for both the biotic and abiotic affects on P biogeochemical pools and cycles. Because the biological, chemical, and physical properties of soils vary from site to site, the results from soil P tests will also vary.

Despite this variability, there are common chemical mechanisms that determine P intensity in the soil solution. These mechanisms include P dissolution-precipitation (mineral equilibrium), sorption-desorption (ligand exchange reactions), immobilization-mineralization (biologically influenced), and oxidation-reduction reactions (Pierzynski *et al.*, 2005a). With acknowledgement of the complex chemical environment that governs P intensity in the soil solution and the variety of P species present, it is the goal of this dissertation to determine the long-term effects of P fertilization on soil P pools in pine plantations. The specific objectives of this study were:

1. Determine the long-term effects of fertilization on P pools using the Hedley sequential fractionation procedure (Chapter 2).
2. Determine the long-term effects of fertilization on organic P compounds using  $^{31}\text{P}$ -NMR spectroscopy (Chapter 3).
3. Determine oxalate exchangeable versus dissolvable P pools in the bulk soil and biologically available P pools in the ectomycorrhizal rhizosphere of a mature loblolly pine forest (Chapter 4).

#### *Soil Phosphorus Pools and Silvicultural Treatments*

Plant growth is typically limited by N and P availability in the soil environment. While the absolute quantities of these nutrients in forests soils may be large and appear sufficient to support robust plant growth, the actual pools of soluble reactive inorganic P are markedly smaller and typically growth limiting. The simple and economically viable solution in agriculture has been to apply inorganic fertilizers to meet plant growth requirements. Whereas inorganic N is influenced by soil microbes and plants, it can also be volatilized and rapidly lost after fertilization. The fate of inorganic P ( $\text{P}_i$ ) and organic P ( $\text{P}_o$ ) is typically considered to be regulated by plants, soil microbes, and the P fixation capacity of the soil (Turner and Lambert, 1988; Yuan and Lavkulich, 1994). The loss of P from a undisturbed ecosystems is very modest due to the strong retention to the soil minerals, and minimal erosion losses (Patric et al., 1984).

The strong sorption of P anions to soil colloids, particularly Fe- and Al-oxides, in addition to plant and microbial uptake, is responsible for a substantial reduction of  $\text{P}_i$  in soil water over time (Tiessen *et al.*, 1984). It is common in agriculture to fertilize yearly with a low dose of  $\text{P}_i$  to increase plant yields. Yet, a single application of just  $56 \text{ kg P ha}^{-1}$  can substantially increase growth of pine stands over a 20 year rotation (Pritchett and Comerford, 1982; Allen *et al.*, 1990).

Phosphorus fertilization of *Pinus radiata* in New Zealand showed increased labile P during the second rotation (Ballard, 1978; Gentle et al., 1986). Identifying and quantifying the labile P pools in the soil environment is one method of estimating if P will be growth limiting.

Soil P fixation capacity is also important for the retention and long-term supply of applied P and will influence the cycling of P<sub>o</sub> pools (Turner and Lambert, 1988). Soils with high concentrations of Fe- and Al-oxides and clays will retain larger amounts of P fertilizer. Sorbed Pi is gradually released from soil colloids and incorporated into the living biomass. This uptake shifts soil P pools from Pi to Po as the site develops. The P<sub>o</sub> concentration in soils ranges up to 70 % of total P in many forest soils (Turner and Lambert, 1988). The increase in the P<sub>o</sub> pool suggests that biological cycling becomes more important as the stand develops, a hypothesis that was suggested by Wells and Jorgensen (1975). This also suggests that soil microbes have the ability to actively regulate the pools of P in the soil.

The importance of the organic P fraction was documented in 6-yr-old pine plantations on spodosols in Florida (Polglase *et al.*, 1992a,b). Higher P mineralization in laboratory incubations showed that P fertilization increased organic matter quality. In the field, mineralization in the surface 5 cm of soil supplied 0 and 25% of the annual P requirements in unfertilized and fertilized plots respectively. Higher lab and field mineralization rates with fertilization were attributed to rapid recycling of P in readily mineralizable compounds. In contrast, slow growth in nutrient deficient control plots translated to slower biological cycling of phosphorus.

The increased P mineralization noted above may be part of a “priming effect” on nutrient cycling mediated by fertilization, as P content in biomass is generally much higher than the amount applied (Harding and Jokela, 1994). Fertilization can increase total P returns in litterfall

from 150 to 400% (Dalla-Tea and Jokela, 1991; Piatek and Allen, 2001). Release of P from decomposing needles is strongly and positively correlated to the needle concentration of Pi (Polglase *et al.*, 1992b), which increases with fertilization (Polglase *et al.*, 1992c). Organic P inputs via fine-root turnover may decrease slightly with fertilization based on studies documenting small reductions in fine-root production with fertilization of loblolly pine (Albaugh *et al.*, 1998). However, the net soil input of Po is probably much greater following fertilization.

Vegetation control can enhance P availability in young stands by allocating available nutrients to the pine trees (Allen *et al.*, 2000). In the longer term, the effects of vegetation control on P availability are less certain. In general, hardwood litter decomposes and releases P faster than pine litter (Piatek and Allen, 2001). Thus, the P release rate from decomposing forest floor should be higher without vegetation control. However, while the rate may be higher, the actual total P released may be similar (or less). Vegetation control increased litter production by 36 % in 8-yr-old pine plantations, resulting in 20% greater P return and equivalent total P released (Lockaby *et al.*, 1995). Greater litter production in the pure pine system reflects the higher nutrient use efficiency of pines compared to hardwoods.

Mechanical site preparations can have multiple effects on P availability. Shearing and piling can remove greater than 60% of the total site P by scraping harvest residues, forest floor, and mineral soil into windrows (Tew *et al.*, 1986). Such large removals may explain growth reductions observed with windrowing on P deficient sites (Fox *et al.*, 1988). On sites with apparently adequate soil P, windrowing followed by disking can enhance productivity with this effect being attributed primarily to vegetation control (Allen *et al.*, 2000). Treatments such as bedding on wet sites or subsoiling on dry sites may enhance P availability by increasing available rooting volume.

Depending on what resources limit productivity on a given site, growth responses to combinations of mechanical site preparation, vegetation control, and fertilization will vary widely. For example, bedding and P fertilization on a poorly drained, nutrient deficient, soil resulted in a synergistic growth response, while the same treatments on a well drained, nutrient deficient soil resulted in a less than additive response (Allen, 1996). In the latter case, the bedding response was lower on the well-drained soil because aeration was a non-limiting resource. Growth responses to fertilization can be wide ranging, depending on the inherent level of soil fertility and the buffering capacity (Kelting, 2000).

#### *Phosphorus Reactions in Soils*

The concentration of plant available Pi in soil solution is controlled by chemical and biochemical processes including: dissolution/precipitation (mineral equilibrium), mineralization/immobilization (biological manipulation), and oxidation/reduction reactions (Pierzynski *et al.*, 2005). The effects of P sorption/desorption have been studied in various forest soils (Ballard, 1980; Fox *et al.*, 1990; Beauchemin *et al.*, 1996; Kelly and Kelly, 2001). Depending on the nature of the soil, Pi sorption in soils is primarily controlled by calcium carbonates in alkaline soils, or Fe- and Al- oxides and hydrous oxides in acidic soils (Sample *et al.*, 1980; Delgado and Torrent, 2000; Pierzynski *et al.*, 2005). The P anions act as Lewis bases and forming mostly inner- and some outer-sphere complexes with Lewis acids. The P anions are rapidly adsorbed to the surface minerals such as gibbsite and goethite via ligand exchange reactions of the orthophosphate ion with OH<sup>-</sup> and/or H<sub>2</sub>O on the mineral surfaces (Geelhoed *et al.*, 1997). Labile P pools being taken up by plants and microbes are constantly replenished by

other P pools maintaining a portion of orthophosphate in solution according Le Chatelier's Principle.

The P content in younger soils is primarily supplied by the dissolution of apatite minerals,  $\text{Ca}(\text{X})(\text{PO}_4)_6$  with  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{OH}^-$ , or  $(\text{CO}_3)^{2-}$  substituting for X (Pierzynski et al., 2005). With age and weathering the importance of Al- and Fe- sorbed P increases (Parfitt et al., 1975). The sorption mechanisms for alumino-silicates and hydrous oxides are similar enough that scientists use the same sorption mechanisms for kaolinite, gibbsite, and pseudoboehmite (Muljadi *et al.*, 1966a, b, c). This suggested that the sorption mechanisms were similar in these two sorbents. The P sorption index (PSI) is often generated using a single-point P isotherm with a solution containing  $50 \mu\text{mol P g}^{-1}$  soil (Sims, 2000a). This procedure was performed on 42 acid and alkaline soils, and found to be well correlated ( $r = 0.97$ ) with complete P sorption isotherm but has the advantage of being completed rapidly. The procedure was adapted by Penn, Mullins, and Zelazny (2005) for use in a P desorption study.

In fertilized soils, the reaction of P cannot be limited to sorption reactions on colloidal surfaces. The dissolution of P into the soil environment causes precipitation reactions as a result of the high concentration of P in solution around a dissolving P granule (Sample *et al.*, 1980; Hedley and McLaughlin, 2005). The area immediately around a P granule (the exact distance is a function of pH, P sorption, volumetric water, bulk density) saturates adsorption sites and therefore the solution around the granule becomes supersaturated (Hedley and McLaughlin, 2005). The supersaturated area immediately around a P granule can cause the dissolution of clay-sized particles which brings into solution Si, Fe, Al, Mn, Mg, Ca, K, and solubilize soil organic carbon (Moody *et al.*, 1995; Hedley and McLaughlin, 2005). The type of P fertilizer applied and the soil which it is applied to greatly affects the soluble by-products. Moody et al.

(1995) showed that diammonium phosphate (DAP) is less likely to dissolve clay particles and therefore precipitated less Fe- and Al-P minerals and greater amounts of Mg- and Ca-P minerals compared to monoammonium phosphate (MAP). This was attributed to the higher (pH ~ 8) around the DAP granules compared to MAP (pH ~ 3.5).

### *Hedley Sequential Fractionation*

Soil scientists have long understood that the buffering capacity of the soil determines the size of the labile P pools. While plant available inorganic orthophosphate ions are present in the soil solution, the absolute quantity is typically minuscule and is influenced by soil biota. A proportion of the total P at a site may be present in the form of organic P compounds. The Hedley fractionation procedure (Hedley *et al.*, 1982) attempts to identify the P<sub>o</sub> pools and distinguish between labile P compounds and moderately labile P pools that would contribute over time to plant available P.

Tiessen and Moir (1993) modifications of the Hedley sequential fractionation procedure uses a variety of chemical solutions of increasing ionic strength to divide the P content into pools of decreasing biological availability (Fig 2-1). The six sequential fractions are: 1) Deionized water (DiH<sub>2</sub>O) anion exchange membrane - P; 2) NaHCO<sub>3</sub> - P; 3) NaOH - P; 4) 1M HCl – P; 5) Conc. HCl – P; 6) H<sub>2</sub>SO<sub>4</sub> - P which when summed give Hedley Total P. The ionic strengths of these solutions are hypothesized to represent the ability of plant roots to bring the P ions into solution for plant uptake (Tiessen and Moir, 1993).

The anion exchange membranes represent P<sub>i</sub> that is freely exchanged by the soil solution. The bicarbonate liberated P is also considered labile because it represents the P brought into solution by alteration of the rhizosphere pH by root respiration (Tiessen and Moir, 1993). These

two fractions represent P that is cycled over a short time period represented by a single growing season (Bowman and Cole, 1978; Cross and Schlesinger, 1995; Johnson et al., 2003).

The remaining P fractions are extracted with 0.1 M NaOH, followed by an extraction with 1 M HCl, hot concentrated HCl, and a final extraction with 5ml H<sub>2</sub>O<sub>2</sub> and concentrated H<sub>2</sub>SO<sub>4</sub>. The P pools extracted by these solutions would represent P pools which are moderately labile to highly recalcitrant (Tiessen and Moir, 1993). The P<sub>i</sub> extracted in the bicarbonate and NaOH solutions represent a continuum of P associated with Fe- and Al-oxides with increasing pH (8.5-13). The NaOH extractable P<sub>o</sub> is available over a longer time period. The P<sub>i</sub> extractions from 1 M HCl are likely Ca-Pi (Tiessen and Moir, 1993). The hot conc. HCl extract and H<sub>2</sub>O<sub>2</sub> /H<sub>2</sub>SO<sub>4</sub> solutions liberate P from more resistant soil organic compounds and occluded P compounds. These P fractions represent Hedley P fractions that have been traditionally considered recalcitrant (Tiessen and Moir, 1993).

An advantage of the Hedley fractionation procedure is the attempt to quantify P<sub>o</sub> pools. Studies have demonstrated that P<sub>o</sub> compounds are returned to the soil and become the dominate P species present (Walker and Syers, 1976; Smeck, 1985; Magid *et al.*, 1996). This shift from P<sub>i</sub> to P<sub>o</sub> compounds is important to tree growth because there is increasing evidence that P<sub>o</sub> compounds contribute to plant available (labile) P pools (Johnson *et al.*, 2003; Turner *et al.*, 2005; George *et al.*, 2006). For example, Richter *et al.* (2006) reported that the Hedley slowly cycling P<sub>o</sub> pools were depleted after conversion of the site from a cotton field to a pine forest in 1957.

#### *Organic P and <sup>31</sup>P-NMR Spectroscopy*

The soil P<sub>o</sub> pools represented in the Hedley fractionation do not yield any useful information on the species of P<sub>o</sub> present and limited information on how much of the P<sub>o</sub> is biological

available (Condon et al., 2005). A common belief is that the  $P_o$  extracted in the NaOH fraction of the Hedley fractionation is moderately labile and latter solutions Le Chatelier's Principle are largely unavailable to plants. However, Liu et al. (2004) recently showed that a decrease in the NaOH- $P_o$  pool in the rhizosphere of a *Pinus radiata* planted in an allophanic soil increased the NaOH- $P_i$  pool. There were no effects on the resin extracted  $P_i$  content of the bulk soil or rhizosphere suggesting the biological activity is responsible for these changes in the short-term. Likewise, other research has show that the residual P pools can be influenced by the soil biota (Chen et al., 2002).

The use of liquid state  $^{31}P$  nuclear magnetic resonance ( $^{31}P$  NMR) spectroscopy in NaOH extracts is becoming more routine because of its' ability to identify different  $P_o$  compounds (Newman and Tate, 1980; Turner *et al.*, 2003; Turner and Richardson, 2004). Organic P pools can represent 20-90% of the total P present in most mineral soils (Condon et al., 2005).

The advantage of  $^{31}P$  NMR is that it produces separate signals which can identify and quantify orthophosphate  $P_i$ , orthophosphate monoester and diester  $P_o$ , phosphonates, polyphosphates, and pyrophosphates compounds in the soil which are of varying degrees of plant availability (Condon et al., 1997). Orthophosphate esters are sub-classified as monoesters with one C moiety per P, and diester orthophosphates with two C moieties per P. The orthophosphate esters are stable in the natural range of most natural soils, they are hydrolyzed in the presences of phosphates enzymes (Fox and Comerford, 1992a; Alvarez *et al.*, 2004; He *et al.*, 2004).

The most abundant monoesters in soils are inositol phosphates that occur in various stereoisomeric forms, *myo*, *scyllo*, *d-chiro*, and *neo* of differing biotic availability (Turner *et al.*, 2002; Turner *et al.*, 2005). The monoesters also include sugar phosphates, phosphoproteins, and mononucleotides (Condon et al., 2005). The monoester phosphates bond very tightly to soil

colloids and become very resistant to decomposition. Therefore, high concentrations of monoesters can accumulate in soils with a large P sorption capacity.

The diester phosphates, including nucleic acids, phospholipids, teichoic acid (unique to forests soils), and aromatic compounds are found in much large quantities in acidic forest soils and are generally considered more labile (Cade-Menun *et al.*, 2000). The greater availability of diester phosphates are attributed to breaking one of the two R-O-P bonds, greater solubility, and therefore greater movement into the soil solution (Magid *et al.*, 1996; Condron *et al.*, 2005).

Phosphonates are organic orthophosphate compounds with C-P bonds and were first reported in natural soils by Newman and Tate (1980). The C-P bond is more resistant to hydrolysis and oxidation than C-O-P bonds making their functions as a P storage compound in biotic systems less likely (Quin and Quin, 2001).

Recent studies have shown how soil biota has influenced the biologically available P<sub>o</sub> pools (Condron *et al.*, 1996; Solomon *et al.*, 2002; Turner *et al.*, 2005). Inositol hexa- and pentakisphosphates, monoester phosphates, can be strongly absorbed to soil colloids and are generally thought to be less labile. Diester phosphates and scyllo-inositol hexakisphosphate are believed to contribute to labile P pools in forest soils (Condron *et al.*, 1996; Cade-Menun *et al.*, 2000; Condron *et al.*, 2005; Turner *et al.*, 2005). <sup>31</sup>P NMR spectroscopy can identify these changing P<sub>o</sub> pools. Changes that the Hedley fractionation procedure cannot detect.

The role monoester phosphates play in rapidly cycling P<sub>o</sub> pools has recently been revised. Chen *et al.* (2004) measured P<sub>o</sub> in a variety of soils planted with radiata pine and ryegrass in pots in a greenhouse. They found that orthophosphate monoesters were the predominate P<sub>o</sub> in a variety of soils. Their results also showed that myo-inositol hexakisphosphates (IP) were mineralized in soils planted with radiata pine inoculated with the ectomycorrhizal fungus

*Rhizopogon rubescens*. The mineralization of IP accounted for 18-100% of the mineralized orthophosphate monoesters. Despite the strong sorption capacity of soils for IP, it cannot simply be considered a recalcitrant P pool.

#### *Low-Molecular-Mass-Organic Acids and P Sorption*

The bioavailability of P from mineral surfaces depend upon desorption/dissolution reactions. These desorption/dissolution reactions depend predominately upon a ligand being exchanged with an inner-sphere P anion. Inner-sphere complexes are believed to form covalent bonds. Some low-molecular-mass-organic acids such as oxalate have the ability to promote the dissolution of the mineral surface and the release of P from the colloidal surface. A wide variety of naturally occurring organic acids have been identified in many different soils (Stevenson, 1967).

However not all organic acids have the same ability to assist in the dissolution of P from colloidal surfaces (Fox et al. 1990). Low-molecular-mass-organic acids (LMMOA), specifically oxalate, have the ability to promote the dissolution of P from colloidal surfaces (Illmer and Schinner, 1992; Wallander, 2000; Sato and Comerford, 2006). Sato and Comerford (2006) using a Brazilian Ultisol (Kandiudult) showed that P desorption can be separated from P dissolution when oxalate and citrate anion ligand concentrations exceeded  $1 \mu\text{mol g}^{-1}$  soil. The soil was first saturated with P and then lab tests were performed to distinguish between desorption and dissolution P pools.

While lab tests on sieved air-dried soils may approximate field conditions, air drying may increase  $\text{NaHCO}_3$  extractable  $\text{P}_o$  content by as much as 60%, especially in soils with  $\text{pH} < 5.3$  (Turner, 2005). The changes were possibly attributed to the lysing of microbial cells and the

disruption of organic matter coatings on colloidal surfaces (Pritchett, 1979; Turner, 2005). The effect of air drying soils may therefore result in an increase in the concentrations of P released during soil tests.

The rhizosphere may also alter P desorption/dissolution characteristics compared to the bulk soil. The ectomycorrhizal rhizosphere (ECMR) with its' associated microflora may have many different phosphatases and organic acids that would influence the buffering capacity and quantity of P sorbed to colloidal surfaces compared to the bulk soil. Ligands like the organic acid oxalate may solubilize metal cations and relocate them outside the ECMR.

Even at very low ligand concentrations,  $< 1.0 \mu\text{mol g}^{-1}$  soil, continuous secretion of LMMOA may promote dissolution of amorphous minerals over the course of weeks to months. It has been observed that ectomycorrhizal (ECM) fine roots in the nursery (personal observation) and from field grown roots results in the rejuvenation of morphologically "old" appearing roots by the same or different ECM fungi (Durall et al., 1994). Rejuvenation would serve to increase the time in which the ECM rhizosphere has had to affect P desorption/dissolution.

Sato and Comerford (2006) used anion exchange membranes to estimate disequilibria-desorbable and ligand-desorbable P. Disequilibria-desorption P is brought into solution by the removal of inorganic P from solution. Ligand-desorbable P is P released into solution by the release of P from the soil surface. Their research suggests that in a Brazilian Kandiudult even at very low concentrations of citrate and oxalate ligands,  $1 \mu\text{mol anion g}^{-1}$ , the P released was due to ligand-dissolution of the mineral surface. Phosphorus continued to be released after 7 hrs. in a low P native soil at  $10 \mu\text{mol anion g}^{-1}$ . Sato and Comerford (2006) concluded that disequilibria-desorbable and ligand-desorbable P could thus be operationally defined and quantified.

Citrate and oxalate differ in the ability to dissolve different minerals. Fox et al. (1990) determined the different abilities of a variety of organic acids to forming stable complexes with Al. Sato and Comerford (2006) noted that the concentration of Fe released into solution was twice as larger when citrate was used compared to oxalate. The destruction of poorly and noncrystalline (amorphous) materials in soils for the characterization of clay mineralogy in carried out by acid ammonium oxalate in the dark (AOD) for 2 hrs. (Jackson et al., 2005). Depending upon the mineralogy of the soil, AOD at 2 hrs. has minor effects on clay minerals in soil. Selective dissolution of amorphous materials may yield information on the affects of ECM fungi on occluded P pools.

Ectomycorrhizal production of oxalate in the environment is extremely difficult to measure. However, their effect on inorganic P pools and therefore long-term P availability may be a measurable parameter. Cromack et al. (1979) found that oxalate concentrations in the mycorrhizal mats of *Hysterangium crassum* were  $0.44 \text{ mol m}^{-2}$ . Estimates of oxalate concentration in soils typically range from zero to over  $100 \text{ } \mu\text{mol anion g}^{-1}$  soil (Lapeyrie et al., 1987; Fox and Comerford, 1990b; Hees et al., 2002). ECM fungi and soil microbes such as *Penicillium* and *Pseudomonas* spp. have been proven to be a source of oxalate in both labs and field experiments (Illmer and Schinner, 1992; van Hees et al., 2000; Arvieu et al., 2003; Casarin et al., 2003; van Hees et al., 2003). Additionally, ECM colonization of fine roots increases fine root longevity (Smith and Read, 1997). The growing tip of a fine root is believed to be the area of maximum nutrient uptake and biological activity. Ectomycorrhizal roots may exude enough oxalate (in addition to other ligands) to surpass the  $1.0 \text{ } \mu\text{mol anion g}^{-1}$  required to dissolve amorphous minerals (Sato and Comerford, 2006). When considering ECM fine roots have increased longevity, and that water films in the rhizosphere reduce the volume of solution in

contact with the surrounding soil, it is possible that higher anion concentrations are found in the ECM rhizosphere of native soils.

The heterogeneity of the soil environment adds to the complexity of P cycling. Phosphorus sorption and precipitation reactions are also influenced by the soil organic carbon (SOC). As soils weather, colloidal surfaces become coated by the Al- and Fe- poorly crystalline material and SOC. Soil organic carbon, especially at sites with low cation exchange capacity, can serve as a nutrient source as the SOC decomposes, and help to increase the quantity of nutrients with increasing cation exchange capacity (CEC). Because of the many P dissolution/precipitation, sorption/desorption, and immobilization/mineralization reactions occurring, using the soil as a bioassay may be most appropriate to measure changes in P pools in the rhizosphere and as affected of microbial organisms.

The addition of P fertilizer to soils results in precipitation and adsorption of P to reactive colloids including Fe- and Al-oxides surfaces on poorly to well crystalline minerals. The P may also be sorbed to Ca and Mg cations at the soil surface among other cations supplied by decomposition of forest litter. Additionally, SOC coats mineral surfaces which could limit the precipitation/adsorption reactions with amorphous surfaces. The SOC may contribute metal ions at the soil surface with the decomposition of plant materials which could act as metal bridges to sorb P.

Naturally occurring organic acids such as oxalate, citrate, malonate, and ketogluconate have been identified in a variety of soils (Pierzynski, 2000; Essington, 2004). Eick et al. (1999) showed that oxalate dissolution of goethite is limited by adsorbed oxyanions. However Fe dissolution was increased at pH 6 possibly due to protonation of goethite with oxyanions adsorption which in turn increased the adsorption of oxalate. This test was performed in the lab

and at an oxalate concentration of 5 mM. However not all organic acids have the same ability to assist in the dissolution of P from colloidal surfaces (Fox et al. 1990a).

Advances in measurements of rhizosphere chemistry and knowledge of soil microbes has shown biota accessing P pools once believed to be nonlabile (Cajuste *et al.*, 1996; Singh *et al.*, 2001; Xu *et al.*, 2005; Johnson and Loeppert, 2006; Sato and Comerford, 2006). The traditional views of Al- and Fe- associated inorganic P pools in the soil as a highly recalcitrant P sink may no longer be valid.

The rhizosphere may alter P desorption/dissolution characteristics compared to the bulk soil. The ECM rhizosphere with the associated microflora may have many different phosphatases and organic acids that would influence the buffering capacity and quantity of P sorbed to colloidal surfaces compared to the bulk soil. Ligands, like oxalate, may solubilize metal cations and then move them outside the ECM rhizosphere. Several studies have attributed the mobilization of mineral cations to low molecular weight anions like oxalate from mycorrhizal fungi, rhizosphere exudates, and soil bacteria (Fox and Comerford, 1990a; Illmer and Schinner, 1992; van Hees *et al.*, 2000; Wallander *et al.*, 2002; van Hees *et al.*, 2004; van Scholl *et al.*, 2006). If the ECM rhizosphere release enough ligands to access P pools adsorbed to Fe and Al (hydr)oxides and occluded P pools by promoting the dissolution of poorly crystalline Fe and Al minerals this pool of biologically available P (BAP) could be calculated as:

$$\text{BAP } (\mu\text{mol g}^{-1}) = \text{P}_{\text{bulk soil}} (\mu\text{mol g}^{-1}) - \text{P}_{\text{ECM rhizosphere}} (\mu\text{mol g}^{-1})$$

## Literature Cited

- Albaugh, T.J., H.L. Allen, P.M. Dougherty, L.W. Kress, and J.S. King. 1998. Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions. *Forest Science* 44:317-328.
- Allen, H.L. 1996. Effects of site preparation and early fertilization and weed control on 14-yr loblolly pine growth. College of Forest Resources, North Carolina State University, Raleigh, NC.
- Allen, H.L., P.M. Dougherty, and R.G. Campbell. 1990. Manipulation of water and nutrients -- Practice and opportunity in Southern U.S. pine forests. *Forest Ecology and Management* 30:437-453.
- Allen, H.L., D.L. Kelting, K.B. Piatek, and S.B. Jeffries. 2000. Long-term forest site productivity in third rotation loblolly pine plantations with contrasting silvicultural treatments. Paper presented at the Long-term Soil Productivity Conference. USFS, Alexandria, LA.
- Alvarez, M., R. Godoy, W. Heyser, and S. Hartel. 2004. Surface-bound phosphatase activity in living hyphae of ectomycorrhizal fungi of *Nothofagus obliqua*. *Mycologia* 96:479-487.
- Arvieu, J.C., F. Leprince, and C. Plassard. 2003. Release of oxalate and protons by ectomycorrhizal fungi in response to P-deficiency and calcium carbonate in nutrient solution. *Annals of Forest Science* 60:815-821.
- Ballard, R. 1978. Effect of first rotation phosphorus applications on fertilizer requirements of second rotation pine. *New Zealand Journal of Forestry Science*. 8:135-145.
- Ballard, R. 1980. Phosphorus nutrition and fertilization of forest trees. In: *Role of Phosphorus in Agriculture*. editors F. E. Khasawneh, E. C. Sample, E. J. Kamprath. p.763-804.
- Beauchemin, S., R.R. Simard, and D. Cluis. 1996. Phosphorus sorption-desorption kinetics of soil under contrasting land uses. *Journal of Environmental Quality*. 25:1317-1325.
- Bowman, R.A., and C.V. Cole. 1978. An exploratory method for fractionation of organic phosphorus from grassland soils. *Soil Science*. 125:95-101.
- Cade-Menun, B.J., S.M. Berch, C.M. Preston, and L.M. Lavkulich. 2000. Phosphorus forms and related soil chemistry of podzolic soils on northern Vancouver Island. I. A comparison of two forest types. *Canadian Journal of Forest Research* . 30:1714-1725.
- Cajuste, L.J., R.J. Laird, B. Cajuste, Jr., and B.G. Cuevas. 1996. Citrate and oxalate influence on phosphate, aluminum, and iron in tropical soils. *Communications in Soil Science and Plant Analysis*. 27:1377-1386.
- Casarin, V., C. Plassard, G. Souche, and J.-C. Arvieu. 2003. Quantification of oxalate ions and protons released by ectomycorrhizal fungi in rhizosphere soil. *Agronomie* 23:461-469.
- Chen, C.R., L.M. Condon, M.R. Davis, and R.R. Sherlock. 2002. Phosphorus dynamics in the rhizosphere of perennial ryegrass (*Lolium perenne* L.) and radiata pine (*Pinus radiata* D. Don.). *Soil Biology and Biochemistry* 34:487-499.
- Chen, C.R., L.M. Condon, B.L. Turner, N. Mahieu, M.R. Davis, Z.H. Xu, and R.R. Sherlock. 2004. Mineralisation of soil orthophosphate monoesters under pine seedlings and ryegrass. *Australian Journal of Soil Research* 42:189-196.
- Condon, L.M., M.R. Davis, R.H. Newman, and I.S. Cornforth. 1996. Influence of conifers on the forms of phosphorus in selected New Zealand grassland soils. *Biology and Fertility of Soils* 21:37-42.

- Condrón, L.M., E. Frossard, R.H. Newman, P. Tekely, and J.L. Morel. 1997. Use of  $^{31}\text{P}$  NMR in the study of soils and the environment. *In* M. A. Nannny, et al., eds. Nuclear Magnetic Resonance Spectroscopy in Environmental Chemistry. Oxford University Press Inc., New York. p. 247-271.
- Condrón, L.M., B.L. Turner, and B.J. Cade-Menun. 2005. Chemistry and dynamics of soil organic phosphorus. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. p. 87-121. p 87-122.
- Conner, R.C., and A.J. Hartsell. 2002. Forest Area and Conditions, pp. 357-402, *In* D. N. Wear and J. G. Greis, (eds.) Southern Forest Resource Assessment. Southern Research Station, Asheville, NC.
- Cromack Jr., K., P. Sollins, W.C. Graustein, K. Speidel, A.W. Todd, G. Spycher, C.Y. Li, and R.L. Todd. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. *Soil Biology and Biochemistry* 11:463-468.
- Cross, A.F., and W.H. Schlesinger. 1995. A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64:197-214.
- Dai, K.H., M.B. David, G.F. Vance, and A.J. Krzyszowska. 1996. Characterization of phosphorus in a spruce-fir spodosol by phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Science Society of America Journal* 60:1943-1950.
- Dalla-Tea, F., and E.J. Jokela. 1991. Needlefall, canopy light interception and productivity of young intensively managed slash and loblolly pine stands. *Forest Science* 37:1298-1313.
- Delgado, A., and J. Torrent. 2000. Phosphorus forms and desorption patterns in heavily fertilized calcareous and limed acid soils. *Soil Science Society of America Journal* 64:2031-2037.
- Durall, D.M., J.D. Marshall, M.D. Jones, R. Crawford, and J.M. Trappe. 1994. Morphological changes and photosynthate allocation in ageing *Hebeloma crustuliniforme* (Bull.) Quel. and *Laccaria bicolor* (Maire) Orton mycorrhizas of *Pinus ponderosa* Dougl. ex. Laws. *New Phytologist* 127:719-724.
- Eick, M.J., J.D. Peak, and W.D. Brady. 1999. The Effect of Oxyanions on the Oxalate-Promoted Dissolution of Goethite. *Soil Science Society of America Journal* 63:1133-1141.
- Essington, M.E. 2004. *Soil and Water Chemistry: An integrative approach*. CRC Press, New York.
- Fox, T.R., and N.B. Comerford. 1990a. Influence of oxalate loading on phosphorus and aluminum solubility in spodosols. *Soil Science Society of America Journal* 56:290-294.
- Fox, T.R., and N.B. Comerford. 1990b. Low-molecular-weight organic acids in selected forest soils of the southeastern USA. *Soil Science Society of America Journal* 54:1139-1144.
- Fox, T.R., and N.B. Comerford. 1992. Rhizosphere phosphatase activity and phosphatase hydrolysable organic phosphorus in two forested spodosols. *Soil Biology and Biochemistry* 24:579-583.
- Fox, T.R., L.A. Morris, and R.A. Maimone. 1988. The impact of windrowing on the productivity of a rotation age loblolly pine plantation, *In* USDA, (ed.). Forest Service General Technical Report, SO-74.
- Fox, T.R., N.B. Comerford, and W.W. Mcfee. 1990. Kinetics of Phosphorus Release from Spodosols - Effects of Oxalate and Formate. *Soil Science Society of America Journal* 54:1441-1447.

- Geelhoed, J.S., G.R. Findenegg, and W.H. VanRiemsdijk. 1997. Availability to plants of phosphate adsorbed on goethite: experiment and simulation. *European Journal of Soil Science* 48:473-481.
- Gentle, S.W., F.R. Humphreys, and M.J. Lambert. 1986. Continuing response of *Pinus radiata* to phosphate fertilizers over two rotations. *Forest Science* 32:822-829.
- George, T.S., B.L. Turner, P.J. Gregory, B.J. Cade-Menun, and A.E. Richardson. 2006. Depletion of organic phosphorus from Oxisols in relation to phosphatase activities in the rhizosphere. *European Journal of Soil Science* 57:47-57.
- Gil-Sotres, F., W. Zech, and H.G. Alt. 1990. Characterization of phosphorus fractions in surface horizons of soils from Galicia (N.W. Spain) by  $^{31}\text{P}$  NMR spectroscopy. *Soil Biology and Biochemistry*. 22 (1):75-79.
- Harding, R.B., and E.J. Jokela. 1994. Long-term effects of forest fertilization on site organic matter nutrients. *Soil Science Society of America Journal* 58:216-221.
- He, Z., T.S. Griffin, and C.W. Honeycutt. 2004. Enzymatic hydrolysis of organic phosphorus in swine manure and soil. *Journal of Environmental Quality*. 33:367-372.
- Hedley, M.J., and M.J. McLaughlin. 2005. Reactions of phosphate fertilizers and by-products in soils. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. p. 181-252 p. 181-252.
- Hedley, M.J., J. Stewart, and B.S. Chauhan. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Science Society of America Journal* 46:970-976.
- Illmer, P., and F. Schinner. 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biology and Biochemistry*. 24:389-395.
- Jackson, M.L., C.H. Lim, and L.W. Zelazny. 2005. Oxides, Hydroxides, and Aluminosilicates. *In* A. L. Page, et al., eds. *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods.* , 2nd. ed. American Society of Agronomy-Soil Science Society of America, Madison, WI. p. 101-150.
- Johnson, A.H., J. Frizano, and D.R. Vann. 2003. Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. *Oecologia* 135:487-499.
- Johnson, S.E., and R.H. Loeppert. 2006. Role of organic acids in phosphate mobilization from iron oxide. *Soil Science Society of America Journal* 70:222-234.
- Kelly, J.M., and J.K. Kelly. 2001. Phosphorus and potassium uptake kinetics in red maple seedlings. *Forest Science*. 47:397-402.
- Kelting, D.L. 2000. Loblolly pine growth responses to early fertilization. College of Natural Resources, North Carolina State University, Raleigh, NC.
- Lapeyrie, F., G.A. Chilvers, and C.A. Bhem. 1987. Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus* (Batsch. ex Fr.) Fr. *New Phytologist* 106:139-146.
- Liu, Q., P. Loganathan, M.J. Hedley, and M.F. Skinner. 2004. The mobilisation and fate of soil and rock phosphate in the rhizosphere of ectomycorrhizal *Pinus radiata* seedlings in an Allophanic soil. *Plant and Soil* 264:219-229.
- Lockaby, B.G., J.H. Miller, and R.G. Clawson. 1995. Influences of community composition on biogeochemistry of loblolly pine (*Pinus taeda*) systems. *American Midland Naturalist* 134:176-184.

- Magid, J., H. Tiessen, and L.M. Condron. 1996. Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. *In* G. Piccolo, ed. Humic substances in terrestrial ecosystems. Elsevier, Amsterdam. p. 429-466
- Moody, P.W., D.G. Edwards, and L.C. Bell. 1995. Effect of banded fertilizers on soil solution composition and short-term root-growth .2. Mono-ammonium and di-ammonium phosphates. *Australian Journal of Soil Research* 33:689-707.
- Muljadi, D., A.M. Posner, and J.P. Quirk. 1966a. The mechanism of phosphate adsorption by kaolinite, gibbsite, and pseudoboehmite. Part 1. The isotherm and the affect of pH on adsorption. *Journal of Soil Science* 17:219-229.
- Muljadi, D., A.M. Posner, and J.P. Quirk. 1966b. The mechanism of phosphate adsorption by kaolinite, gibbsite, and pseudoboehmite. Part 2. The location of the adsorption sites. . *Journal of Soil Science* 17:230-237.
- Muljadi, D., A.M. Posner, and J.P. Quirk. 1966c. The mechanism of phosphate adsorption by kaolinite, gibbsite, and pseudoboehmite. Part 3. The effect of temperature on the adsorption. *Journal of Soil Science* 17:238-247.
- Newman, R.H., and K.R. Tate. 1980. Soil characterized by <sup>31</sup>P nuclear magnetic resonance. *Communications in Soil Science and Plant Analysis* 11:835-842.
- Patric, J.H., J.O. Evans, and J.D. Helvey. 1984. Summary of sediment yield data from forested land in the United States. *Journal of Forestry* 82:101-104.
- Parfitt, R., R.J. Atkinson, and R.C. Smart. 1975. The mechanism of phosphate fixation on iron oxides. *Soil Science Society of America Journal* 39:837-841.
- Penn, C.J., G.L. Mullins, and L.W. Zelazny. 2005. Mineralogy in relation to phosphorus sorption and dissolved phosphorus losses in runoff. *Soil Science Society of America Journal* 69:1532-1540.
- Piatek, K.B., and H.L. Allen. 2001. Are forest floors in mid-rotation stands of loblolly pine (*Pinus taeda*) a sink for nitrogen and phosphorus. *Canadian Journal of Forest Research* 31:1164-1174.
- Pierzynski, G., R.W. McDowell, and J.T. Sims. 2005. Chemistry, cycling, and potential movement of inorganic phosphorus in soils. *In* J. T. Sims and A. N. Sharpley, eds. Phosphorus: Agriculture and the Environment American Society of Agronomy, Madison, WI. p. 53-86
- Pierzynski, G.M. 2000. Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters North Carolina State University.
- Polglase, P.J., E.J. Jokela, and N.B. Comerford. 1992a. Phosphorus, nitrogen, and carbon fractions in litter and soil of southern pine plantations. *Soil Science Society of America Journal* 56:566-573.
- Polglase, P.J., E.J. Jokela, and N.B. Comerford. 1992b. Nitrogen and phosphorus release from decomposing needles of southern pine plantations. *Soil Science Society of America Journal* 56:914-920.
- Polglase, P.J., N.B. Comerford, and E.J. Jokela. 1992c. Mineralization of nitrogen and phosphorus from soil organic matter in southern pine plantations. *Soil Science Society of America Journal* 56:921-927.
- Pritchett, W.-L. 1979. Properties and management of forest soils Wiley, New York
- Pritchett, W.L., and N.B. Comerford. 1982. Long-term response to phosphorus fertilization on selected southeastern coastal plain soils. *Soil Science Society of America Journal* 46:640-644.

- Quin, L.D., and G.S. Quin. 2001. Screening for carbon-bound phosphorus in marine animals by high resolution  $^{31}\text{P}$ -NMR spectroscopy: Coastal and hydrothermal vent invertebrates. *Comparative Biochemistry and Physiology* 128:173-185.
- Richter, D.D., H.L. Allen, J. Li, D. Markewitz, and J. Raikes. 2006. Bioavailability of slowly cycling soil phosphorus: major restructuring of soil P fractions over four decades in an aggrading forest. *Oecologia* 150:259-271.
- Sample, E.C., R.J. Soper, and G.J. Racz. 1980. Reactions of phosphate fertilizers in soils. In: *Role of phosphorus in agriculture*. eds F. E. Khasawneh, E. C. Sample, E. J. Kamprath. p. 263-310.
- Sato, S., and N.B. Comerford. 2006. Organic anions and phosphorus desorption and bioavailability in a humid Brazilian Ultisol. *Soil Science* 171:695-705.
- Sims, J.T. 2000a. A Phosphorus Sorption Index, *In* G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University. p. 22-23.
- Sims, J.T. 2000b. Soil test phosphorus: Mehlich 1. *In* G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University. , p. 15-16.
- Sims, J.T. 2000c. Soil test phosphorus: Mehlich 3. *In* G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University. p. 17-19.
- Singh, K., E. Yoshimura, K. Kanazawa, P.C. Sudhakar, and S. Mori. 2001. Effect of organic acid ligands on nutrient mobilization from volcanic acid soils. *Indian Journal of Agricultural Sciences* 71:760-764.
- Smeck, N.E. 1985. Phosphorus dynamics in soils and landscapes. *Geoderma* 36:185-199.
- Smith, S.E., and D.J. Read. 1997. *Mycorrhizal Symbiosis*. 2nd ed. Academic Press, Inc., San Diego, CA.
- Solomon, D., J. Lehmann, T. Mamo, F. Fritzsche, and W. Zech. 2002. Phosphorus forms and dynamics as influenced by land use changes in the sub-humid Ethiopian highlands. *Geoderma* 105:21-48.
- Stevenson, F.J. 1967. Organic acids in soils. *In* A. D. McLaren and G. H. Peterson, eds. *Soil Biochemistry*. Marcel Dekker, New York. p. 119-146.
- Tate, K.R., and R.H. Newman. 1982. Phosphorus fractions of a clinosequence of soils in New Zealand tussock grassland. *Soil Biology and Biochemistry*. 14:191-196.
- Tew, D.T., L.A. Morris, H.L. Allen, and C.G. Wells. 1986. Estimates of nutrient removal, displacement and loss resulting from harvest and site preparation of a *Pinus taeda* plantation in the piedmont of North Carolina. *Forest Ecology and Management* 15:257-267.
- Tiessen, H., and J.O. Moir. 1993. Characterization of available P by sequential extraction. *In* M. R. Carter, ed. *Soil Sampling and Methods of Analysis*. Lewis Publishers. p. 75-86.
- Tiessen, H., J. Stewart, and C.V. Cole. 1984. Pathways of phosphorus transformations in soils of differing pedogenesis. *Soil Science Society of America Journal* 48:853-858.
- Turner, B.L. 2005. Storage-induced changes in phosphorus solubility of air-dried soils. *Soil Science Society of America Journal* 69:630-633.
- Turner, B.L., and A.E. Richardson. 2004. Identification of scyllo-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Science Society of America Journal* 68:802-808.

- Turner, B.L., N. Mahieu, and L.M. Condon. 2003. Quantification of myo-inositol hexakisphosphate in alkaline soil extracts by solution  $^{31}\text{P}$  NMR spectroscopy and spectral deconvolution. *Soil Science* 168:469-478.
- Turner, B.L., M.J. Paphazy, P.M. Haygarth, and I.D. McKelvie. 2002. Inositol phosphates in the environment. *Philosophical Transactions of the Royal Society of London* 357:449-469.
- Turner, B.L., N. Mahieu, L.M. Condon, and C.R. Chen. 2005. Quantification and bioavailability of scyllo-inositol hexakisphosphate in pasture soils. *Soil Biology and Biochemistry* 37:2155-2158.
- Turner, B.L., L. Condon, S. Richardson, D.A. Peltzer, and V. Allison. 2006. Transformations of soil organic phosphorus during ecosystem development. *ASA-CSSA-Soil Science Society of America Journal 2006 International Annual Meetings*, Indianapolis, IN.
- Turner, J., and M.J. Lambert. 1988. Long-term effects of phosphorus fertilization on forests. *In* B. B. and W. C. H., eds. *Forest site evaluation and long-term productivity*. University of Washington Press, Seattle, WA. p. 125-133.
- van Hees, P.A., U.S. Lundstrom, and R. Giesler. 2000. Low molecular weight organics and their Al complexes in soil solution - composition, distribution, and seasonal variation in three podzolized soils. *Geoderma* 94:173-200.
- van Hees, P.A.W.v., D.L. Jones, and D.L. Godbold. 2002. Biodegradation of low molecular weight organic acids in coniferous forest podzolic soils. *Soil Biology and Biochemistry* 34:1261-1272.
- van Hees, P.A.W., D.L. Godbold, G. Jentschke, and D.L. Jones. 2003. Impact of ectomycorrhizas on the concentration and biodegradation of simple organic acids in a forest soil. *European Journal of Soil Science* 54:697-706.
- van Hees, P.A.W., D.L. Jones, G. Jentschke, and D.L. Godbold. 2004. Mobilization of aluminum, iron and silicon by *Picea abies* and ectomycorrhizas in a forest soil. *European Journal of Soil Science* 55:101-112.
- van Scholl, L., E. Hoffland, and N. van Breemen. 2006. Organic anion exudation by ectomycorrhizal fungi and *Pinus sylvestris* in response to nutrient deficiencies. *New Phytologist* 170:153-163.
- Walker, T.W., and J.K. Syers. 1976. The fate of phosphorus during pedogenesis. *Geoderma* 15:1-19.
- Wallander, H. 2000. Uptake of P from apatite by *Pinus sylvestris* seedlings colonized by different ectomycorrhizal fungi. *Plant and Soil* 222:249-256.
- Wallander, H., L. Johansson, and J. Pallon. 2002. PIXE analysis to estimate the elemental composition of ectomycorrhizal rhizomorphs grown in contact with different minerals in forest soil. *FEMS Microbiology Ecology*. 39:147-156.
- Wells, C.G., and J.R. Jorgensen, (eds.) 1975. *Nutrient cycling in loblolly pine plantations*. Les Presses de L'Université, Laval, Quebec. pp. 137-158.
- Xu, R.K., A.Z. Zhao, and G.L. Ji. 2005. Effect of low molecular weight organic anions on adsorption of potassium by variable charge soils. *Communications in Soil Science and Plant Analysis* 36:1029-1039.
- Yuan, G., and L.M. Lavkulich. 1994. Phosphate sorption in relation to extractable iron and aluminum in spodosols. *Soil Science Society of America Journal* 58:343-346.

## **Chapter 2: HEDLEY PHOSPHORUS POOLS IN SOILS FROM FOUR LOBLOLLY PINE (*Pinus taeda*) PLANTATION IN THE SOUTHEASTERN UNITED STATES.**

### **Abstract**

Phosphorus (P) fertilization increases plant available or labile P pools immediately after fertilization. Studies in the southeastern United States and abroad have demonstrated long-term fertilization effects on P pools and stand growth. I examined soil P pools using the Hedley sequential fractionation procedure and Mehlich-3 soil tests in a long-term study of P fertilization in loblolly pine (*Pinus taeda* L.) plantations installed at four sites in the Coastal Plains of the southeastern United States. The Hedley fractionation procedure partitions the extracted P into six fractions, which can be grouped into labile, moderately labile, and recalcitrant P pools.

Results indicate there is a long-term fertilization effect on P pools in the surface soil 21 years after fertilization; however these effects were limited to the surface horizons. Mehlich-3 extractable P was largest in the 0-10 cm soil depth of the fertilized treatments plots. Hedley labile and moderately labile P pools were also largest in the soil surface and decreased with depth. The decrease with depth reflects the affect of P adsorption reactions that limit the mobility of P in these soils. Mehlich-3 soil test were able to detect long-term changes in soil P pools.

### **Introduction**

The application of nitrogen (N) and phosphorus (P) fertilizers have become routine treatments for intensively managed southern pine plantations in coastal plain sites in the southern United States (Fox *et al.*, 2007). Since the discovery that southern pines respond to P fertilization in the 1960's (Pritchett, 1961; Walker, 1960) more than 6.5 million ha of southern pine plantations

have been fertilized (Albaugh *et al.*, 2007). Additionally, many abandoned farmlands, which typically received P inputs, have been converted to pine plantations. Since 1973, more than 1.25 million ha of land in the southeastern United States have been reforested (Drummond, 2008).

Previous P inputs may have long-term effects on soil P pools and subsequent tree growth. Fertilization can increase total P returns in litter fall from 150 to 400% and P content in the forest floor (Dalla-Tea and Jokela, 1991; Piatek and Allen, 2001). Release of P from decomposing needles is strongly and positively correlated to the needle concentration of inorganic P (Polglase *et al.*, 1992b), which increases with fertilization (Polglase *et al.*, 1992c).

Many plantations in the southern US are entering their second or third rotations after being P fertilized during previous rotations. Results have shown that there is a long-term effect of P fertilization in some forest soils (Ballard, 1978; Comerford *et al.*, 2002b; Fife and Nambiar, 1999; Turner *et al.*, 2002). The application of P fertilizer to these sites has had long-term effects on forest productivity effectively increasing site index. These long-term effects are affected by the capacity of the site to fix a portion of the applied P in a labile form in the soil or forest floor.

The soil P fixation capacity affects the retention and long-term supply of applied P and influences the cycling of P pools (Turner and Lambert, 1988). Soils with high sorption capacities retain larger quantities of P. Iron and aluminum minerals such as goethite and gibbsite and related amorphous compounds are responsible for the fixed P in many soils of the southeastern US. Fixed P is gradually released into the soil solution and incorporated into living biomass but this process is slow occurring over many hundreds to thousands of years (Cross and Schlesinger, 1995), and may not supply adequate P to maintain rapid growth of pine plantations.

Phosphorus taken up by living organisms, plants, and microbes, in the soil will convert a portion of the inorganic P (Pi) into organic P (Po) compounds. These Po compounds are

returned to the soil and over time soil P pools become dominated by Po compounds (Magid et al., 1996; Smeck, 1985; Walker and Syers, 1976). Organic P concentrations can account for 70 % of total P in forest soils (Turner and Lambert, 1988). This shift from Pi to Po compounds is important to tree growth because there is increasing evidence that Po compounds contribute to plant available (labile) P pools (George et al., 2006; Johnson et al., 2003; Turner et al., 2005). For example, Richter *et al.* (2006) reported that the slowly cycling Po pools were depleted after conversion of the site from a cotton field to pine forest in 1957. The importance of Po pools has also been documented in 6-yr-old pine plantations on spodosols in Florida (Polglase et al., 1992a). In this study, mineralization of organic material in the surface 5 cm of soil supplied 0 and 25% of the annual P requirements in unfertilized and fertilized plots respectively.

Standard soil P tests such as Mehlich 1, Mehlich 3, Bray and Kurtz, and Olsen-P have been developed to determine available soil P and the need for P fertilization (Allen and Mallarino, 2006; Wells et al., 1986). In the southern US results from Mehlich 1 test have been used to determine the probability of response of loblolly pine to P fertilization (Wells *et al.*, 1973). These soil tests do not attempt to distinguish between soil Pi and Po pools. The Hedley sequential fractionation procedure attempts to quantify Po and Pi pools in mineral soils (Hedley et al., 1982b). Tiessen and Moir (1993) modifications of the Hedley sequential fractionation procedure use a variety of chemical solutions of increasing ionic strength to divide soil P into pools of decreasing biological availability (Fig 2-1). The six sequential fractions are: 1) DiH<sub>2</sub>O-resin strips - P; 2) NaHCO<sub>3</sub> - Pi and Po; 3) NaOH - Pi and - Po; 4) 1M HCl Pi; 5) Conc. HCl - Pi and - Po; 6) H<sub>2</sub>SO<sub>4</sub> - P which when summed give Hedley total P. Research shows that Hedley labile P pools (DiH<sub>2</sub>O-resin strips P and NaHCO<sub>3</sub> - Pi and - Po) are available over the course of a single growing season (Cross and Schlesinger, 1995; Johnson et al., 2003; Richter et al., 2006;

Tiessen et al., 1984). Hedley moderately labile P pools (NaOH - Po) are labile over a longer period (Richter et al., 2006), and a large portion of the HRP pools (remaining fractions) cycle very slowly.

My goal was to determine the long-term effects of P fertilization on soil P pools. The specific objective of this research project is to quantify the effects of fertilization on Mehlich 3 extractable P and Hedley P pools in four southeastern loblolly pine (*Pinus taeda* L.) plantation soils. My hypotheses are that 22 years after fertilization:

Ha<sub>1</sub>: Hedley labile and moderately labile P pools will be significantly greater in fertilized plots versus controls.

Ha<sub>2</sub>: Mehlich - 3 extractable P will be significantly greater in the fertilized plots versus control.

## **Material and Methods**

### *Study Sites*

The fertilization trials were installed in 1978-1981 to determine long-term impacts of site preparation, fertilization, and vegetation control on growth, yield, and nutrition of *P. taeda*. The four trials sampled in this study were located in the Coastal Plain physiographic province of the southeastern United States. Two sites were located in Virginia and South Carolina and were established on poorly drained soils, and two sites were located in Georgia and Alabama and were established on well-drained soils (Table 2-1).

Site preparation differed at each location according to site-specific conditions and operator preferences. Site preparation treatments were defined as high and low intensity. A control (0 kg ha<sup>-1</sup>) and P fertilizer treatments (56 kg ha<sup>-1</sup>) were applied as diammonium phosphate (DAP) at

the time of planting in 1.8 m bands centered over the planted rows. Vegetation control treatments consisted of two applications of hexazinone, once at planting and the second the following spring. Two treatments at each location were selected to study the long-term effects of fertilization on P availability. The specific treatments sampled for this study were chosen because they represented the maximum and minimum (control) tree height and volume growth response at each location. The growth responses at the individual sites are listed in Table 2-2 (Forest Nutrition Cooperative, 1991; Cooperative, 1996). The growth data represent the last measurements made before the stands were harvested.

### *Experimental Design*

The study was originally installed in 1980 as a randomized complete block with a split-plot design. Each location had four blocks with the site preparation (whole plot) assigned followed by random assignments of fertilizer and vegetation control (subplot) treatments. Site preparation and weed control treatments differed according to landowner preferences. Treatment plots were 160 × 40 m (0.65 ha), with 16 rows of 16 trees planted at 2.4 x 2.4 m spacing. The internal eight rows × eight trees served as the measurements plots

Table 2-1 Study site descriptions and experimental treatments applied.

<i>Site</i>	<i>Soil Series</i>	<i>Soil Type</i>	<i>Surface texture</i>	<i>Subsoil texture</i>	<i>Drainage</i>	<i>Site Index (BA 25)</i>	<i>Treatment</i>	<i>Site Preparation</i>	<i>Fertilizer (kg/ha)</i>
SC 1101	Wahee	Fine, mixed semiactive, thermic Aeric Endoaquults	Fine Sandy Loam	Clay Loam	Poorly	63	Control	Chop	0
							Fertilized	Chop, Bed	56
VA 2602	Grantham	Fine-silty, siliceous, semiactive, thermic Typic Paleaquults	Very Fine Sandy Loam	Sandy Clay Loam	Poorly	69	Control	Chop, Burn	0
							Fertilized	Shear, Pile, Bed	56
AL 2401	Dothan	Fine-loamy kaolinitic, thermic Plinthic Kandiudults	Sandy Loam	Sandy Clay Loam	Well	51	Control	Chop, Burn	0
							Fertilized	Shear, Pile, Disc	56
GA 0701	Wagram	Loamy, kaolinitic, thermic Arenic Kandiudults	Loamy Sand	Sandy Loam	Well	49	Control	Burn	0
							Fertilized	Chop, Burn	56

Table 2-2 Treatment means from four loblolly pine stands in the Coastal Plains province of the southeastern United States.

Site	Stand Age	Treatment	Foliar P conc. %	Height (m)	DBH (cm)	Volume (m <sup>3</sup> /ha)	Site Index (BA 25)
SC 1101	14	Control	0.079a	12a	15a	68a	63
		Fertilized	0.97b***	14b***	18b***	141b***	76
VA 2602	16	Control	0.102a	14a	17a	154a	69
		Fertilized	0.105a	16b***	18b <sup>+</sup>	211b <sup>+</sup>	78
AL 2401	14	Control	0.091a	10a	14a	60a	51
		Fertilized	0.102b**	12b**	15b <sup>*</sup>	109b**	63
GA 0701	10	Control	0.110a	7a	9a	26a	49
		Fertilized	0.120b***	9b***	12b***	47b***	69

Conc. denotes concentration. DBH denotes diameter at breast height. <sup>+</sup> denotes  $p \leq 0.1$ ; <sup>\*</sup> denotes  $p \leq 0.05$ ; <sup>\*\*</sup> denotes  $p \leq 0.01$ ; <sup>\*\*\*</sup> denotes  $p \leq 0.001$ .

In this study, the experimental design was different from the 1980 experiment. The design was a randomized complete block design with two factors (P fertilization x depth). Soil samples were collected at each site from treatment plots that exhibited the maximum and minimum stand growth response to the 1980 treatments. Therefore, treatments selected for sampling in this study include one with P fertilization (maximum stand growth response) and one without fertilization (minimum stand growth response) designated as the control in this study. While P fertilization rates were similar at each site, site preparation and weed control treatments differed. Sites were therefore analyzed separately in order to prevent any confounding affects of site preparation and weed control treatments.

#### *Soil Physical and Chemical Analysis*

Soil samples were collected within the plots in 2001 using a 7.2 cm inside diameter auger. Soils were sampled at five depths (0-10, 10-20, 20-40, 40-60, and 60-100 cm) at nine or more locations in the SC, GA, and VA sites. Soil samples collected from the AL site were collected at four locations within the treatment plots. The individual samples were air dried, sieved (< 2 mm), and stored in plastic bags. Soil samples within each plot were composited by depth. Then composited samples were oven dried and weigh for moisture corrections.

Soil samples were analyzed for Mehlich-3 extractable P (M-3) (Sims (2000)). The M-3 solution is composed of 0.2 M acetic acid ( $\text{CH}_3\text{COOH}$ ), 0.25 M ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), 0.015 M ammonium fluoride ( $\text{NH}_4\text{F}$ ), 0.013 M nitric acid ( $\text{HNO}_3$ ), and 0.001 M ethylenediaminetetraacetic (EDTA) ( $\text{HO}_2\text{CCH}_2)_2\text{NCH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CO}_2\text{H})_2$ ). Draco (P

free) activated charcoal was added to the soil to obtain a colorless solutions. The solutions were then filtered (2.5  $\mu\text{m}$ ), and P was measured on a spectrophotometer at 880 nm using the molybdate-ascorbic acid procedure (Murphy and Riley, 1962).

Hedley P fractions were determined following the procedure of Tiessen and Moir (1993). The Hedley fractionation procedure uses six sequential extraction solution of differing ionic strength to divide the P content into pools of decreasing biological availability (Fig. 2-1). The sequential fractions are (1) deionized water with resin strips – P; (2)  $\text{NaHCO}_3$  pH 8.5– Pi and –Po; (3)  $\text{NaOH}$  – Pi and –Po; (4) 1M  $\text{HCl}$  – P; (5) hot concentrated  $\text{HCl}$  – Pi and –Po; and (6)  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$  - Pi. These fractions are partitioned into Hedley labile ( $\text{DiH}_2\text{O}$ -Pi,  $\text{NaHCO}_3$  – Pi, and  $\text{NaHCO}_3$  – Po), moderately labile ( $\text{NaOH}$  – Po), and recalcitrant ( $\text{NaOH}$  – Pi, 1M  $\text{HCl}$  – Pi, hot concentrated  $\text{HCl}$  – Pi and –Po;  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$  - Pi) P pools. The assignments are based upon to the chemical strength of the P bonds and the plants ability to access those P pools (Johnson et al., 2003; Richter et al., 2006; Tiessen et al., 1984). Henceforth Hedley labile P, Hedley moderately labile P, and Hedley recalcitrant P will be referred to HLP, HMLP, and HRP respectively.

The extraction procedure was as follows; a 0.5 g sample of sieved air-dried soil was added to a 100 ml centrifuge tube and weighed (Fig. 2-1). Anion exchange membranes were charged by shaking the membranes in two separate baths of 0.5 M  $\text{NaHCO}_3$  over a 16-hr. period. Then the membranes were rinsed in three baths of  $\text{DiH}_2\text{O}$ . Next 30 ml of  $\text{DiH}_2\text{O}$  and 600  $\text{mm}^2$  of anion exchange membranes (AEM) were added to the tube, capped, and placed on a reciprocating shaker (~100 rpm). After 16 hrs., the membrane was removed, and any adhering soil was rinsed back into the tube. The AEMs were

placed in separate vials and 15 ml of 0.5M HCl was added to eluted the sorbed Pi into solution which was passed through a 0.45  $\mu\text{m}$  filter and refrigerated until analysis.

Following the removal of the AEM, the tubes were centrifuged for 20 min. ( $\sim 3500$  g). The water was decanted through a 0.45  $\mu\text{m}$  filter and the soil and or any precipitated SOC ( $\text{NaHCO}_3$  and  $\text{NaOH}$  solutions) retained by the filter was rinsed back into the tube with the 0.5 M  $\text{NaHCO}_3$  solution. The tubes and soil were placed on a scale and brought to 30 ml (by weight) with  $\text{NaHCO}_3$  solution. The tubes were then capped and shaken for 16 hrs. (Fig. 2-1). Following the 16-hr. shake, the  $\text{NaHCO}_3$  solution was extracted and filtered as described above. Next, the  $\text{NaOH}$  was extracted and filtered as described above; followed by the 1 M HCl solutions which were also centrifuged and filtered.

After the samples were extracted with 1 M HCl, the samples were placed in a water bath at  $80^\circ\text{C}$  with 10 ml of conc. HCl for 10 min. The sample solution was held at  $80^\circ\text{C}$  for at least 10 min. Then they were removed from the water bath, vortexed, and an additional 5 ml of conc. HCl was added. The samples were vortex every 15 min. while cooling for one hr.. The tubes were then centrifuged for 15 min. and the HCl was decanted into a separate preweighted 100 ml centrifuged tube. The soil samples were rinsed twice with 10 ml  $\text{DiH}_2\text{O}$ , centrifuged for 15 min., and the rinse solution was added to the decanted conc. HCl solution. Those solutions were brought to 50 ml and saved for analysis.

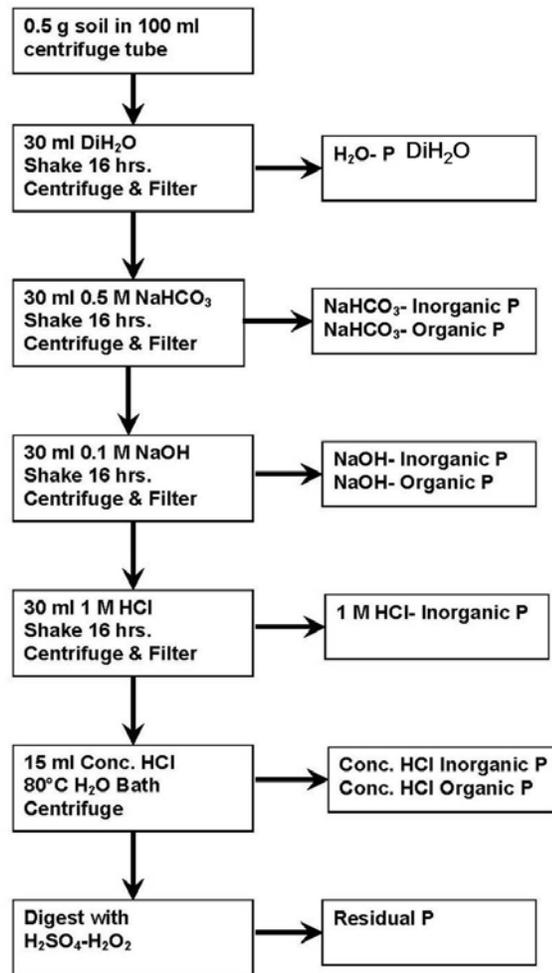


Figure 2-1. Schematic representation of Hedley sequential fractionation procedure.

After the hot conc. HCL digest the soils were quantitatively transferred into 75 ml block digestion tubes using a pipette and DiH<sub>2</sub>O. After the samples were transferred, 2 boiling chips were added and a majority of the water was evaporated off on a block digest at 120°C. Next, 5ml of conc. H<sub>2</sub>SO<sub>4</sub> was added to the soils and the block digest was slowly (over approximately four hrs.) heated to 360°C. The samples were then removed from the heated block and allowed to cool to room temperature. After cooling 0.5 ml of 50% H<sub>2</sub>O<sub>2</sub> was added to the soil samples. The samples were then vortexed, returned to the block digest, and heated until no further water vapor was released. The tubes were then removed allowed to cool and H<sub>2</sub>O<sub>2</sub> was added again. This procedure was repeated until the SOC was oxidized, which occurred when the solution changed from darkly colored to clear. This occurred after about 10 additions of 50% H<sub>2</sub>O<sub>2</sub>. After the final addition, the sample was heated to 360°C for 30 min. to ensure all the H<sub>2</sub>O<sub>2</sub> was digested. The solution was then cooled, brought to 75 ml, and an aliquot was saved for analysis.

Initial attempts to measure Pi concentrations in the first two extracted solutions revealed P to be below the detection limits. Therefore I measured Hedley P pools on the fine soil fraction passing through a 0.15 mm sieve (100 mesh) as suggested by Sommers and Nelson (1972). Extractions with sieved soil samples allow the detection of Pi from the DiH<sub>2</sub>O-Pi, NaHCO<sub>3</sub> – Pi, and NaHCO<sub>3</sub> – Po solutions. The concentration of P measured was corrected back to the whole soil equivalent by:

$$P \text{ mg kg}^{-1} = \frac{P \text{ concentration (mg L}^{-1}) \times \text{Soil fines mg (2.0 < mm)} \times \text{Extracting solution L}}{\text{Soil fines mg (0.15 < mm)} \times \text{Extracted soil kg}}$$

The Pi solutions were analyzed by molybdate-ascorbic acid procedure for dissolved orthophosphate (Murphy and Riley, 1962). The organic P (Po) concentrations in selected fractions were determined as the difference between total P measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) and Pi. In order to measure the Pi concentration in the NaHCO<sub>3</sub> and NaOH solutions, aliquots of the samples were acidified with 0.9 M H<sub>2</sub>SO<sub>4</sub> to a pH < 2. After the gas in the solutions had bubbled off, the vials were capped, chilled at 4°C overnight to precipitate the humic acids. The solutions were then decanted with an autopipetter and measured for molybdate reactive Pi concentration.

The molybdate-ascorbic acid procedure requires that the solutions be near neutral pH. Therefore the pH of each aliquot was adjusted to a neutral pH with *p*-nitrophenol indicator and 0.01 M NaOH or 0.5 M H<sub>2</sub>SO<sub>4</sub> (Tiessen and Moir, 1993). The Pi concentrations of the Hedley extracts were measured on a spectrophotometer at 712 nm, which limits interference of dissolved fulvic acids (Tiessen and Moir, 1993).

### *Statistical Analysis*

Statistical analyses were performed using the MIXED model for all extractions except deionized water with restricted/residual maximum likelihood estimation method in SAS software (SAS Institute, Cary, NC). The two factors tested were fertilization (0, 56 kg P/ha) and soil depth (0-10, 10-20, 20-40, 40-60, 60-100 cm). The Satterthwaite option was employed to calculate the correct degrees of freedom. When necessary, data were natural log transformed to meet the assumption of a normal distribution. For all test generalized least squared means were calculated and analyzed for treatment effects.

## Results

### *Hedley P Pools*

There was no significant effect of fertilization on total Hedley extractable P at the four sites (Tables 2-3, 2-4, 2-5, 2-6). Total Hedley extractable P was greatest in the surface soil at two of the four sites studied SC and VA (Fig. 2-2). At the study site in GA, total Hedley extractable P was slightly higher at the 10-20 cm depth than in the 0-10 cm depth, although the difference was not significant. Total Hedley extractable P in the surface soil at the two poorly drained soils from SC and Va was approximately  $30 \text{ mg kg}^{-1}$ . This was almost twice as large as the total Hedley extractable P in the surface soil of the two well drained soils from Al and GA that average about  $13 \text{ mg kg}^{-1}$ . Both these two soils have an arenic surface that is more than 20 cm thick (Table 2-1). Total Hedley extractable P decreased with soil depth (Tables 2-3, 2-4, 2-5, 2-6). This decrease was greater at the two poorly drained sites. Total Hedley extractable P in the subsoil below 40 cm was similar among the four sites and ranged from 6 to  $15 \text{ mg kg}^{-1}$  across the four sites. Total Hedley extractable P above 20 cm soil depth, is largely influenced by increases in the HLP and HMLP pools at all sites except GA.

Concentrations of HLP pools were larger in the surface depths of the poorly drained sites compared to well drained sites. The HLP was greatest in the surface soil of the VA site, averaging  $8 \text{ mg kg}^{-1}$  and somewhat less in the SC site which was  $4 \text{ mg kg}^{-1}$ . Hedley labile P concentrations declined through the soil profile.

For example, the concentrations of HLP in VA declined from  $5 \text{ mg P kg}^{-1}$  at the soil surface (0-10 cm depth) to  $0.75 \text{ mg P kg}^{-1}$  in the 60-100 cm soil depth. In contrast, the concentrations of HLP pools in the surface soil depth at the well-drained sites in Al and

GA were both approximately  $1.4 \text{ mg kg}^{-1}$ . Additionally the concentrations of HLP in the well drained sites remain relatively constant throughout the profile.

In the surface soil and 10-20 cm soil depth the concentration of HMLP pools from SC, VA, and AL were equal to or greater than HRP pools (Fig. 2-2). Concentrations of HMLP were typically greater than HLP at all depths and across the four sites. The HMLP concentrations in the surface soil are approximately 12, 11, and  $6 \text{ mg kg}^{-1}$  at the SC, VA, and AL sites respectively. Hedley moderately labile P pools from GA, about  $1.5 \text{ mg P kg}^{-1}$ , was much less than the HMLP pools at the other three sites. The concentrations of HMLP were highest in the soil surface of poorly drained soils and decreased with depth.

Hedley recalcitrant P pools at all four sites were relatively constant throughout the profile. This trend was most apparent from soils at the VA site where the HRP pools were approximately  $13 \text{ mg P kg}^{-1}$  throughout the entire profile (Fig. 2-2). Above 20 cm soil depth, the HRP pools at SC, VA, and AL sites accounted for approximately half of the Hedley total P. Below the 20 cm depth, the concentration of HRP pools to represent > 70% of the Hedley total extractable P pools.

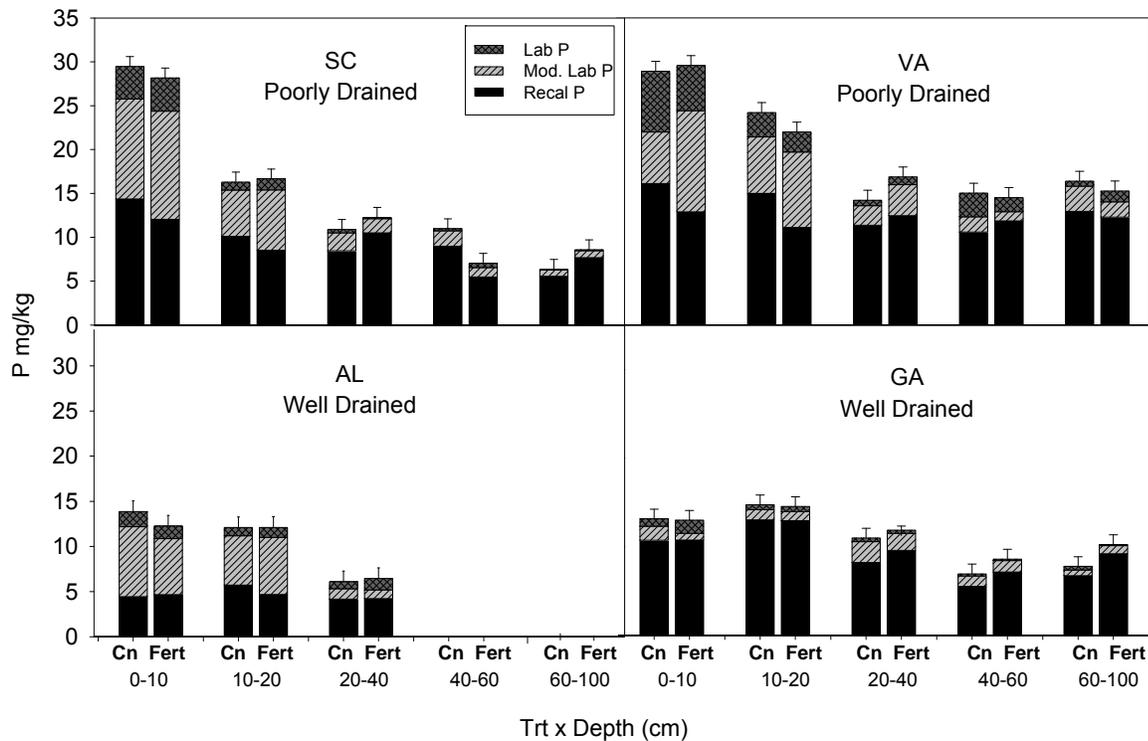


Figure 2-2. Hedley extractable phosphorus pools ( $\text{mg kg}^{-1}$ ) from Coastal Plain soils supporting loblolly pine plantations in the southeastern US. Labile P ( $\text{DiH}_2\text{O-Pi}$ ,  $\text{NaHCO}_3 - \text{Pi}$ , and  $\text{NaHCO}_3 - \text{Po}$ ). Moderately labile P ( $\text{NaOH} - \text{Po}$ ). Recalcitrant P ( $\text{NaOH} - \text{Pi}$ ,  $1\text{M HCl} - \text{Pi}$ , hot concentrated  $\text{HCl} - \text{Pi}$  and  $-\text{Po}$ ;  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2 - \text{Pi}$ ). Error bars represent the standard error of the mean for total Hedley extractable P. Cn= control, Fert = fertilized, Trt = treatment.

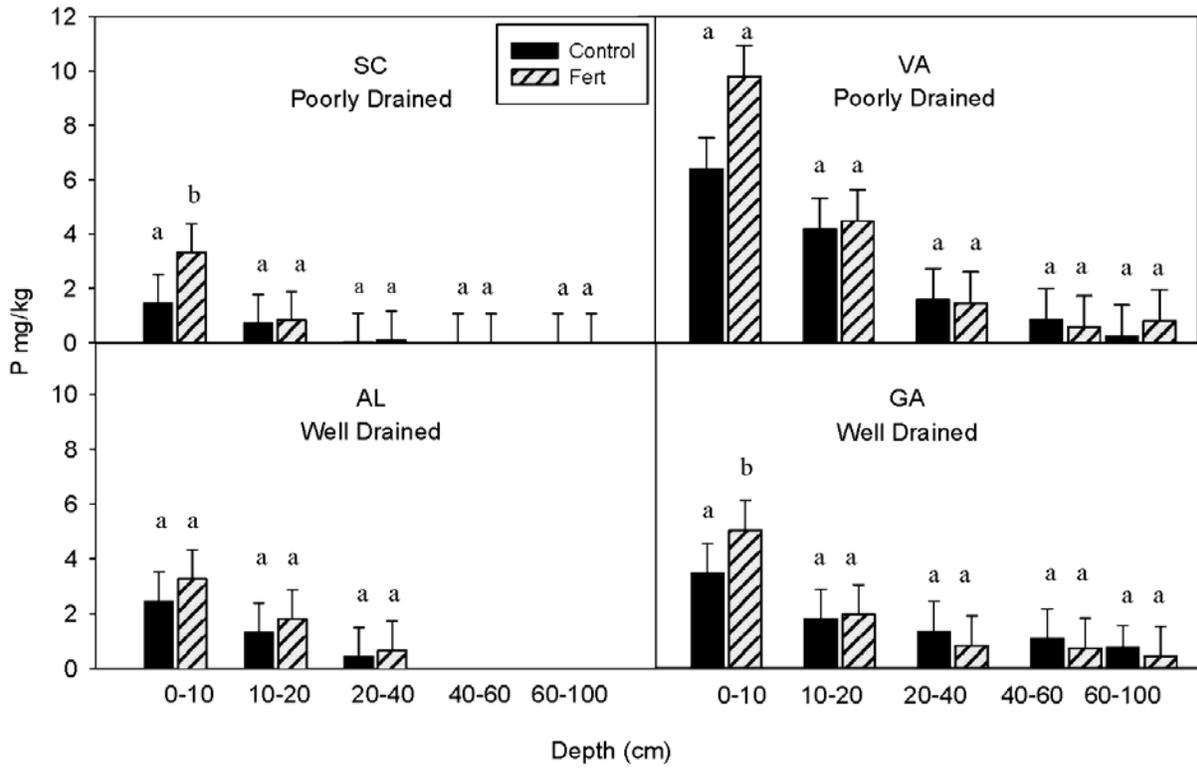


Figure 2-3. Mehlich 3 extractable phosphorus ( $\text{mg kg}^{-1}$ ) from Coastal Plains soils supporting loblolly pine plantations in the southeastern US. Values represent least square means and the bars are the standard error of the least square means. Bars with different letters are significantly different at the  $p < 0.1$ .

Table 2-3 Georgia Type 3 table test of fertilization and depth treatment effects on Hedley Labile (A) Moderately Labile (B) Recalcitrant (C) and Total (D) P pools.

Table A.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	2	18	0.88	0.4327
Fert	1	18	0.03	0.8709
Depth	4	18	9.16	0.0003
Fert*Depth	4	18	1.57	0.2242

Table B.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	2	18	2.65	0.0978
Fert	1	18	0.35	0.5619
Depth	4	18	4.85	0.0079
Fert*Depth	4	18	1.17	0.3567

Table C.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	2	18	7.61	0.0040
Fert	1	18	3.67	0.0714
Depth	4	18	9.62	0.0002
Fert*Depth	4	18	0.65	0.6329

Table D.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	2	18	7.27	0.0048
Fert	1	18	2.76	0.1140
Depth	4	18	10.07	0.0002
Fert*Depth	4	18	0.62	0.6515

Table 2-4 South Carolina Type 3 table test of fertilization and depth treatment effects on Hedley Labile (A) Moderately Labile (B) Recalcitrant (C) and Total (D) P pools.

Table A.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	3	26	2.30	0.1012
Fert	1	26	1.18	0.2866
Depth	4	26	87.06	<.0001
Fert*Depth	4	26	1.04	0.4076

Table B.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	3	26	0.73	0.5418
Fert	1	26	0.00	0.9939
Depth	4	26	67.89	<.0001
Fert*Depth	4	26	0.90	0.4808

Table C.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	3	26	2.05	0.1318
Fert	1	26	0.40	0.5302
Depth	4	26	3.01	0.0365
Fert*Depth	4	26	1.04	0.4064

Table D.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	3	26	1.56	0.2237
Fert	1	26	0.23	0.6386
Depth	4	26	48.53	<.0001
Fert*Depth	4	26	3.07	0.0339

Table 2-5 Alabama Type 3 table test of fertilization and depth treatment effects on Hedley Labile (A) Moderately Labile (B) Recalcitrant (C) and Total (D) P pools.

Table A.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	2	10	1.42	0.2860
Fert	1	10	0.50	0.4974
Depth	2	10	1.93	0.1949
Fert*Depth	2	10	0.67	0.5350

Table B.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	2	10	1.51	0.2663
Fert	1	10	0.15	0.7104
Depth	2	10	54.95	<.0001
Fert*Depth	2	10	0.52	0.6103

Table C.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	2	10	13.55	0.0014
Fert	1	10	0.23	0.6393
Depth	2	10	1.26	0.3260
Fert*Depth	2	10	0.43	0.6608

Table D.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	2	10	6.50	0.0155
Fert	1	10	0.05	0.8337
Depth	2	10	10.85	0.0031
Fert*Depth	2	10	0.06	0.9441

Table 2-6 Virginia Type 3 table test of fertilization and depth treatment effects on Hedley Labile (A) Moderately Labile (B) Recalcitrant (C) and Total (D) P pools.

Table A.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	3	27	0.07	0.9742
Fert	1	27	0.26	0.6112
Depth	4	27	10.27	<.0001
Fert*Depth	4	27	0.60	0.6644

Table B.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	3	26	0.60	0.6217
Fert	1	26	0.93	0.3438
Depth	4	26	14.46	<.0001
Fert*Depth	4	26	1.59	0.2062

Table C.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	3	26	7.98	0.0006
Fert	1	26	1.28	0.2676
Depth	4	26	1.25	0.3157
Fert*Depth	4	26	0.78	0.5455

Table D.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
block	3	26	4.49	0.0114
Fert	1	26	0.03	0.8742
Depth	4	26	6.48	0.0009
Fert*Depth	4	26	0.45	0.7703

### *Mehlich-3*

Fertilization had a long-term impact on Mehlich 3 extractable P in the surface soil at all four sites. Fertilization resulted in significantly higher concentrations of M-3 P in the 0-10 cm depth at the SC ( $p = 0.0055$ ) and GA ( $p=0.0498$ ) sites. There was a 38% increase in concentration of M-3 P in the 0-10 cm soil depth of fertilized plots in VA ( $p=0.0645$ ). Similarly, a 26% increase in the M-3 P in the 0-10 cm soil depth of fertilized plots in AL ( $p=0.11$ ). The concentrations of M-3 extractable P in the 10-20 cm soil depth at all four sites were unaffected by fertilization. Values ranged from  $<1$  to about  $4 \text{ mg P kg}^{-1}$ . The concentration of M-3 extractable P below 20 cm was less than  $1.5 \text{ mg P kg}^{-1}$  across all four sites.

## **Discussion**

### *Hedley P*

All four sites in this study had significant long-term P fertilization effects on tree height, diameter at breast height, and stand volume (Table 2-2). Additionally, foliar P concentrations remained significantly higher in response to a single P fertilization at the GA, SC, and AL sites. Foliar P concentrations in the fertilized plots at the SC site were 0.97%, less than the suggested critical levels of 0.11% (Fox *et al.*, 2006). Foliar P concentrations in the fertilized plots at the VA, AL, and GA sites were within 10% of the critical levels. These long-term growth responses to fertilization suggest that there has been an increase in labile P pools at each of these sites in response to fertilization at establishment. Forest managers are interested in determining if there are long-term effects of P fertilization on soil P pools

Results from this study showed no significant increases in HLP, HMLP or HRP as a result of fertilization. However there was a trend of increasing HMLP in the surface 20 cm of poorly

drained sites. In the SC and VA soil the HMLP increased by 17 and 21% respectively. The mineralization of Po is slower in poorly-drained soils due to increased soil moisture and decreased microbial activities (Wright *et al.*, 2001). Generally, soils under agronomic cultivation have reduced Hedley Po pools due to mineralization, increased Hedley Pi pools, and increased plant growth (Hedley *et al.*, 1982a; Liu *et al.*, 2004; Sui *et al.*, 1999; Zhang *et al.*, 2004). However, there can be Po mineralization and a net increase in Hedley Po pools (Beck and Sanchez, 1994).

The relative sizes of Hedley P pools were consistent within soil drainage classes. Poorly drained soils had accumulated relatively higher concentrations of HLP and HMLP compared to well drained soils. Increasing soil moisture limits oxygen and decreases microbial activities, and previous research has show that microbial P decreased with flooding (Wright *et al.*, 2001). The trend towards higher concentrations of HLP and HMLP in the poorly drained soils may be attributed to decreased microbial P mineralization.

The lack of a response of Hedley P pools to a single application of 56 kg P ha<sup>-1</sup> in the forest soils in this study may be due a combination factors. First the fertilizer P added has been taken up by plants and microbes. Results from a variety of studies put the P content of fertilized pine stands at 21-34 kg P ha<sup>-1</sup> (Albaugh *et al.*, 2008; Wells and Jorgensen, 1975; Will *et al.*, 2006). This represents approximately half of the P applied. A portion of the applied P could also have been taken up by other plants. The accumulation of P associated with the litter and humus in the O horizon is not accounted for in the Hedley fractionation. Therefore a large portion of the 56 kg P ha<sup>-1</sup> originally applied is no longer in the soil for extraction by the Hedley procedure.

Second, the Hedley fractionation does not extract total soil P. It may be that a portion of the fertilizer P applied has entered the occluded P pool and was not extracted. The original Hedley

fractionation left up to 60% of the P in the soil (Hedley et al., 1982a). The Tiessen and Moir modification followed in this study also did not remove total P in a Kanhapludult but recovery rates increased (Richter et al., 2006). Zhang *et al.* (2004) reported that in unfertilized plots with annual crop removal, Hedley Pi pools did not decrease over 10 years. Additionally, Bowman *et al.* (1998) demonstrated that 26% of total soil P was occluded and not recoverable in sequential fractionation procedures. To measure total soil P you need a very strong reducing agent to extract P from minerals and occluded pools. This may also be a contributing factor to the lack of a fertilizer response in the Hedley P pools.

Lastly, because of the variability in the results from the Hedley extractions it is difficult to detect a significant change in Hedley P concentrations. For example based upon the variance of the HLP in the 0-10 cm soil depth at the VA site, I would need to test 47 samples to establish a significant difference at  $\alpha=0.05$  between the control and fertilized plot. The sample sizes need to detect a fertilizer effect at  $\alpha=0.05$  for the total Hedley extractable P was  $n > 46$  for all four sites.

### *Mehlich-3*

Mehlich-3 extractable P may be a more sensitive test to long-term changes in soil P than the Hedley fractionation. Mehlich 3 is able to extract a different pool of P than the Hedley procedure. Mehlich 3 uses both fluoride and the organic ligand EDTA for the extraction of P from ligand exchange sites in the mineral soil. While the Hedley procedure did not detect a significant fertilizer effect the Mehlich-3 procedure did.

Mehlich-3 extractable P showed significant fertilizer treatment effects on the surface soil of the SC and GA sites. There was a trend of increasing Mehlich-3 extractable P in the surface soil of the AI ( $p=0.11$ ) and VA ( $p=0.065$ ) sites. This increase was consistent with pine stand growth

at all four sites (Table 1-2). Mehlich-3 is a widely used soil P test in the southeastern United States (Tucker, 1992) because it is rapid and well correlated with crop growth responses to P fertilization (Chilimba et al., 1999; Cox and Barnes, 2002; Indiaty et al., 1997). The results from our tests suggest that the Mehlich-3 procedure can accurately detect long-term changes in soil P pools.

The highest concentration of M-3 extractable P was in the soil surface and decreased dramatically below 20 cm to less than 2 mg P kg<sup>-1</sup> at all four sites. The lack of an increase in the Mehlich 3 extractable P in subsurface depths suggests that P did not leach through the soil profile. The results of both the Hedley and Mehlich-3 soil test demonstrated the importance of maintaining the integrity of the soil surface. Minimizing disturbances to the soil surface includes activities such as raking which could redistribute and pile surface soil at the site. The HLP and HMLP accounted for approximately half of the Hedley total extractable P in the surface depths at the SC, VA, and AL sites and are a potential source of mineralizable Po. Therefore, soil disturbances that redistribute the surface soil could result in a reduction of labile P to newly planted pine rotations.

Recent studies have shown that some portion of HMLP and HRP pools is labile (Gahoonia and others 2000, Liu and others 2004, Liu and others 2006). Results demonstrate that some Po compounds are made labile in the bulk soil by microbes and in the plant rhizosphere by symbiotic microbes like ectomycorrhiza (Cade-Menun and Lavkulich, 1997; George *et al.*, 2006; Parsons *et al.*, 2007). Therefore based on the significant increased in M-3 extractable Pi in the surface soil, and large HLP and HMLP at these sites it does not appear that soil P was reduced at these four sites.

Many sites in the southeastern United States and abroad have also show long-term P fertilization effects on tree growth (Albaugh et al., 2004; Ballard, 1978; Comerford et al., 2002a; Turner et al., 2002). One study of P fertilization 50 years earlier showed that the second rotation stand volume growth increased and soil P pools remained significantly higher than the control plots suggesting that fertilization effectively increased the site index (Turner et al., 2002).

Comerford et al. (2002) argue that a Georgia Coastal Plain loblolly pine plantation operationally fertilized with concentrated super phosphate at  $50 \text{ kg P ha}^{-1}$  29 years earlier had enough P to meet the demand of the following rotation. Comerford et al. (2002b) reported a significant increase in  $P_o$  in the L horizon and total P and  $P_o$  from the F horizon. Additionally, the previous stand was harvested; the site was windrowed, and double bedded incorporating the O horizon into the soil. Results from the fertilized treatment also included increases in labile  $P_i$  in the soil solution and increased P content from seedling bioassays grown in the operationally managed GA plantation soil (Comerford et al., 2002b). Comerford et al. (2002) estimated that a one-year old southern pine stand need upwards of  $2.5 \text{ kg P ha}^{-1}$ . This matches closely the  $2.27 \text{ kg P ha}^{-1}$  leached from the O horizon of a loblolly pine stand before harvest. Hedley labile P pools ranging from  $2\text{-}10 \text{ mg kg}^{-1}$  at these sites are similar to previous studies (Kitayama *et al.*, 2000; Lawrence and Schlesinger, 2001). Based on these results, our sites would not need P fertilization at establishment to meet the early demand of the following rotation if the litter is incorporated into the soil.

The soil surface is very dynamic, influenced by plant roots, fauna such as worms and microarthropods, bacteria, and fungi. The  $P_i$  applied to the sites over 20 years ago was rapidly incorporated into biogeochemical P pools. The cycling of P among these different pools is not linear or constant. Soil properties, soil biota, and silvicultural prescriptions can influence the

cycling of P among these different pools (Field *et al.*, 2003; Lindo and Visser, 2003). Plant roots and microbes are involved in the rapid cycling of labile P pools at the soil surface when conditions are favorable. Hedley moderately labile P concentrations were twice as large as labile P pools and nearly equal to HRP pools in the poorly drained soils of SC and VA. The mineralization of Po was slower in poorly-drained soils due to the increased soil moisture and decreased microbial activities (Wright *et al.*, 2001). Silvicultural operations, like bedding, that increase seedling survival and soil properties like bulk density and aeration may make conditions more favorable for the mineralization of HMLP pools. The increased mineralization of HMLP may also be a potential source of Pi for new planted seedlings.

Based upon bulk density values obtained from the Natural Resources Conservation Service I calculated the quantity of HLP pools at each site (Fig. 2-4). For loblolly pine in control and fertilized plots, P uptake of 4 and 6 kg P ha<sup>-1</sup> yr<sup>-1</sup> were recorded in an eight year old stand (Albaugh *et al.*, 2008). Based upon Albaugh et al. (2008) if the P demands of the stands in this study were only meet by the HLP pools the entire labile P pool would be absorbed down to 100 cm. This is an unlikely scenario. However HLP and HMLP combined would easily meet the annual demands of the stands in the surface soil depths alone.

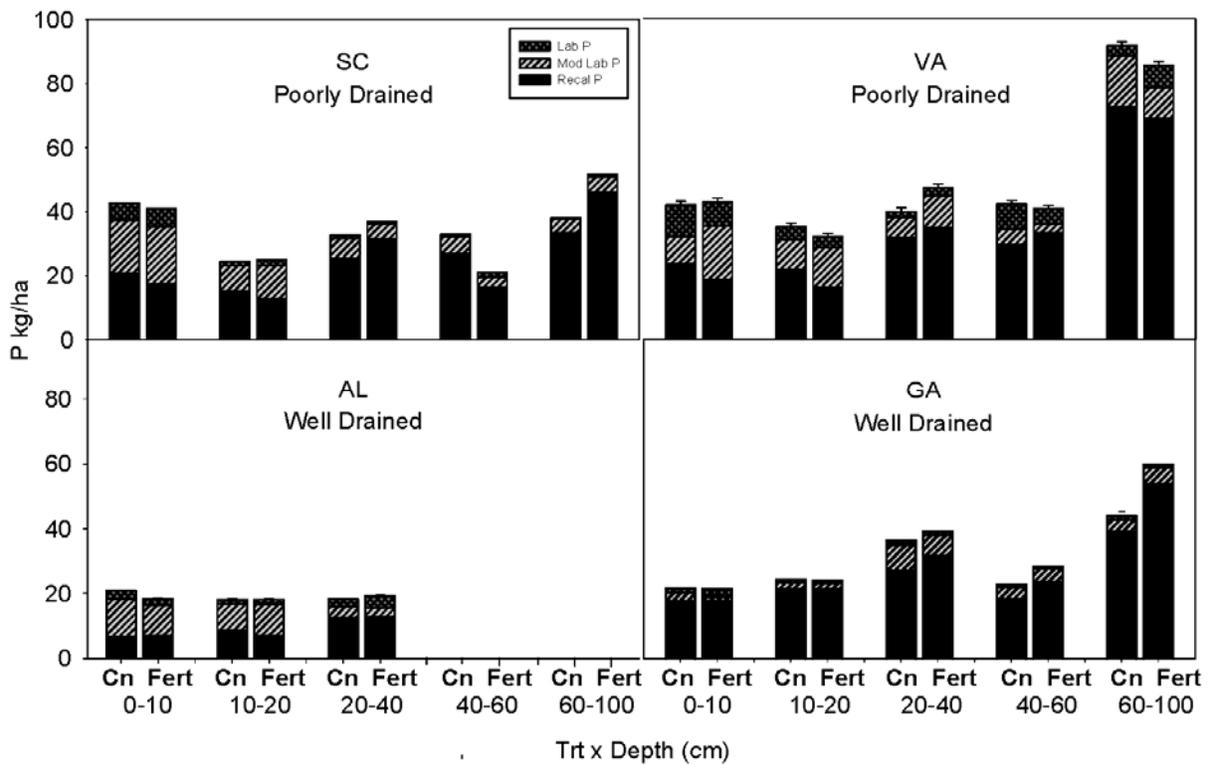


Figure 2-4 Total Hedley extractable phosphorus ( $\text{kg ha}^{-1}$ ) from Coastal Plains soils supporting loblolly pine plantations in the southeastern US. Labile P ( $\text{DiH}_2\text{O-Pi}$ ,  $\text{NaHCO}_3\text{-Pi}$ , and  $\text{NaHCO}_3\text{-Po}$ ). Moderately labile P ( $\text{NaOH-Po}$ ). Recalcitrant P ( $\text{NaOH-Pi}$ ,  $1\text{M HCl-Pi}$ , hot concentrated  $\text{HCl-Pi}$  and  $\text{-Po}$ ;  $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2\text{-Pi}$ ). Values represent least square means. Error bars represent the standard error of the least square means for Hedley total extractable P. Cn= control, Fert = fertilized, Trt = treatment.

## Conclusion

Phosphorus fertilization at  $56 \text{ kg ha}^{-1}$  of elemental P (applied as DAP) has had a long-term affect on M-3 extractable P pools at our sites in the Coastal Plain provinces of the southeastern United States. The M-3 extractable P approached the critical value 20 years after P fertilization in the VA and GA sites. Mehlich-3 soil tests have EDTA and  $\text{F}^-$  in the extracting solution. Based upon a growth response of the stand and significant increases in M-3 extractable P, our results demonstrate that the M-3 extraction is able to quantify labile P.

Results from this study also demonstrate that HLP and HMLP pools in this study are larger than the demand of newly planted pine seedling ( $0.6 - 2.5 \text{ kg P ha}^{-1}$ ). According to Comerford et al. (2002), conservation and incorporation of the litter layer into the soil surface depths will also increase P pools available to the newly planted seedlings. Results from this study suggest that land managers can delay P fertilization applications on sites previously fertilized at similar or higher rates in the Coastal Plain province of the US. Therefore, fertilizer applications are not required at stand establishment. While each manager should make decisions based upon their own unique forested site and financial circumstances, the increasing cost of fertilizers and operating cost means that delaying the application and rates of fertilization should have a net positive effect for most land owners.

## Literature Cited

- Albaugh, T.J., H.L. Allen, and T.R. Fox. 2007. Historical Patterns of Forest Fertilization in the Southeastern United States from 1969 to 2004. *Southern Journal of Applied Forestry* 31:129-137.
- Albaugh, T.J., H.L. Allen, and T.R. Fox. 2008. Nutrient use and uptake in *Pinus taeda*. *Tree Physiology* 28:1083-98.
- Albaugh, T.J., H.L. Allen, P.M. Dougherty, and K.H. Johnsen. 2004. Long term growth responses of loblolly pine to optimal nutrient and water resource availability. *Forest Ecology and Management* 192:3-19.
- Allen, B.L., and A.P. Mallarino. 2006. Relationships between extractable soil phosphorus and phosphorus saturation after long-term fertilizer or manure application. *Soil Science Society of America Journal* 70:454-463.
- Ballard, R. 1978. Effect of first rotation phosphorus applications on fertilizer requirements of second rotation pine. *New Zealand Journal of Forestry Science*. 8:135-145.
- Beck, M.A., and P.A. Sanchez. 1994. Soil phosphorus fraction dynamics during 18 years of cultivation on a typic paleudult. *Soil Science Society of America Journal* 58:1424-1431.
- Bowman, R.A., J.B. Rodriguez, and J.R. Self. 1998. Comparison of methods to estimate occluded and resistant soil phosphorus. *Soil Science Society of America Journal* 62:338-342.
- Cade-Menun, B.J., and L.M. Lavkulich. 1997. A comparison of methods to determine total, organic, and available phosphorus in forest soils. *Communications in Soil Science and Plant Analysis*. 28 (9/10):651-663.
- Chilimba, A.D.C., S.K. Mughogho, and J. Wendt. 1999. Mehlich 3 or modified Olsen for soil testing in Malawi. *Communications in Soil Science and Plant Analysis*. 30:1231-1250.
- Comerford, N.B., M. McLeod, and M. Skinner. 2002a. Phosphorus form and bioavailability in the pine rotation following fertilization, P fertilization influences P form and potential bioavailability to pine in the subsequent rotation. *Forest Ecology and Management* 169:203-211.
- Comerford, N.B., M. McLeod, and M. Skinner. 2002b. Phosphorus form and bioavailability in the pine rotation following fertilization - P fertilization influences P form and potential bioavailability to pine in the subsequent rotation. *Forest Ecology and Management* 169:203-211.
- Cooperative, F.N. 1991. Effects of site preparation, fertilization, and weed control on the growth and nutrition of old loblolly pine. Raleigh, NC. North Carolina State and Virginia Tech. .
- Cooperative, F.N. 1996. Effects of site preparation and early fertilization and weed control on 14-year old loblolly pine growth. Raleigh, NC. North Carolina State and Virginia Tech. .
- Cox, F.R., and J.S. Barnes. 2002. Peanut, corn, and cotton critical levels for phosphorus and potassium on Goldsboro soil. *Communications in Soil Science and Plant Analysis*. 33:1173-1186.
- Cross, A.F., and W.H. Schlesinger. 1995. A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64:197-214.
- Dalla-Tea, F., and E.J. Jokela. 1991. Needlefall, canopy light interception and productivity of young intensively managed slash and loblolly pine stands. *Forest Science* 37:1298-1313.
- Drummond, M.A. 2008. Souther Coastal Plain, *In T. R. L. W. Acevedo*, ed. Status and trends of eastern United States Land Cover. USGS.

- Field, J.P., K.W. Farrish, and E.A. Carter. 2003. Soil and nutrient losses following site preparation burning in a harvested loblolly pine site. *Transactions of the ASAE* 46:1697-1703.
- Fife, D.N., and E.K.S. Nambiar. 1999. Response to phosphorus application of second rotation radiata pine on podsolised sands from planting to first thinning: implications for management. *Australian Forestry*. 62:109-119.
- Fox, T.R., E.J. Jokela, and H.L. Allen. 2007. The development of pine plantation silviculture in the southern United States. *Journal of Forestry* 105:337-347.
- Fox, T.R., H.L. Allen, T.J. Albaugh, R. Rubilar, and C.A. Carlson. 2006. Forest Fertilization in Southern Pine Plantations. *Better Crops* 90:12-15.
- George, T.S., B.L. Turner, P.J. Gregory, B.J. Cade-Menun, and A.E. Richardson. 2006. Depletion of organic phosphorus from Oxisols in relation to phosphatase activities in the rhizosphere. *European Journal of Soil Science* 57:47-57.
- Hedley, M.J., J.W.B. Stewart, and B.S. Chauhan. 1982a. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Journal - Soil Science Society of America Journal* 46:970-976.
- Hedley, M.J., J. Stewart, and B.S. Chauhan. 1982b. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Science Society of America Journal* 46:970-976.
- Indiati, R., C. Izza, A. Figliolia, B. Felici, E. Coppola, C. Buondonno, and A. Buondonno. 1997. Comparing test for soil fertility. III. Evaluation of phosphate availability in Alfisols by Olsen, Mehlich 3, electro-ultrafiltration, and the paper-strip methodology procedures. *Communications in Soil Science and Plant Analysis*. 28:997-1009.
- Johnson, A.H., J. Frizano, and D.R. Vann. 2003. Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. *Oecologia* 135:487-499.
- Kitayama, K., N. Majalap-Lee, and S. Aiba. 2000. Soil phosphorus fractionation and phosphorus-use efficiencies of tropical rainforests along altitudinal gradients of Mount Kinabalu, Borneo. *Oecologia*. 123 (3):342-349.
- Lawrence, D., and W.H. Schlesinger. 2001. Changes in soil phosphorus during 200 years of shifting cultivation in Indonesia. *Ecology* 82:2769-2780.
- Lindo, Z., and S. Visser. 2003. Microbial biomass, nitrogen and phosphorus mineralization, and mesofauna in boreal conifer and deciduous forest floors following partial and clear-cut harvesting. *Canadian Journal of Forest Research* 33:1610-1619.
- Liu, Q., P. Loganathan, M.J. Hedley, and M.F. Skinner. 2004. The mobilisation and fate of soil and rock phosphate in the rhizosphere of ectomycorrhizal *Pinus radiata* seedlings in an Allophanic soil. *Plant and Soil* 264:219-229.
- Magid, J., H. Tiessen, and L.M. Condron. 1996. Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. , p. 429-466, *In* G. Piccolo, ed. *Humic substances in terrestrial ecosystems*. Elsevier, Amsterdam.
- Murphy, J.F., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27:31-36.
- Parsons, K.J., V.D. Zheljzakov, J. MacLeod, and C.D. Caldwell. 2007. Soil and Tissue Phosphorus, Potassium, Calcium, and Sulfur as Affected by Dairy Manure Application in a No-Till Corn, Wheat, and Soybean Rotation. *Agronomy Journal* 99:1306-1316.

- Piatek, K.B., and H.L. Allen. 2001. Are forest floors in mid-rotation stands of loblolly pine (*Pinus taeda*) a sink for nitrogen and phosphorus? *Canadian Journal of Forest Research* 31:1164-1174.
- Polglase, P.J., N.B. Comerford, and E.J. Jokela. 1992a. Mineralization of nitrogen and phosphorus from soil organic matter in southern pine plantations. *Soil Science Society of America Journal* 56:921-927.
- Polglase, P.J., E.J. Jokela, and N.B. Comerford. 1992b. Nitrogen and phosphorus release from decomposing needles of southern pine plantations. *Soil Science Society of America Journal* 56:914-920.
- Polglase, P.J., E.J. Jokela, and N.B. Comerford. 1992c. Phosphorus, nitrogen, and carbon fractions in litter and soil of southern pine plantations. *Soil Science Society of America Journal* 56:566-573.
- Pritchett, W.L., Llewellyn, W.R., Swinford, K.R. 1961. Response of slash pine to colloidal phosphate fertilization. *Soil Science Society of America Journal* 25:397-400.
- Richter, D.D., H.L. Allen, J. Li, D. Markewitz, and J. Raikes. 2006. Bioavailability of slowly cycling soil phosphorus: major restructuring of soil P fractions over four decades in an aggrading forest. *Oecologia* 150:259-271.
- Sims, J.T. 2000. Soil test phosphorus: Mehlich 3. In G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University. p. 17-19.
- Smeck, N.E. 1985. Phosphorus dynamics in soils and landscapes. *Geoderma* 36:185-199.
- Sommers, L.E., and D.W. Nelson. 1972. Determination of total phosphorus in soils: a rapid perchloric acid digestion procedure. *Proceeding of Soil Science Society of America* 3:902-904.
- Sui, Y., M.L. Thompson, and C. Shang. 1999. Fractionation of phosphorus in a Mollisol amended with biosolids. *Soil Science Society of America Journal* 63:1174-1180.
- Tiessen, H., and J.O. Moir. 1993. Characterization of available P by sequential extraction. In M. R. Carter, ed. *Soil Sampling and Methods of Analysis*. Lewis Publishers. p. 75-86.
- Tiessen, H., J.W.B. Stewart, and C.V. Cole. 1984. Pathways of phosphorus transformations in soils of differing pedogenesis. *Soil Science Society of America Journal*, vol 48:853-858.
- Tucker, M.R. 1992. Determination of phosphorus by Mehlich 3 extractant. In S. J. Donohue, ed. *Reference soil and media diagnostic procedure for the southern region of the United States*. Va. Agric. Exp. Station, Blacksburg, VA. p. 9-12.
- Turner, B.L., N. Mahieu, L.M. Condron, and C.R. Chen. 2005. Quantification and bioavailability of scyllo-inositol hexakisphosphate in pasture soils. *Soil Biology and Biochemistry* 37:2155-2158.
- Turner, J., and M.J. Lambert. 1988. Long-term effects of phosphorus fertilization on forests. In B. B. and W. C. H., eds. *Forest site evaluation and long-term productivity*. University of Washington Press, Seattle, WA. p. 125-133.
- Turner, J., M.J. Lambert, and F.R. Humphreys. 2002. Continuing growth response to phosphate fertilizers by a *Pinus radiata* plantation over fifty years. *Forest Science*. 48:556-568.
- Walker, L.C. 1960. Fertilizing southern pine: A roundup of observations and concepts. In P. D. Burns, ed. *Southern Forest Soils*. Louisiana State Univ., Baton Rouge. p. 86-95.
- Walker, T.W., and J.K. Syers. 1976. The fate of phosphorus during pedogenesis. *Geoderma* 15:1-19.

- Wells, C.G., and J.R. Jorgensen, (eds.) 1975. Nutrient cycling in loblolly pine plantations, pp. 1-137-158. Les Presses de L'Université, Laval, Quebec.
- Wells, C.G., D.M. Crutchfield, N.M. Berenyyi, and C.B. Davey. 1973. Soil and foliar guidelines for phosphorus fertilization of loblolly pine. USDA For Serv Res SE US Dep Agric For Serv Southeast For Exp Stn 110.
- Wells, C.G., J.R. Craig, M.B. Kane, and H.L. Allen. 1986. Foliar and soil tests for the prediction of phosphorus response in loblolly pine. *Soil Science Society of America Journal* 50:1330-1335.
- Will, R.E., D. Markewitz, R.L. Hendrick, D.F. Meason, T.R. Crocker, and B.E. Borders. 2006. Nitrogen and phosphorus dynamics for 13-year-old loblolly pine stands receiving complete competition control and annual N fertilizer. *Forest Ecology and Management* 227:155-168.
- Wright, R.B., B.G. Lockaby, and M.R. Walbridge. 2001. Phosphorus availability in an artificially flooded southeastern floodplain forest soil. *Soil Science Society of America Journal* 65:1293-1302.
- Zhang, T.Q., A.F. MacKenzie, B.C. Liang, and C.F. Drury. 2004. Soil Test Phosphorus and Phosphorus Fractions with Long-Term Phosphorus Addition and Depletion. *Soil Science Society of America Journal* 68:519-528.

# CHAPTER 3: LONG-TERM EFFECTS OF FERTILIZATION ON ORGANIC PHOSPHORUS POOLS IN A LOBLOLLY PINE (*Pinus taeda*) PLANTATION DETERMINED BY $^{31}\text{P}$ NMR SPECTROSCOPY

## Abstract

Phosphorus (P) is one of the most limiting nutrients in forest soils. Phosphorus fertilization increases labile P after fertilization; however, it is uncertain how inorganic P fertilization influences soil organic P pools over time in forest soils. The goal of this project was to determine how fertilization affects organic P pools 28 years after fertilization in the southeastern US. Surface soil samples (0-5 cm) were collected in a *Pinus taeda* plantation established in poorly drained acid sandy soil. I used solution  $^{31}\text{P}$  nuclear magnetic resonance (NMR) spectroscopy to characterize NaOH-EDTA extractable P. Total NaOH-EDTA extractable P ranged from  $0.1 \text{ mg L}^{-1}$  in the control plots to  $5.1 \text{ mg L}^{-1}$  in fertilized plots. The extracts in both treatments were dominated by inorganic orthophosphate. Orthophosphate monoesters were the dominant organic P species present in both treatments. Control plots had very low quantities of monoester P in comparison to fertilized plots. Results suggest that there has a moderate increase in organic P pools in the fertilized treatments. Fertilization significantly increased the P content of the litter layer by 118%. The increase in the P content of the litter layer may be limiting the accumulation of organic P in the mineral soil.

## Introduction

Plant growth is typically limited by N and P availability in the soil. While the total quantities of these nutrients in forest soils may be large and appear sufficient to support robust plant growth, the pools of plant available or labile P are markedly smaller and typically growth limiting. Yet, a single application of  $56 \text{ kg P ha}^{-1}$  can substantially increase the growth of pine

stands in poor quality soils (Allen et al., 1990; Kyle et al., 2005; Pritchett and Comerford, 1982). Phosphorus fertilization of *Pinus radiata* in New Zealand has also increased labile P concentrations into the second rotation (Ballard, 1978; Gentle et al., 1986). Whereas inorganic N can be volatilized and rapidly lost after fertilization, the fate of inorganic P ( $P_i$ ) and organic P ( $P_o$ ) is typically regulated by plants, soil microbes, and the P fixation capacity of the soil (Turner and Lambert, 1988; Yuan and Lavkulich, 1994). The loss of P from soils predominately occurs with removal of crops although P losses through erosion, subsurface, and surface runoff is possible in disturbed ecosystems (Pierzynski *et al.*, 2005).

Over time,  $P_o$  pools come to represent a larger portion of the total P in the mineral soil (Tiessen et al., 1984; Walker and Syers, 1976). This increase in  $P_o$  suggests that biological cycling becomes more important as the stand ages: a hypothesis that was suggested by Wells and Jorgensen (1975). There are a variety of chemical fractionation procedures used to partition P into different chemical pools (Chang and Jackson, 1957; Hedley *et al.*, 1982). Over the long term, P cycling is controlled by geochemical cycling, however biological effects on P pools control P availability in the short term (Cross and Schlesinger, 1995). Sequential fractionation procedures are not able to reliably quantify labile  $P_o$  pools (Tiessen and Moir, 1993).

While naturally occurring P in the soil originates from apatite minerals, they can be slowly converted from  $P_i$  forms to  $P_o$ . Organic P can represent 20-90% of the total P present in the mineral soil (Condrón et al., 2005). Understanding what  $P_o$  species are present and how soil biota influence  $P_o$  pools may be important for predicting the quantity of labile P pools. The strength of the P bonds and changes in P species over time leads to information about P cycling. The use of nuclear magnetic resonance spectroscopy (NMR) can identify different P species and

quantify the extractable pool of Po (Newman and Tate, 1980; Turner and Richardson, 2004; Turner et al., 2003b).

Data collected from  $^{31}\text{P}$  NMR spectroscopy can identify and quantify orthophosphate Pi, orthophosphate monoester and diester Po, phosphonates, polyphosphates, and pyrophosphates compounds in the soil which are of varying degrees of plant availability (Condrón et al., 1997).

Orthophosphate esters are subclassified as monoesters with one C moiety per P, and diester orthophosphates with two C moieties per P. Orthophosphate esters are stable in the natural range of most natural soils. They are hydrolyzed in the presences of phosphatases enzymes (Alvarez et al., 2004; Fox and Comerford, 1992; He et al., 2004).

The most abundant monoester in soils are inositol phosphates and occur in various stereoisomeric forms, *myo*, *scyllo*, *d-chiro*, and *neo* of differing biotic availability (Turner et al., 2002a; Turner et al., 2005b). The monoesters also include sugar phosphates, phosphoproteins, and mononucleotides (Condrón et al., 2005). The monoester phosphates bond very tightly to soil colloids and become very resistant to decomposition.

The diester phosphates, including nucleic acids, phospholipids, teichoic acid (unique to forests soils), and aromatic compounds are found in much large quantities in acid forest soils and are generally considered more labile (Cade-Menun *et al.*, 2000). The greater availability of diester phosphates are attributed to breaking one of the two R-O-P bonds, greater solubility, and therefore greater movement into the soil solution (Condrón et al., 2005; Magid et al., 1996).

Phosphonates are organic orthophosphate compounds with C-P bonds and were first reported in natural soils by Newman and Tate (1980). The C-P bond is more resistant to hydrolysis and oxidation than C-O-P bonds making their functions as a P storage compound in biotic systems less likely (Quin and Quin, 2001). The phosphonates accumulate in soils that are wet (Tate and

Newman, 1982), cold, and acidic (Cade-Menun et al., 2000; Dai et al., 1996; Gil-Sotres et al., 1990).

The soil biota (bacteria, fungi, nematodes and protozoa) can account for 5-100 mg P kg<sup>-1</sup> soil, 5-15% of the total P, and are generally highest in fertilized agricultural soils (Jakobsen et al., 2005). Mycorrhizal fungi absorb P and produce polyphosphate granules. These granules help regulate the osmotic potential during movements of solutes through hyphal stands (Bücking and Heyser, 1999; Rasmussen et al., 2000). The role of soil biota in the transformation of litter to a variety of Po compounds is evident when enzymatic degradation is retarded under adverse environmental conditions (Condrón et al., 1990; Tate and Newman, 1982).

Recent studies have shown how the soil biota can influence Po pools (Condrón et al., 1996; Solomon et al., 2002; Turner et al., 2005). Inositol phosphates are monoester P compounds that can be strongly adsorbed to soil colloids and has been considered recalcitrant (Brady and Weil, 2002). However, some species of phytate such as scyllo-inositol hexakisphosphate and diester phosphates contribute to labile P pools in forest soils (Cade-Menun et al., 2000; Condrón et al., 2005; Condrón et al., 1996; Turner et al., 2005). The role of monoester phosphates in rapidly cycling Po pools has been questioned in light of recent pot studies with radiata pine in which myo- inositol hexakisphosphates were degraded but not in pots with ryegrass (Chen *et al.*, 2004). Determining how P fertilization may affect Po pools in the mineral soil may help land managers make better predictions on the long-term effects of P fertilization on site quality.

Results from chapter two indicated a trend of increasing Po pools associated with the Headley moderately labile P (HMLP) pools in the poorly drained soils of VA. The trend was most pronounced in the 0-10 cm soil depth (Fig. 2-2). Poorly drained soils with high soil moisture levels reduce oxygen concentrations and decrease microbial activity (Wright *et al.*, 2001). The

trend towards higher concentrations of Po in the HMLP in the poorly drained soils may be attributed to decreased microbial Po mineralization. Based upon the trend of increasing HMLP concentration in the VA surface soil, I tested for a long-term fertilization effects on Po compounds using liquid state  $^{31}\text{P}$  NMR spectroscopy.

The objective of this study is to determine if there is a long-term effect of P fertilization on Po pools in a loblolly pine plantation. The use of  $^{31}\text{P}$  NMR spectroscopy will yield a more detailed understanding of soil Po pools in this VA pine plantation. I hypothesize that the NaOH/EDTA extractable P will be larger in the fertilized plots compared to the control. Additionally, I hypothesize that the fertilized plots will have higher diester and monoester phosphates compared to the control plots.

## **Materials and Methods**

Soils samples were collected from a long-term study (27 yrs. old) that examined silviculture treatment effects on loblolly pine (*Pinus taeda*) plantations. The site (RW 072602) was located in the Coastal Plain physiographic province of Virginia (Table 3-1). This study was installed in March 1980. Fertilizer treatments were zero (control) or 56 kg P ha<sup>-1</sup> (fertilized) applied as diammonium phosphate centered on the planted row 2 months after seedling establishment. Treatment plots were 160 x 40 m (0.64 hectares), with 16 rows of 16 trees planted at 2.4 x 2.4 m spacing. The internal eight rows × eight trees served as the measurements plots.

The forest floor and 10 cm of the surface soil were collected from each plot. The sample locations were randomly selected within each plot. The L (Oi) and F (Oe) horizons were collected from three locations at each plot. The litter layer was collected within a 930 cm<sup>2</sup> area.

There was little to no H (Oa) layer and therefore where present it was sampled with the F horizon.

Eighteen soil cores (110 cm<sup>3</sup>) were randomly collected at 0-5 cm and 5-10 cm deep using a bulk density hammer. The cores were immediately placed in plastic freezer bags and stored over a bed of ice in a cooler. Samples returned to the lab were kept in a 4°C cold room until processing. Previous research has shown that soil drying and rewetting has resulted in the release of both Pi and Po extractable pools presumably from the lysing of microbial cells, degradation of organic ligands, and physical disruption of Po coating soil particles (Sparling et al., 1985; Turner, 2005; Turner and Haygarth, 2003).

Three composite soil samples were made per treatment by randomly combining six cores per sample. The fine earth fraction of the soil samples were sieved (< 2mm) in the cold room and stored until P extraction for <sup>31</sup>P NMR analyses.

The extraction and <sup>31</sup>P NMR analysis followed the procedures of previous researchers (Cade-Menun and Preston, 1996; Turner et al., 2003a). Triplicate five g samples of field-moist soil were extracted with 100 ml of 0.25 M NaOH and 0.05M EDTA on a reciprocating shaker. Extractions at room temperature resulted in sample solutions exceeding 40°C due to heat from the shaker. Subsequent <sup>31</sup>P NMR analysis revealed samples with little to degraded Po species.

Table 3-1 Study site description and experimental treatments.

Site	Soil Series	Soil Taxonomy	Surface Texture	Drainage	Treatment	Elemental P* kg ha <sup>-1</sup>
VA Coastal Plains	Grantham	Fine-silty, siliceous, semiactive, thermic Typic Paleaquults	Very Fine Sandy Loam	Poorly Drained	Control	0
					Fertilized	56

\*Phosphorus was applied as diammonium phosphate.

Therefore, samples were extracted in the cold room at 4°C. Solution temperatures were approximately 27°C. After a 16 h extraction, the samples were centrifuged at 10,000 g for 30 min. Aliquots were taken for determination of total P by ICP. Equal volumes of the supernatants from the triplicate subsamples were combined and immediately frozen (-80°C). After at least 24 hrs. in the freezer, the samples were lyophilized.

The freeze dried NaOH-EDTA extract were dissolved in 0.9 ml of 1M NaOH with 0.1 M EDTA (pH > 12) and 0.1 ml D<sub>2</sub>O (deuterium oxide for signal lock) and transferred to a 5-mm diameter NMR tubes. The spectra were obtained at 500 MHz on a Jeol 500 MHz NMR (JEOL USA, Inc., 11 Dearborn Rd., Peabody, MA 01960 USA) operating at 202.468 MHz with a 5 mm broadband probe. The spectral window was centered at 0 and set 50 ppm because most P compounds in environment samples fall within -25 and 25 ppm (Cade-Menun, 2005). The pulse angle was set to 90° with a 0.80 s acquisition time, delay time of 4.4 sec, and the samples were spun at 10 Hz (Cade-Menun, 2005; Turner et al., 2003c). Samples were subjected to approximately 10,000 scans in order to increase the signal to noise ration. The assignment of P species based upon the chemical shifts reported by Turner *et al.* (2003a).

Mehlich-3 extractable P (M-3) results are reported from previous research (Chapter 2). Soil samples were collected within the plots in 2001 using a 7.2 cm inside diameter auger. Soils were randomly sampled at five depths (0-10, 10-20, 20-40, 40-60, and 60-100 cm). The samples were air dried, sieved (< 2 mm), and stored in plastic bags. Individual soil samples within each plot were composited by depth and passed through a 2 mm sieve several times. Then composited samples were oven dried and weighed for moisture corrections.

Mehlich-3 extractions followed the procedures of Sims (2000). Draco (P free) activated charcoal was added to the soil to obtain a color-less solutions. The solutions were then filtered

(2.5  $\mu\text{m}$ ), and P was measured on a spectrophotometer at 880 nm using the molybdate-ascorbic acid procedure (Murphy and Riley, 1962).

## **Results**

Fertilization had a significant effect ( $p < 0.001$ ) on the P concentrations in the forest floor (Fig. 3-1). The effect of forest floor layer (Oi and Oe) on the P concentrations of the two layers of the forest floor was significant ( $p = 0.052$ ). In the fertilized plots the P concentration in the litter and fragmented layers of the forest floor increased by 32 and 55%, respectively.

Fertilization also had a significant effect ( $p = 0.035$ ) on the P content in the forest floor. The average P content in the control plot was 3.9  $\text{kg ha}^{-1}$  compared to 8.5  $\text{kg ha}^{-1}$  in the fertilized plots. This is a 118% increase in the P content of the forest floor and accounts for 8% of the P fertilizer applied in 1980.

Mehlich 3 extractable  $\text{P}_i$  decreased significantly with depth ( $p < 0.001$ ). Fertilization had a significant ( $p = 0.065$ ) effect on Mehlich 3 extractable P in the 0-10 cm soil depth only. The M-3 extractable  $\text{P}_i$  increased by 38% in the 0-10 cm soil depth of the fertilized plots (Fig. 3-2). The M-3 extractable P concentration decreased by more than 50% moving from the 0-10, 10-20, and 20-40 cm depth. Due to the large increase in Mehlich 3 extractable P in the 0-10 cm soil depth and a large decrease with depth, further soil analyses focused on the surface soil depth.

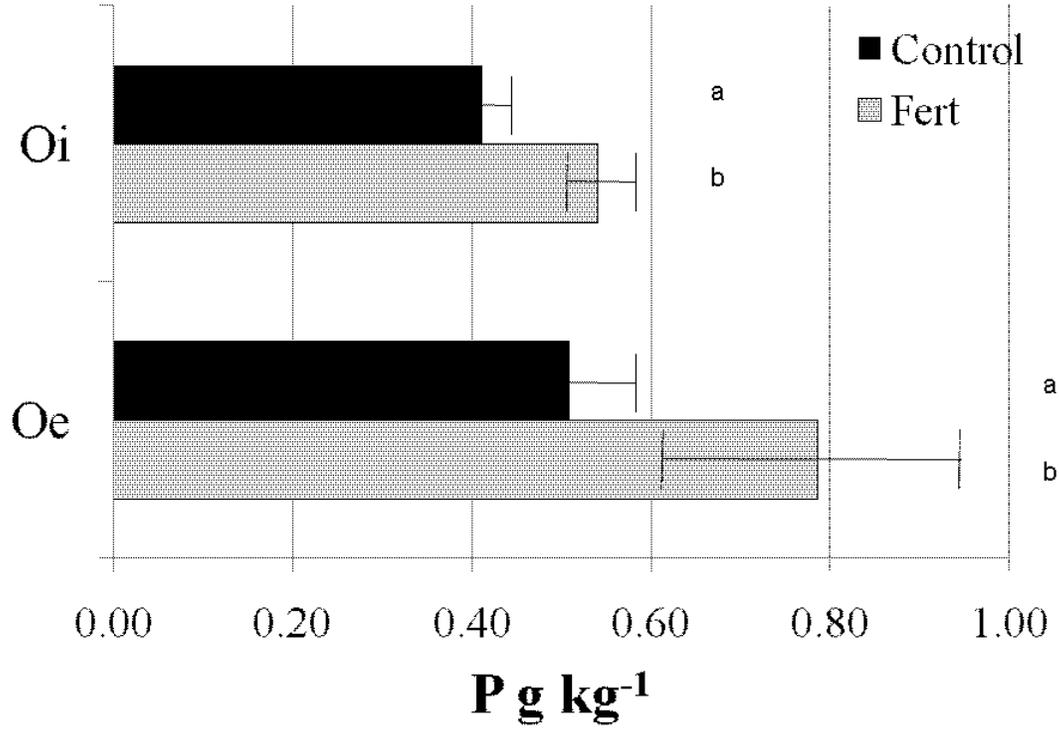


Figure 3-1 Long-term response of the litter (Oi) and fragmented (Oe) horizons of the litter layer to P fertilization (56 kg ha<sup>-1</sup>) 27 years after application. Values are means (n=3) and error bars represent standard deviation. Bars with different letters represent significant effects of fertilization at the  $p < 0.05$ .

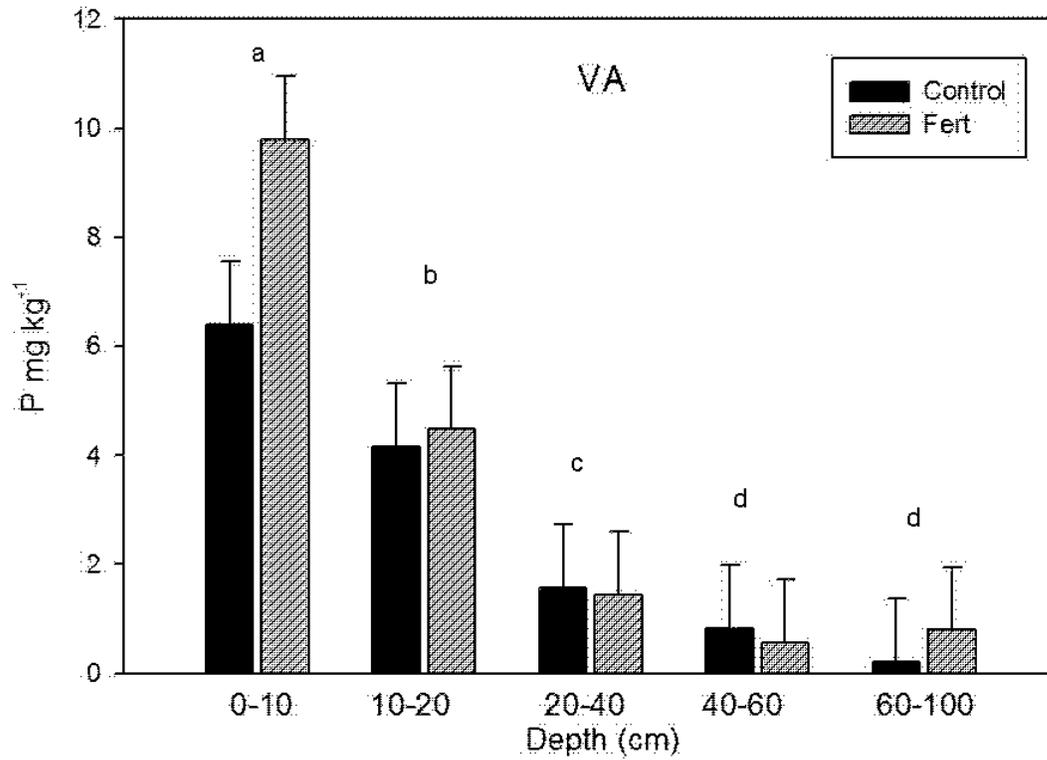


Figure 3-2 Mehlich 3 extractable phosphorus ( $\text{mg kg}^{-1}$ ) determined colorimetrically from Grantham soils series supporting loblolly pine in the southeastern US. Values represent least square means. Error bars represent the standard error of the least square means. Bars with different letters represent significant effects of depth at the  $p < 0.05$ .

The total NaOH/EDTA extractable P from the soil was significantly higher ( $p = 0.0468$ ) in the fertilized plots 27 years after fertilization (Table 3-2). The total P concentration in the 0-5 cm soil depth of the fertilizer plots was 21% larger than the control plots (Fig. 3-3). In both the 0-5 and 5-10 cm soil depths the total P concentration in the fertilized plots was greater than the control plots. The P concentration increased 49% in response to fertilization within the 5-10 cm soil depth.

Soil depth also had a significant ( $p = 0.005$ ) effect on total NaOH/EDTA extractable P. The mean P concentration in the 0-5 cm soil depth was  $3.99 \text{ mg L}^{-1}$  compared to  $3.25 \text{ mg L}^{-1}$  5-10 cm deep, a 22% decrease with depth. In the control plots, there was a 28% decrease in total P concentration moving from the surface soil to 5-10 cm soil depth (Fig. 3-3). However, there was only an 11% difference in P concentrations in the 0-5 and 5-10 cm soil depths in the fertilized plots.

The P content in the fertilized treatment increased by  $29 \text{ kg ha}^{-1}$ . The significant ( $p = 0.047$ ) increase in NaOH/EDTA extractable P pools in response to fertilization resulted in a 32% increase in soil P content. The largest increase was  $18 \text{ kg P ha}^{-1}$  in the 5-10 cm soil depth of the fertilizer plots (Table 3-2).

Table 3-2 Long-term effects of P fertilization on total extractable phosphorus (0.25 M NaOH + 0.05 M EDTA) determined by ICP-AES from Grantham soil series in the Coastal Plains of Virginia. Values are means (n=18) with standard deviation in parenthesis. Values with different letters represent significant effect of fertilization at the  $p < 0.05$ .

Soil Depth (cm)	Treatment	P mg/L	P kg/ha
0-5	Control	3.61 (0.7)a	52 (10)a
	Fertilized	4.34 (1.3)b	63 (20)b
5-10	Control	2.61 (1.06)a	38 (15)a
	Fertilized	3.89 (2.69)b	56 (39)b

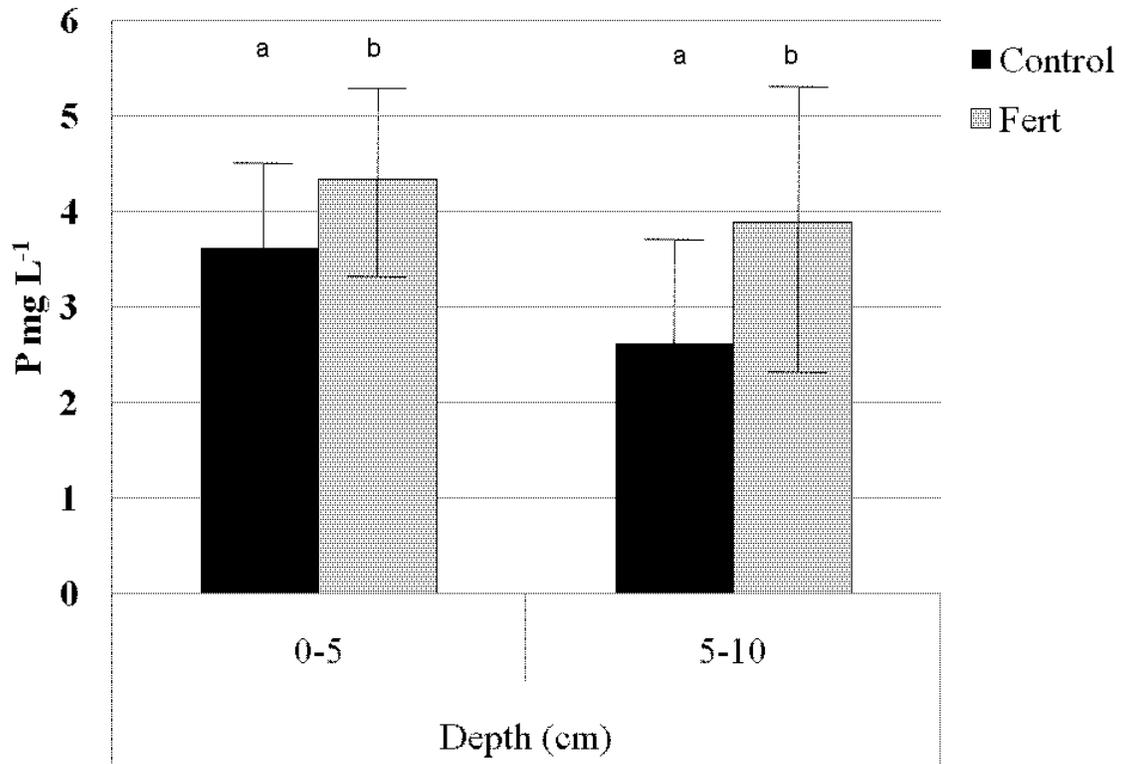


Figure 3-3 Long-term fertilization affects on total extractable P (0.25M NaOH + 0.05M EDTA) from the surface 10 cm of a Grantham soil series. Values are means (n=18) and error bars represent standard deviation. Bars with different letters represent significant effects of fertilization at the  $p < 0.05$ .

### *NMR Spectra*

Solution state  $^{31}\text{P}$ -NMR spectra from the control and fertilized treatment plots revealed that the peak corresponding to the orthophosphate anion had the highest intensity (Fig. 3-4). Orthophosphate in the soil solution is typically below  $0.1 \text{ mg L}^{-1}$  because it rapidly sorbs to mineral surfaces. The large signal of orthophosphate in the soil extraction represents inorganic P released from mineral surfaces and any Po species mineralized during the extraction procedure.

Monoester Po compounds represented the only pool of Po species detected in the extraction of the control and fertilized plots (Fig. 3-4). The chemical shift of monoester P species is typically found from 3-6 ppm (Turner et al., 2003a). The chemical shift of monoester P compounds in the control and fertilizer samples ranged from 4.2 to 5.2 ppm (Table 3-3).

The highest concentration of Po was found in the fertilizer treatments. Monoester P pools in the fertilizer treatment accounted for 55% of the NaOH/EDTA extractable P (Table 3-4). The chemical shift of monoester P in the fertilizer treatments were predominately occurring at 4.7 and 4.2 ppm. Addition Po species were near 5.1 and 5.3 ppm. No other Po compounds were detected in the  $^{31}\text{P}$  NMR spectra (Fig. 3-2).

The average concentration of monoester P pools in the control plots however, only representing 41% of the NaOH/EDTA extractable P (Table 3-4). The Po peaks identified in the  $^{31}\text{P}$  NMR spectra of the control plots had chemical shift ranging from 4.32 to 4.73 ppm. The Po compounds in the control plots accounted for less of the NaOH/EDTA extractable P. There were fewer Po peaks identified in the control plots.

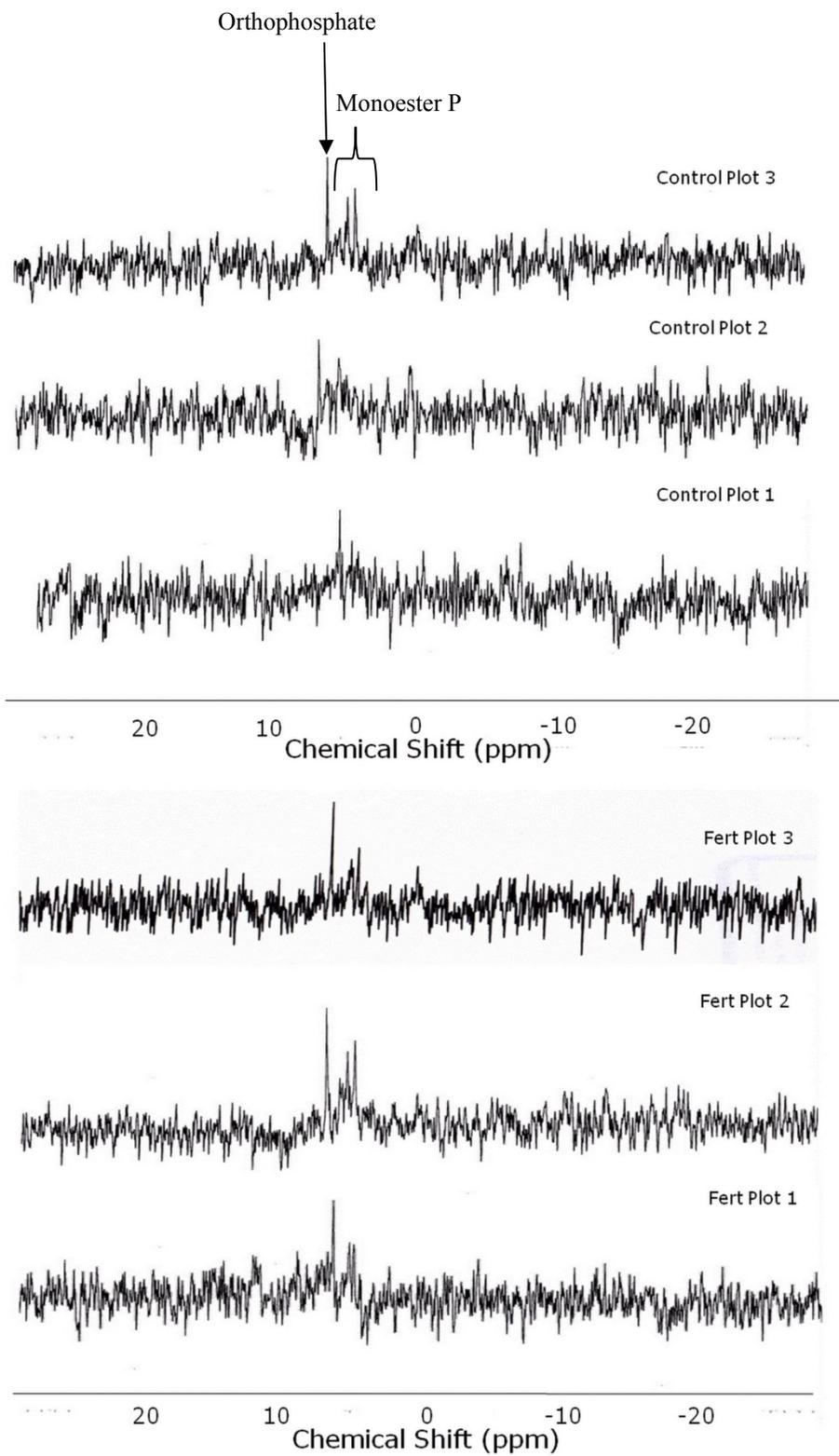


Figure 3-4 Solution  $^{31}\text{P}$  NMR spectra from 0.25M NaOH & 0.05M EDTA extracts from fertilized and control plots.

Table 3-3 Chemical shift and proportion of P compounds determined by NaOH/EDTA extraction and solution  $^{31}\text{P}$  NMR spectroscopy. Phosphorus was extracted from a loblolly pine fertilization field trail installed on an eastern Virginia Grantham soil series.

Treatment	Plot	Chemical Shift ppm		Proportion of Total P (%)	P mg L <sup>-1</sup>
		Orthophosphate	Monoesters		
Control	1	6.1575		100	2.4
	2	6.1636		63	2.8
			4.7389	37	1.7
	3	6.1575		48	2.3
			4.7236	15	0.7
			4.2253	37	1.8
Fertilized	1	6.237		39	1.6
			5.05	25	1
			4.75	36	1.5
	2	6.1881		45	2.5
			5.2556	8	0.4
			4.7144	15	0.8
		4.1947	32	1.8	
3	6.1697		54	2.4	
		4.7206	6	0.3	
		4.2192	40	1.7	

Table 3-4 Phosphorus pools determined by  $^{31}\text{P}$  NMR spectroscopy. Values are the mean (n=3) and S.E.M. Values with different letters represent significant effects of fertilization at the  $p < 0.05$ .

	Ortho P %	Monoester P %	P mg/L		Total P
			Ortho P	Monoester P	
Control	59	41	2.5 (0.3)a	1.4 (1.2)a	3.9 (1.3)a
Fertilized	45	55	2.1 (0.5)a	2.5 (0.5)a	4.6 (0.7)b

## Discussion

Phosphorus fertilization at  $56 \text{ kg ha}^{-1}$  in 1980 has had a long-term effect on P pools at this site. Confirming my hypothesis that P pools in the litter layer and NaOH/EDTA extractable P increased in response to P fertilization. The accumulation of P in the litter layer in pine forest over time is also consistent with previous research (Comerford et al., 2002; Harding and Jokela, 1994; Piatek and Allen, 2001; Saggar et al., 1998; Staaf and Berg, 1982). Turner et al. (2002) reported increased Radiata pine growth and P pools in the soil and litter layer in responses to P fertilization treatments over the course of 50 years and two plantation rotations. The P taken up by the growing stand increases leaf area and is returned to the soil, in part, via P enriched litter fall to the forest floor (Albaugh et al., 2008; Polglase et al., 1992b). The higher P content in the litter layer of the fertilized plots from this site may reflect the increased P uptake by the crop trees followed by mineralization and uptake of P from the enriched litter layer by plants and soil microbes (Bending and Read, 1995; Colpaert and vanTichelen, 1996; Conn and Dighton, 2000).

Previous research extracting P from the litter layer using trichloroacetic acid has shown that the increase in the P content of the forest floor with fertilization affected both  $P_i$  and  $P_o$  fractions (Comerford et al., 2002; Polglase et al., 1992a). The use of NMR spectroscopy has also shown that the forest floor has a greater variety and higher concentration of  $P_o$  compounds in comparison to the mineral soil (Cade-Menun, 2005). While total NaOH/EDTA extractable P increased the soil P pools, NMR results from this study detected only minor changes in soil  $P_o$  pools.

Based upon the NMR analysis, there was no significant increase in  $P_o$  compounds in the fertilized plots. The concentration of orthophosphate generally increases in response to fertilization and decreases in the variety of  $P_o$  compounds (Cade-Menun, 2005; Hawkes et al.,

1984). Diester P compounds are degraded more rapidly than monoester P in cultivated soils (Condron *et al.*, 1990). While monoester P compounds typically increase in the mineral soil over time and with cultivation because they chemisorb to mineral surfaces more readily than other Po compounds (Condron *et al.*, 2005; Condron *et al.*, 1990; Zhang *et al.*, 1999). There was a little if any changes in monoester P pools in the fertilized plots in this study.

The lack of a significant increase in Po compounds may be attributed to a combination of factors including increased mineralization of Po compounds in the soil. Many studies show increased monoester P mineralization in soils colonized with pine roots and ectomycorrhiza (Chen *et al.*, 2000; Chen *et al.*, 2004; Colpaert *et al.*, 1997; Phillips and Fahey, 2006). The lack of a significant increase in Po in the mineral soil may have been prevented by an increase in the mineralization of monoester P compounds. Additionally acidic soils (pH < 5.5) are not favorable to earthworms that would increase pedoturbation (Brady and Weil 2002). Limited incorporation of the P enriched litter layer into the surface soil in the fertilized sites could preclude the rapid increase in Po compounds. The forest floor has a greater variety and higher concentration Po compounds (Cade-Menun, 2005). While there is limited evidence that soil fauna contribute directly to the leaching of C and Pi to the soil (Bardgett and Chan, 1999; Chamberlain *et al.*, 2006) no research has shown that Po from the litter layer is rapidly incorporated into the mineral. In fact studies several studies suggest that little if any P in the litter layer is leached into the mineral horizon (Gressel *et al.*, 1996; Piatek and Allen, 2001).

The increase in the forest floor P content may potentially contribute to the long-term effects of P fertilization on subsequent forest growth and soil P pools. Comerford *et al.* (2002) grew loblolly pine seedlings in soils collected from a long-term P fertilizer study in GA. Their results showed an increase in the P concentration of the seedlings and it was suggested that the

incorporation of the litter layer into the mineral soil was responsible for P increase. In the same study, Comerford et al. (2002) predicted that the newly planted pine stand would have enough residual P from the previous fertilization to meet the seedling demand early in the rotation.

In 1980, diammonium phosphate (DAP) fertilizer was broadcast across the treatment plots and P<sub>i</sub> would be taken up by soil microbes and plants. In the acid soils of the southeastern US the majority of DAP not taken up by the biota could form covalent chemical bonds with mineral soils predominately Al and Fe. Only a very limited amount of the 56 kg ha<sup>-1</sup>P applied would be expected to leach deep into the soil profile because of the P sorption capacity of Ultisols like the Grantham series soil at this site. Despite this surface application, the increase in the 5-10 cm soil depth of the fertilized plots suggest there has been a limited amount of downward movement of the P pools.

Based upon the results of the NaOH/EDTA extraction in this study, I recovered approximately 51% (28 kg ha<sup>-1</sup>) of the applied P in the mineral soil. I also recovered 4 kg ha<sup>-1</sup> of P in the litter layer at this site. Previous long-term P research has shown that a majority of the P applied stayed in the surface horizons or was taken up by the forest stand (Turner *et al.*, 2002b). Based on stand growth response to P fertilization at this site, approximately 6 kg P ha<sup>-1</sup> was taken up. The remaining 18 kg P ha<sup>-1</sup> applied was most likely distributed among different P pools at the site. These P pools include understory plant and microbial biomass and P pools not desorbed from minerals surfaces during the extraction with NaOH/EDTA.

## **Conclusion**

The results of this study show that there has been a long-term effect of fertilization on soil P pools at this site. While extractable soil P pools and the P in the litter increased, <sup>31</sup>P NMR analysis demonstrated that the diversity and quantity of monoester P compounds in the surface

soil changes more slowly in response to P fertilization. Further research into the effects of stand harvesting and site preparation on Po pools is important in determining how monoester P compounds at this site may affect labile P pools.

## Literature Cited

- Albaugh, T.J., H.L. Allen, and T.R. Fox. 2008. Nutrient use and uptake in *Pinus taeda*. *Tree Physiology* 28:1083-98.
- Allen, H.L., P.M. Dougherty, and R.G. Campbell. 1990. Manipulation of water and nutrients -- Practice and opportunity in Southern U.S. pine forests. *Forest Ecology and Management* 30:437-453.
- Alvarez, M., R. Godoy, W. Heyser, and S. Hartel. 2004. Surface-bound phosphatase activity in living hyphae of ectomycorrhizal fungi of *Nothofagus obliqua*. *Mycologia* 96:479-487.
- Ballard, R. 1978. Effect of first rotation phosphorus applications on fertilizer requirements of second rotation pine. *New Zealand Journal of Forestry Science*. 8:135-145.
- Bardgett, R.D., and K.F. Chan. 1999. Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in montane grassland ecosystems. *Soil Biology and Biochemistry* 31:1007-1014.
- Bending, G.D., and D.J. Read. 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants VI. Activities of nutrient mobilizing enzymes in birch litter colonized by *Paxillus involutus* (Fr.) Fr. *New Phytologist* 130:411-417.
- Brady, N.C., and R.R. Weil. 2002. *The nature and properties of soils*. 13th ed. Prentice Hall, Upper Saddle River.
- Bücking, H., and W. Heyser. 1999. Elemental composition and function of polyphosphates in ectomycorrhizal fungi – an X-ray microanalytical study. *Mycological Research* 103:31-39.
- Cade-Menun, B.J. 2005. Characterizing phosphorus in environmental and agricultural samples by  $^{31}\text{P}$  nuclear magnetic resonance spectroscopy. *Talanta* 66:359-371.
- Cade-Menun, B.J., and C.M. Preston. 1996. A comparison of soil extraction procedures for  $^{31}\text{P}$  NMR spectroscopy. *Soil Science*. 161:770-785.
- Cade-Menun, B.J., S.M. Berch, C.M. Preston, and L.M. Lavkulich. 2000. Phosphorus forms and related soil chemistry of podzolic soils on northern Vancouver Island. I. A comparison of two forest types. *Canadian Journal of Forest Research* 30:1714-1725.
- Chamberlain, P.M., N.P. McNamara, J. Chaplow, A.W. Stott, and H.I.J. Black. 2006. Translocation of surface litter carbon into soil by Collembola. *Soil Biology and Biochemistry* 38:2655-2664.
- Chang, S.C., and M.L. Jackson. 1957. Fractionation of soil phosphorus. *Soil Science* 84:133-144.
- Chen, C.R., L.M. Condon, M.R. Davis, and R.R. Sherlock. 2000. Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. *Plant and Soil*. 220:151-163.
- Chen, C.R., L.M. Condon, B.L. Turner, N. Mahieu, M.R. Davis, Z.H. Xu, and R.R. Sherlock. 2004. Mineralisation of soil orthophosphate monoesters under pine seedlings and ryegrass. *Australian Journal of Soil Research* 42:189-196.
- Colpaert, J.V., and K.K. vanTichelen. 1996. Decomposition, nitrogen and phosphorus mineralization from beech leaf litter colonized by ectomycorrhizal or litter-decomposing basidiomycetes. *New Phytologist* 134:123-132.
- Colpaert, J.V., A. VanLaere, K.K. VanTichelen, and J.A. VanAssche. 1997. The use of inositol hexaphosphate as a phosphorus source by mycorrhizal and non-mycorrhizal Scots Pine (*Pinus sylvestris*). *Functional Ecology* 11:407-415.

- Comerford, N.B., M. McLeod, and M. Skinner. 2002. Phosphorus form and bioavailability in the pine rotation following fertilization - P fertilization influences P form and potential bioavailability to pine in the subsequent rotation. *Forest Ecology and Management* 169:203-211.
- Condon, L., E. Frossard, R.H. Newman, P. Tekely, and J.L. Morel. 1997. Use of  $^{31}\text{P}$  NMR in the study of soils and the environment. *In* M. A. Nannny, et al., eds. *Nuclear Magnetic Resonance Spectroscopy in Environmental Chemistry*. Oxford University Press Inc., New York. p. 247-271.
- Condon, L.M., B.L. Turner, and B.J. Cade-Menun. 2005. Chemistry and dynamics of soil organic phosphorus. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. p. 87-121.
- Condon, L.M., M.R. Davis, R.H. Newman, and I.S. Cornforth. 1996. Influence of conifers on the forms of phosphorus in selected New Zealand grassland soils. *Biology and Fertility of Soils* 21:37-42.
- Condon, L.M., E. Frossard, H. Tiessen, R.H. Newman, and J.W.B. Stewart. 1990. Chemical nature of organic phosphorus in cultivated and uncultivated soils under different environmental conditions. *Journal of Soil Science* 41:41-50.
- Conn, C., and J. Dighton. 2000. Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. *Soil Biology Biochemistry* 32:489-496.
- Cross, A.F., and W.H. Schlesinger. 1995. A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64:197-214.
- Dai, K.H., M.B. David, G.F. Vance, and A.J. Krzyszowska. 1996. Characterization of phosphorus in a spruce-fir spodosol by phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Science Society of America Journal* 60:1943-1950.
- Fox, T.R., and N.B. Comerford. 1992. Rhizosphere phosphatase activity and phosphatase hydrolysable organic phosphorus in two forested spodosols. *Soil Biology Biochemistry* 24:579-583.
- Gentle, S.W., F.R. Humphreys, and M.J. Lambert. 1986. Continuing response of *Pinus radiata* to phosphate fertilizers over two rotations. *Forest Science* 32:822-829.
- Gil-Sotres, F., W. Zech, and H.G. Alt. 1990. Characterization of phosphorus fractions in surface horizons of soils from Galicia (N.W. Spain) by  $^{31}\text{P}$  NMR spectroscopy. *Soil Biology and Biochemistry* 22 (1):75-79.
- Gressel, N., J.G. McColl, C.M. Preston, R.H. Newman, and R.F. Powers. 1996. Linkages between phosphorus transformations and carbon decomposition in a forest soil. *Soil Biology and Biochemistry* 33:97-123.
- Harding, R.B., and E.J. Jokela. 1994. Long-term effects of forest fertilization on site organic matter nutrients. *Soil Science Society of America Journal* 58:216-221.
- Hawkes, G.E., D.S. Powlson, E.W. Randall, and K.R. Tate. 1984. A  $^{31}\text{P}$  nuclear magnetic resonance study of the phosphorus species in alkali extracts of soils from long-term field experiments. *Journal of Soil Science* 35:34-45.
- He, Z., T.S. Griffin, and C.W. Honeycutt. 2004. Enzymatic hydrolysis of organic phosphorus in swine manure and soil. *Journal of environmental quality* 33:367-372.

- Hedley, M.J., J. Stewart, and B.S. Chauhan. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Science Society of America Journal* 46:970-976.
- Jakobsen, I., M.E. Leggett, and A.E. Richardson. 2005. Rhizosphere microorganisms and plant phosphorus uptake. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc.
- Kyle, K.H., L.J. Andrews, T.R. Fox, W.M. Aust, J.A. Burger, and G.H. Hansen. 2005. Long-term effects of drainage, bedding, and fertilization on growth of loblolly pine (*Pinus taeda* L.) in the Coastal Plain of Virginia. *Southern Journal of Applied Forestry* 29:205-214.
- Magid, J., H. Tiessen, and L.M. Condron. 1996. Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. , p. 429-466, *In* G. Piccolo, ed. Humic substances in terrestrial ecosystems. Elsevier, Amsterdam.
- Murphy, J.F., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27:31-36.
- Newman, R.H., and K.R. Tate. 1980. Soil characterized by <sup>31</sup>P nuclear magnetic resonance. *Communications in Soil Science and Plant Analysis* 11:835-842.
- Phillips, R.P., and T.J. Fahey. 2006. Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects. *Ecology* 87:1302-1313.
- Piatek, K.B., and H.L. Allen. 2001. Are forest floors in mid-rotation stands of loblolly pine (*Pinus taeda*) a sink for nitrogen and phosphorus? *Canadian Journal of Forest Research* 31:1164-1174.
- Pierzynski, G., R.W. McDowell, and J.T. Sims. 2005. Chemistry, cycling, and potential movement of inorganic phosphorus in soils. *In* J. T. Sims and A. N. Sharpley, eds. Phosphorus: Agriculture and the Environment American Society of Agronomy, Madison, WI. p. 53-86.
- Polglase, P.J., N.B. Comerford, and E.J. Jokela. 1992a. Mineralization of Nitrogen and Phosphorus from Soil Organic-Matter in Southern Pine Plantations. *Soil Science Society of America Journal* 56:921-927.
- Polglase, P.J., E.J. Jokela, and N.B. Comerford. 1992b. Nitrogen and phosphorus release from decomposing needles of southern pine plantations. *Soil Science Society of America Journal* 56:914-920.
- Pritchett, W.L., and N.B. Comerford. 1982. Long-term response to phosphorus fertilization on selected southeastern coastal plain soils. *Soil Science Society of America Journal* 46:640-644.
- Quin, L.D., and G.S. Quin. 2001. Screening for carbon-bound phosphorus in marine animals by high resolution <sup>31</sup>P-NMR spectroscopy: Coastal and hydrothermal vent invertebrates. *Comparative Biochemistry and Physiology* 128:173-185.
- Rasmussen, N., D.C. Lloyd, R.G. Ratcliffe, P.E. Hansen, and I. Jakobsen. 2000. <sup>31</sup>P NMR for the study of P metabolism and translocation in arbuscular mycorrhizal fungi. *Plant and Soil* 226:245-253.
- Saggar, S., R.L. Parfitt, G. Salt, and M.F. Skinner. 1998. Carbon and phosphorus transformations during decomposition of pine forest floor with different phosphorus status. *Biology and Fertility of Soils* 27:197-204.

- Sims, J.T. 2000. Soil test phosphorus: Mehlich 3. *In* G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University. p. 17-19.
- Solomon, D., J. Lehmann, T. Mamo, F. Fritzsche, and W. Zech. 2002. Phosphorus forms and dynamics as influenced by land use changes in the sub-humid Ethiopian highlands. *Geoderma* 105:21-48.
- Sparling, G.P., K.N. Whale, and A.J. Ramsay. 1985. Quantifying the contribution from the soil microbial biomass to the extractable P levels of fresh and air-dried soils. *Australian Journal of Soil Research* 23:613-621.
- Staaf, H., and B. Berg. 1982. Accumulation and release of plant nutrients in decomposing scots pine needle litter. Long-term decomposition in a Scots Pine forest. II. *Canadian Journal of Botany* 60:1561-1568.
- Tate, K.R., and R.H. Newman. 1982. Phosphorus fractions of a clinosequence of soils in New Zealand tussock grassland. *Soil Biology and Biochemistry* 14 (3):191-196.
- Tiessen, H., and J.O. Moir. 1993. Characterization of available P by sequential extraction. p. 75-86, *In* M. R. Carter, ed. *Soil Sampling and Methods of Analysis*. Lewis Publishers.
- Tiessen, H., J.W.B. Stewart, and C.V. Cole. 1984. Pathways of phosphorus transformations in soils of differing pedogenesis. *Soil Science Society of America Journal* 48:853-858.
- Turner, B.L. 2005. Storage-induced changes in phosphorus solubility of air-dried soils. *Soil Science Society of America Journal* 69:630-633.
- Turner, B.L., and P.M. Haygarth. 2003. Changes in Bicarbonate-extractable Inorganic and Organic Phosphorus by Drying Pasture Soils. *Soil Science Society of America Journal* 67:344-350.
- Turner, B.L., and A.E. Richardson. 2004. Identification of scyllo-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Science Society of America Journal* 68:802-808.
- Turner, B.L., N. Mahieu, and L.M. Condon. 2003a. Phosphorus-<sup>31</sup> Nuclear Magnetic Resonance Spectral Assignments of Phosphorus Compounds in Soil NaOH-EDTA Extracts. *Soil Science Society of America Journal* 67:497-510.
- Turner, B.L., N. Mahieu, and L.M. Condon. 2003b. Quantification of myo-inositol hexakisphosphate in alkaline soil extracts by solution <sup>31</sup>P NMR spectroscopy and spectral deconvolution. *Soil Science* 168:469-478.
- Turner, B.L., B.J. Cade-Menun, and D.T. Westermann. 2003c. Organic phosphorus composition and potential bioavailability in semi-arid arable soils of the western United States. *Soil Science Society of America Journal* 67:1168-1179.
- Turner, J., M.J. Lambert, and F.R. Humphreys. 2002b. Continuing growth response to phosphate fertilizers by a *Pinus radiata* plantation over fifty years. *Forest Science*. 48:556-568.
- Turner, B.L., M.J. Paphazy, P.M. Haygarth, and I.D. McKelvie. 2002a. Inositol phosphates in the environment. *Philosophical Transactions of the Royal Society of London* 357:449-469.
- Turner, B.L., N. Mahieu, L.M. Condon, and C.R. Chen. 2005. Quantification and bioavailability of scyllo-inositol hexakisphosphate in pasture soils. *Soil Biology and Biochemistry*. v. 37, no. 11:2155-2158.
- Turner, B.L., L. Condon, S. Richardson, D.A. Peltzer, and V. Allison. 2006. Transformations of soil organic phosphorus during ecosystem development. *ASA-CSSA-Soil Science Society of America Journal* 2006 International Annual Meetings, Indianapolis, IN.

- Turner, J., and M.J. Lambert. 1988. Long-term effects of phosphorus fertilization on forests. p. 125-133, *In* B. B. and W. C. H., eds. Forest site evaluation and long-term productivity. University of Washington Press, Seattle, WA.
- Walker, T.W., and J.K. Syers. 1976. The fate of phosphorus during pedogenesis. *Geoderma* 15:1-19.
- Wright, R.B., B.G. Lockaby, and M.R. Walbridge. 2001. Phosphorus availability in an artificially flooded southeastern floodplain forest soil. *Soil Science Society of America Journal* 65:1293-1302.
- Yuan, G., and L.M. Lavkulich. 1994. Phosphate sorption in relation to extractable iron and aluminum in spodosols. *Soil Science Society of America Journal* 58:343-346.
- Zhang, T.Q., A.F. Mackenzie, and F. Sauriol. 1999. Nature of soil organic phosphorus as affected by long-term fertilization under continuous corn (*Zea mays* L.): A  $^{31}\text{P}$  NMR study. *Soil Science* 164:662-670.

## **CHAPTER 4: COMPARISON OF OXALATE EXCHANGEABLE PHOSPHORUS IN THE BULK SOIL AND BIOLOGICALLY AVAILABLE PHOSPHORUS POOLS IN THE ECTOMYCORRHIZAL RHIZOSPHERE OF A MATURE LOBLOLLY PINE (*Pinus taeda*) PLANTATION.**

### **Abstract**

Plant growth is typically limited by nitrogen (N) and phosphorus (P) availability in the soil environment. Over time, P becomes is sorbed to poorly crystalline Fe and Al oxides. Some plants and microbes secrete ligands such as low-molecular-mass organic acids to increase P uptake in soils with low available P. Agerer (2001) suggested that ectomycorrhiza can be grouped based upon the exploration strategies of their emanating hyphae. In this study, I measured the quantity of Fe, Al, and P released with increasing concentrations of oxalate in order to distinguish between oxalate exchangeable versus dissolvable P pools. The extraction of P with oxalate was also used in order to determine if there were long-term effects of fertilization on soil P pools. Additionally, I extracted P from the ectomycorrhizal rhizosphere to test for differences in the biologically available P pools among three ectomycorrhizal rhizospheres.

The quantity of Fe and Al desorbed exceeded  $\text{CuCl}_2$  exchangeable concentrations when oxalate exceeded  $20 \mu\text{mol g}^{-1}$  soil. Concurrently, the concentration of P desorbed from mineral surfaces increased significantly when oxalate exceeded  $20 \mu\text{mol g}^{-1}$  soil. This increase in metal desorption and P release indicated a shift from oxalate exchange to oxalate dissolution reactions in the bulk soil. There were only small changes in oxalate exchangeable P concentrations ( $0.014 \mu\text{mol g}^{-1}$ ) in the bulk soil following fertilization. This suggests there has been no long-term effect of fertilization on exchangeable P pools. However, there was a significant increase ( $0.06 \mu\text{mol g}^{-1}$ , 60%) in oxalate dissolvable P pools in response to fertilization. The ectomycorrhizal rhizosphere was depleted of P from oxalate dissolvable P pools. Fertilization increased

biologically available P pools by 396%. These results suggest that although the long-term effect of fertilization at this site is an increase in dissolvable P pools, the ectomycorrhizal rhizosphere has access to this dissolvable pool of P.

## **Introduction**

Soil microbes and plants can modify the soil environment to increase P uptake by exuding organic ligands such as oxalate and citrate, as well as a variety of phosphatases to mineralize organic P (Casarin et al., 2003; Illmer and Schinner, 1992; Smith and Read 1997; van Hees et al., 2000). Predictions about the long-term effects of P fertilization in forest soils are difficult due to the complexity of the P cycle in soils. Additionally, we have an incomplete understanding of what P compounds contribute to the labile P pools and how soil biota, such as mycorrhizal fungi, influence the biologically available P pools. Recent research has demonstrated that soil microbes access P pools once believed to be nonlabile (Cajuste et al., 1996; Johnson and Loeppert, 2006; Sato and Comerford, 2006; Singh et al., 2001; Xu et al., 2005).

The addition of P fertilizer to acid soils results in precipitation and adsorption of P to reactive soil colloids including Fe- and Al-oxides surfaces. The long-term fate of P applied to soils is that P is co-precipitated with poorly crystalline Fe, Al, and Si and/or complexed with soil organic matter (Hedley and McLaughlin, 2005). The bioavailability of P sorbed to mineral surfaces depends in part upon desorption/dissolution reactions. These desorption/dissolution reactions depend predominately upon ligand exchange of P anions from inner-sphere complexes. Inner-sphere complexes form covalent bonds, and low-molecular-mass-organic acids such as oxalate have the ability to promote the desorption of P from colloidal surfaces through ligand exchange reactions (Fox et al. 1990). A wide variety of naturally occurring organic acids have been

identified in different soils that may also enter into ligand exchange reactions with P and oxides (Fox et al., 1990; Stevenson, 1967).

Sato and Comerford (2006) used anion exchange membranes and organic ligand to estimate disequilibria-desorbable and ligand-desorbable P pools. Disequilibria-desorption P is brought into solution by the removal of inorganic P from solution following the Le Châtelier's principle. Using anion exchange membranes P was exhaustively extracted from the mineral surface representing the disequilibria-desorption pool of P. Ligand-desorbable P is P released into solution by the release of P from the soil surface. Their research suggests that in a Brazilian Kandiudult, at low concentrations of citrate and oxalate ligands ( $1\mu\text{mol g}^{-1}$ ) P is released from the mineral surface through ligand exchange reactions. While a ligand concentration of  $10\mu\text{mol g}^{-1}$  resulted in the dissolution of the mineral surface and P release. Therefore ligand-desorbable P could thus be operationally defined and quantified (Sato and Comerford, 2006).

Even at very low ligand concentrations,  $< 1.0\mu\text{mol g}^{-1}$  soil continuous secretion of LMMOA may promote dissolution of amorphous minerals over the course of weeks to months. When oxalate forms an inner sphere complex with Al on the oxide surface, there is a concurrent weakening of the bond strength of the AL bonds inside the mineral surface. This weakening over time may promote the dissolution of the mineral surface. It has been observed that ECM fine roots in the nursery (personal observation) and from field grown roots results in the rejuvenation of morphologically “old” appearing roots by the same or different ECM fungi (Durall et al., 1994). Rejuvenation would serve to increase the time in which the ECMR has had to affect P desorption/dissolution in the rhizosphere.

*In situ* measurements of organic acids in the ECMR difficult to quantify; however, its effects on Pi pools and therefore long-term P availability may be a measurable parameter. Oxalate

concentration in soils range from 0 to over 100  $\mu\text{mol g}^{-1}$  soil (Fox and Comerford, 1990; van Hees et al., 2002; Lapeyrie et al., 1987). ECM fungi and soil microbes, such as *Penicillium* and a *Pseudomonas* spp. are a proven source of oxalate in both labs and field experiments (Arvieu et al., 2003; Casarin et al., 2003; Illmer and Schinner, 1992; van Hees et al., 2000; van Hees et al., 2003). Cromack et al. (1979) found that oxalate concentrations in the mycorrhizal mats of *Hysterangium crassum* were 0.44 mol m<sup>-2</sup>.

Additionally, ECM colonization of fine roots increases fine root longevity (Smith and Read, 1997). The growing tip of a fine root is believed to be the area of maximum nutrient uptake and biological activity. Ectomycorrhizal roots may be exuding enough oxalate (among other ligands) to surpass the ligand concentration ligands required to dissolve amorphous minerals. The colonization of fine roots by ECM increases longevity of the fine roots by increasing its resistance to soil drying (Bending and Read, 1995). If during the process of soil drying, water films in the rhizosphere are reduced, the volume of solution in contact with the surrounding soil, it is possible that the concentration of organic ligands found in the ECMR increases to levels greater than those found in the bulk soils.

Not all organic acids have the same ability to assist in the desorption of P from colloidal surfaces (Fox et al., 1990; Illmer and Schinner, 1992; Sato and Comerford, 2006; Wallander, 2000). Citrate and oxalate differ in the ability to dissolve different minerals. This would be important when determining P released from occluded materials and in determining the long-term availability of P at different sites with different mineralogy. Sato and Comerford (2006) noted that Fe released by citrate was over 100% greater than oxalate. The dissolution of poorly and noncrystalline (amorphous) materials in soils for the characterization of clay mineralogy is carried out by acid ammonium oxalate in the dark (AOD) for 2 hrs. (Jackson et al., 2005).

Depending upon the soil type AOD at 2 hrs. has minor effects on clay minerals in soil. Selective dissolution of amorphous associated Fe minerals is more effective with the citrate-dithionite-bicarbonate extractions (Jackson et al., 2005).

The rhizosphere could be expected to alter P desorption/dissolution characteristics compared to the bulk soil. The ECMR with the associated microflora may have many different phosphatases and organic acids that would influence the buffering capacity and quantity of P sorbed to colloidal surfaces compared to the bulk soil. Ligands, like oxalate may have the ability to solubilize metal cations and then move them outside the ECMR. Several studies have attributed the mobilization of mineral cations to low molecular weight anions like oxalate from mycorrhizal fungi, rhizosphere exudates, and soil bacteria (Fox and Comerford, 1992; Illmer and Schinner, 1992; van Hees et al., 2000; van Hees et al., 2004; van Scholl et al., 2006; Wallander et al., 2002). Biologically available P pools in the ECMR has been calculated as:

$$\text{BAP } (\mu\text{mol g}^{-1}) = \text{P}_{\text{bulk soil}} (\mu\text{mol g}^{-1}) - \text{P}_{\text{ECMR}} (\mu\text{mol g}^{-1})$$

at oxalate concentration where ligand desorbable P pool in the bulk soil exceeds the concentrations of P in the ECMR ( $100 \mu\text{mol P g}^{-1}$  in this study). This would occur after the concentration of Fe and Al cations exceeds the exchangeable rates designating dissolution of amorphous minerals.

A new classification system was devised in an attempt to delineated ECM into groups based upon their “exploration type” (Agerer, 1997; Agerer, 2001; Agerer, 2006). Briefly, this classification system groups ECM species based upon the exploration of the hyphae into the soil from the ECM tips. The hypothesis is that ECM species acquire nutrients differently according to their morphological structures and that functional diversity may be implied by this diversity.

No research has been published that has tested whether this classification of ECM based upon morphology can be used to infer ECMR effects on P pools.

The ability to determine the difference in the ligand exchangeable P pool versus ligand dissolution P pools may help elucidate the long-term effects of fertilization and ectomycorrhizal rhizosphere effects on extractable Pi pools. Therefore the objectives of this experiment are to 1) quantify the amount of oxalate required to distinguish between ligand exchangeable P pools vs. ligand dissolution P pools and 2) quantify the concentration of ligand exchangeable and ligand dissolution P pools as affected by fertilization and in the ECMR 3) determine the quantity of ECM biologically available P in the ECMR

## **Materials and Methods**

### *Study Site*

The soil was collected from a *Pinus taeda* plantation located in Sussex County in the Coastal Plain of VA. This site is a part of a long-term experiment investigating silvicultural treatment effects on forest growth. The VA site was installed in March 1980 as a split plot design (Table 4-1). The whole-plot treatment was site prep and sub-plots treatments of fertilizer and weed control. The fertilizer treatment was 0 or 56 kg P ha<sup>-1</sup> applied as diammonium phosphate after planting. Treatment plots were 160 x 40 m (0.64 ha), with 16 rows of 16 trees planted at 2.4 x 2.4 m spacing. The internal eight rows × 8 trees served as the measurements plots.

Table 4-1 Experimental treatments imposed on a loblolly pine plantation growing on a Grantham soil series in coastal VA.

<i>Soil</i>	<i>Treatment</i>	<i>Site Prep</i>	<i>Fertilizer</i>	<i>Soil Depth (cm)</i>
Fine-silty, siliceous, semiactive, thermic Typic Paleaquults	Control	Chop, Burn	0	0-5
	Fertilized	Chop, Burn	56 kg P ha <sup>-1</sup> *	0-5

\*Applied as diammonium phosphate.

### *Soil Collection, Processing, and Storage*

Eighteen soil cores (5 cm dia. x 5 cm deep) were randomly collected within the planted rows of each fertilizer treatment plot using a bulk density hammer. The leaf litter was carefully removed from the soil surface and the sample cores were collected from the upper 5 cm of the A horizon. Soil cores collected were immediately stored in an ice chest and then kept in a 4°C cold room.

Previous research has shown that soil drying and rewetting has resulted in the release of both Pi and Po extractable pools presumably from the lysing of microbial cells, degradation of organic ligands, and physical disruption of Po coating soil particles (Sparling et al., 1985; Turner, 2005; Turner and Haygarth, 2003). Therefore, samples remained in the cold room or were stored over ice in the lab during processing.

The rhizosphere soils were collected by gently removing the roots from the soil cores. Soil falling away from the fine roots was considered a part of the bulk soil. Because of limited quantities of ECMR soil, only a limited number of analyses described in subsequent sections were possible. Fine roots were then gently examined under a dissecting scope and analyzed for the presence of an ectomycorrhizal mantle and emanating fungal hyphal. The soil adhering to the roots were removed by gentle brushed away from the fine roots under a dissecting scope (25 x magnification) using a fine-tip paint brush. Care was taken to minimize damage to the fungal mantle and separate and hyphal mass from the liberated rhizosphere soil. The ECMR soil samples were delineated into two exploration types based upon their morphological features. This included “contact” type with very few to no emanating extra-radical fungal hyphae, and “short distance” exploration type. A third “dead ECM” rhizosphere type was delineated. Dead ECM root tips exhibited a distinct fungal mantle, lost turgor, and appeared wilted.

The 18 soil cores (110 cm<sup>2</sup>) per treatment plot were randomly composited into three bulk soil samples. One composite sample from the control treatment was contaminated and discarded. The fine earth fraction of the bulk soil samples were sieved (<2 mm) without air drying. The sample size of the rhizosphere soils were unbalanced (“contact” n=2, “short distance” n=4, “dead ECM” n=4). Samples remained in cold storage to limit microbial activities while minimizing disruption of organic matter coating on colloids.

### *Bulk Soil Chemical Analyses*

Bulk soil samples were analyzed for total C by dry combustion using the VarioMAX<sup>®</sup> CNS analyzer. Soil organic carbon was calculated as 1.7 x total C (Essington, 2004). Citrate dithionite bicarbonate (CBD) extractable and acid ammonium oxalate in the dark (AOD) Fe and Al contents were measured by ICP following the procedures of Jackson et al. (2005). The bulk soil samples were measured for pH in water (1:2), exchangeable Fe and Al (0.5 M CuCl<sub>2</sub>) and exchangeable P, Fe, and Al (1 N ammonium acetate at soil pH 4.0) (Sparks et al., 1996). The Mehlich-3 extractable P of the bulk soils was also determined (Sims, 2000a). Phosphorus sorption index was measured (Sims, 2000b). The Mehlich-3 extractable P (filtered through a 2.5 μm Whatmann 42) and P concentrations in the PSI extracts (filtered through a 0.45 μm Millipore<sup>™</sup> membrane) were determined by the molybdate ascorbic-acid method (Sims, 2000a).

Results from Chapter three were used for the total P and organic P content of the site extracted using 0.25 M NaOH and 0.05 M EDTA (Cade-Menun and Preston, 1996; Turner *et al.*, 2003). This extract was used to determine the concentration of Fe and Al associated with humic and fulvic acid fractions. The addition of EDTA likely chelated some portion of Fe and Al bound to poorly crystalline mineral surfaces. Despite this artificial increase, it was interpreted as

the maximum concentration of Fe and Al chemically bound to the humic and fulvic acid fractions.

#### *Ligand Exchangeable vs. Dissolution Phosphorus in Bulk Soil*

Ligand exchangeable versus ligand dissolution P pools were measured following the modified procedures of Sato and Comerford (2006). Soil stored at field moisture levels at 4°C was added to 30 ml syringes with the opening closed by parafilm. Next 20 ml of potassium oxalate solutions, were added at 0, 0.02, 0.2, 2, 20, 100, 200  $\mu\text{mol oxalate g}^{-1}$  soil. The oxalate solution was adjusted using HCl to pH 4 in order to match the pH of the soil. The tubes were shaken for 25 seconds and then filtered through a 0.45  $\mu\text{m}$  Millipore<sup>TM</sup> membrane. The concentration of Fe, Al, Mn, and P were determined by ICP. The dissolved fulvic acids were quantified by measuring the E4/E6 ratio using a spectrophotometer (Chen et al., 1977).

#### *Total Oxalate Desorbable P in Bulk and Rhizosphere Soil*

The total oxalate desorbable P was determined in the bulk soil and the ECMR. Preliminary test comparing 500 and 1000  $\mu\text{mol oxalate g}^{-1}$  soil in the bulk soil determined that 500  $\mu\text{mol g}^{-1}$  soil was adequate. Soil stored at field moisture level at 4°C was placed in a 100 ml centrifuge tube with 50 ml of potassium oxalate, equivalent to 500  $\mu\text{mol oxalate g}^{-1}$  soil. The pH of the solution was adjusted with HCl to match the soil pH. The tubes were shaken for 7 hrs. and then centrifuged at  $\sim 2500$  g for 15 min.. The solutions were filtered at 0.45  $\mu\text{m}$  and P was measured by ICP.

#### *Biologically Available P Pools*

Biologically available phosphorus (BAP) pools were determined in the ECMR soil. There was a very limited quantity of ECMR soil which prohibited the extraction of P at all oxalate concentrations used in the bulk soil. Therefore, P extractions in the rhizosphere was limited to total oxalate desorbable P ( $500 \mu\text{mol g}^{-1}$  soil), and oxalate concentration that surrounded exchangeable versus dissolvable Fe pools (20 and  $100 \mu\text{mol g}^{-1}$  soil respectively). Biologically available P ( $\text{mg kg}^{-1}$ ) was calculated from desorbable P extracted with  $100 \mu\text{mol oxalate g}^{-1}$  soil as follows:

$$\text{BAP } (\mu\text{mol g}^{-1}) = \text{P}_{\text{bulk soil}} (\mu\text{mol g}^{-1}) - \text{P}_{\text{ECMR}} (\mu\text{mol g}^{-1})$$

### *Experimental Design*

The experimental design of this study was a randomized complete block design. Soil sampled in this study was randomly collected within the planted rows from the fertilized and control treatment plots (Table 4-1). The rhizosphere treatments (n=4) are bulk soil (no ECMR) and three ECMR exploration types. The exploration types encountered during the study were contact, short-distance, and dead ectomycorrhizal roots.

### *Statistical Analyses*

Analyses of P concentrations desorbed in the bulk soil and ECMR had a non-normal skewed distribution. Statistical analysis of fertilization (control, fertilized) and oxalate concentration treatment effects on oxalate exchangeable P pools in the bulk soil was tested using the GENMOD model in SAS statistical software (Table 4-2). The response variable (P concentration) in the GENMOD model fit a gamma distribution. The scale parameter was

estimated by maximum likelihood estimate, and the least squared means were calculated and analyzed for treatment effects.

Statistical analysis of fertilization (control, fertilized) and oxalate concentration treatment effects on oxalate dissolvable P pools in the bulk soil was tested using the GENMOD model in SAS statistical software (Table 4-3). The response variable (P concentration) in the GENMOD model fit a gamma distribution. The scale parameter was estimated by maximum likelihood estimate, and the least squared means were calculated and analyzed for treatment effects.

Statistical analysis of fertilizer x rhizosphere treatment effects on P desorption was also tested using the GENMOD model in SAS statistical software because of a nonnormal right-skewed distribution (Table 4-4). The concentration of P extracted was tested based upon two factors fertilization (control, fertilized) and ECMR rhizosphere (bulk soil, ECM1 ECM2 ECM3). The response variable (P concentration) in the GENMOD model fit a gamma distribution. The scale parameter was estimated by maximum likelihood estimate, and the least squared means were calculated and analyzed for treatment effects.

Statistical analyses of total ligand desorbable P was performed using the GLM model in SAS software (Table 4-5). The two factors tested were fertilization (control and fertilized) and ECMR (bulk soil, contact ECM and short ECM). There was not enough soil to test Dead ECM for total ligand desorbable P. The Satterthwaite option was employed to calculate the correct degrees of freedom. The data was natural log transformed to meet the assumption of a normal distribution, and the least squared means were calculated and analyzed for treatment effects.

Table 4-2. Type three table for fertilization and oxalate concentration treatment effects on oxalate exchangeable P pools.

Score Statistics For Type 3 GEE Analysis			
Source	DF	Chi-Square	Pr > ChiSq
Block	2	0.06	0.9724
Fertilizer	1	2.31	0.1285
Oxalate	3	12.15	0.0069
Fert * Oxalate	3	4.07	0.2537

Table 4-3. Type 3 table test of fertilization and oxalate concentration treatment effects on oxalate dissolvable P pools.

Score Statistics For Type 3 GEE Analysis			
Source	DF	Chi-Square	Pr > ChiSq
Block	2	9.66	0.008
Fertilizer	1	4.36	0.0369
Oxalate ( $\mu\text{mol g}^{-1}$ )	2	12.72	0.0017
Fert * Oxalate	2	5.03	0.0809

Table 4-4 Type three table for fertilization and ectomycorrhizal rhizosphere (ECMR) treatment effects on oxalate dissolvable P pools.

Score Statistics For Type 3 GEE Analysis			
Source	DF	Chi-Square	Pr > ChiSq
Block	2	2.43	0.2969
Fertilizer	1	5.32	0.021
EMCR	3	10.42	0.0153
Fert * ECMR	3	6.57	0.0869

Table 4-5. Type three table for fertilization and ectomycorrhizal rhizosphere treatment effects on total oxalate dissolvable P pools.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	2	0.838	0.419	6.79	0.008
Fertilizer	1	0.002	0.002	0.03	0.855
EMCR	2	3.392	1.696	27.46	<.0001
Fert * ECMR	2	0.44	0.22	3.57	0.054

## Results and Discussion

### *Oxalate Exchangeable versus Dissolution Pools*

The difference in ligand exchange versus dissolvable P pools is inconsequential to soil biota (Sato and Comerford, 2006). However, this information may be useful in determining if there are long-term effects of fertilization on biologically available soil P pools. The first step in delineating between ligand exchangeable and dissolution P is determining the concentration of exchangeable Fe and Al. Exchangeable metal cations was determined by three extractions yielding different concentrations of exchangeable Fe and Al (Table 4-6). A high estimate of exchangeable Fe and Al was determined using 0.25 M NaOH and 0.05 M EDTA (NaOH-EDTA). The NaOH-EDTA extractions are used to desorb organic P from mineral surfaces for  $^{31}\text{P}$  NMR analyses (Turner *et al.*, 2005). The EDTA in this solution is capable of dissolving Fe and Al from poorly crystalline mineral surfaces thereby overestimating Fe and Al associated with soil organic carbon (Garcia-Marco *et al.*, 2006; Borggaard, 1983).

The addition of 20  $\mu\text{mol oxalate g}^{-1}$  soil is the loading rate that delineates ligand exchange and dissolution reactions based upon the release of Fe, Al, and FA into the soil solution (Fig. 4-1). The concentration of 0.5 M  $\text{CuCl}_2$  extractable Al (4.6  $\mu\text{mol g}^{-1}$ ) and Fe (0.6  $\mu\text{mol g}^{-1}$ ) was used as the benchmark for delineating ligand exchange versus dissolution reactions. Copper chloride is preferred for determining exchangeable AL and Fe in soils with medium to high amounts of soil organic carbon because the Cu cation has a high affinity for carboxylate sites (Bertsch and Bloom, 1996; Villapando and Graetz, 2001). When oxalate concentration reached 20  $\mu\text{mol g}^{-1}$  both the Fe (1.0  $\mu\text{mol g}^{-1}$ ) and Al (4.9  $\mu\text{mol g}^{-1}$ ) concentrations exceed  $\text{CuCl}_2$  exchangeable Fe and Al concentrations (Fig. 4-1). Concentrations of Fe and Al were less than 0.5  $\mu\text{mol g}^{-1}$  when

the oxalate concentrations were  $\leq 2 \mu\text{mol g}^{-1}$ . This suggest exchangeable Fe and Al cations were present in higher concentrations than the oxalate ligand for desorption.

A third factor supporting the assertion that  $20 \mu\text{mol oxalate g}^{-1}$  soil delineates ligand exchange and dissolution reactions in this soil is the release of fulvic acids from mineral surfaces. The presence of dissolved organic C was visibly present in the solutions with 20, 100, and 200  $\mu\text{mol oxalate g}^{-1}$  soil (Fig. 4-2). The E4/E6 ratio measures the particle size and atomic weight of FA in solution not their concentration (Chen et al., 1977). In this study the E4/E6 ratio is only intended to serve as a measure of presence or absence of FA in solutions (Zhang et al., 1996). Solutions with oxalate concentration less than  $20 \mu\text{mol g}^{-1}$  had no detectable fulvic acids. The solutions with oxalate concentration equal to or greater than  $20 \mu\text{mol oxalate g}^{-1}$  detectable fulvic acids in solution.

Once the oxalate concentration approached  $20 \mu\text{mol g}^{-1}$  soils, exchangeable Fe, Al was chelated, and the additional oxalate began the dissolution of the mineral surface. The dissolution of the mineral surface resulted in the release of fulvic acids as indicated by the E4/E6 ratio. The increase in Fe and Al concentration increased beyond both 0.5M  $\text{CuCl}_2$  and 1 N ammonium acetate (pH 4) exchangeable levels (Table 4-6). The Fe and Al dissolved from the mineral surface and released with FA ( $1 \mu\text{mol g}^{-1}$ ) at  $20 \mu\text{mol oxalate g}^{-1}$  soil was larger than exchangeable concentrations and equivalent to the concentration of Fe in the humic and fulvic acid extraction with NaOH-EDTA. This is additional evidence supporting the assertion that at  $20 \mu\text{mol oxalate g}^{-1}$  soil metal cations are released via ligand promoted dissolution reactions instead of ligand exchange reactions.

Table 4-6 Surface soil (0-5 cm) properties from a loblolly pine plantation on Grantham soil series in coastal VA.

pH <sup>§</sup>	Total C <sup>#</sup> (%)	SOC <sup>#</sup> (%)	Bulk Density (g cm <sup>-3</sup> )	PSI <sup>@</sup> (μmol g <sup>-1</sup> )	Exch. P <sup>†</sup> (μmol g <sup>-1</sup> )	Ext. P <sup>‡</sup> (μmol g <sup>-1</sup> )	Ext. P <sup>‡</sup> (kg ha <sup>-1</sup> )	H & FA Al <sup>@@</sup> (μmol g <sup>-1</sup> )
4.0	4.4	7.5	0.84	30.3	1.4 x 10 <sup>-2</sup>	2.5	33	6.1
H & FA Fe <sup>@@</sup> (μmol g <sup>-1</sup> )	Exch. Fe <sup>†</sup> (μmol g <sup>-1</sup> )	Exch. Al <sup>†</sup> (μmol g <sup>-1</sup> )	CuCl <sub>2</sub> Exch. Fe <sup>‡‡</sup> (μmol g <sup>-1</sup> )	CuCl <sub>2</sub> Exch. Al <sup>‡‡</sup> (μmol g <sup>-1</sup> )	AAO Fe <sup>##</sup> (μmol g <sup>-1</sup> )	AAO Al <sup>##</sup> (μmol g <sup>-1</sup> )	CBD Fe <sup>\$\$</sup> (μmol g <sup>-1</sup> )	CBD Al <sup>\$\$</sup> (μmol g <sup>-1</sup> )
1.0	0.8	3.7	0.6	4.6	14.5	17.3	42.6	23.8

<sup>§</sup> 1:2 in water.

<sup>#</sup> VarioMAX<sup>®</sup> CNS analyzer.

<sup>@</sup> Phosphorus Sorption Index (Sims, 2000b).

<sup>†</sup> 1 N ammonium acetate (pH 4).

<sup>@@</sup> Humic & Fulvic Acid 0.25 M NaOH/0.05 M EDTA extractable.

<sup>‡</sup> 0.25 M NaOH/0.05 M EDTA extractable.

<sup>‡‡</sup> 0.5 M CuCl<sub>2</sub> extractable.

<sup>##</sup> Acid ammonium oxalate extractable.

<sup>\$\$</sup> Citrate-bicarbonate-dithionite extractable.

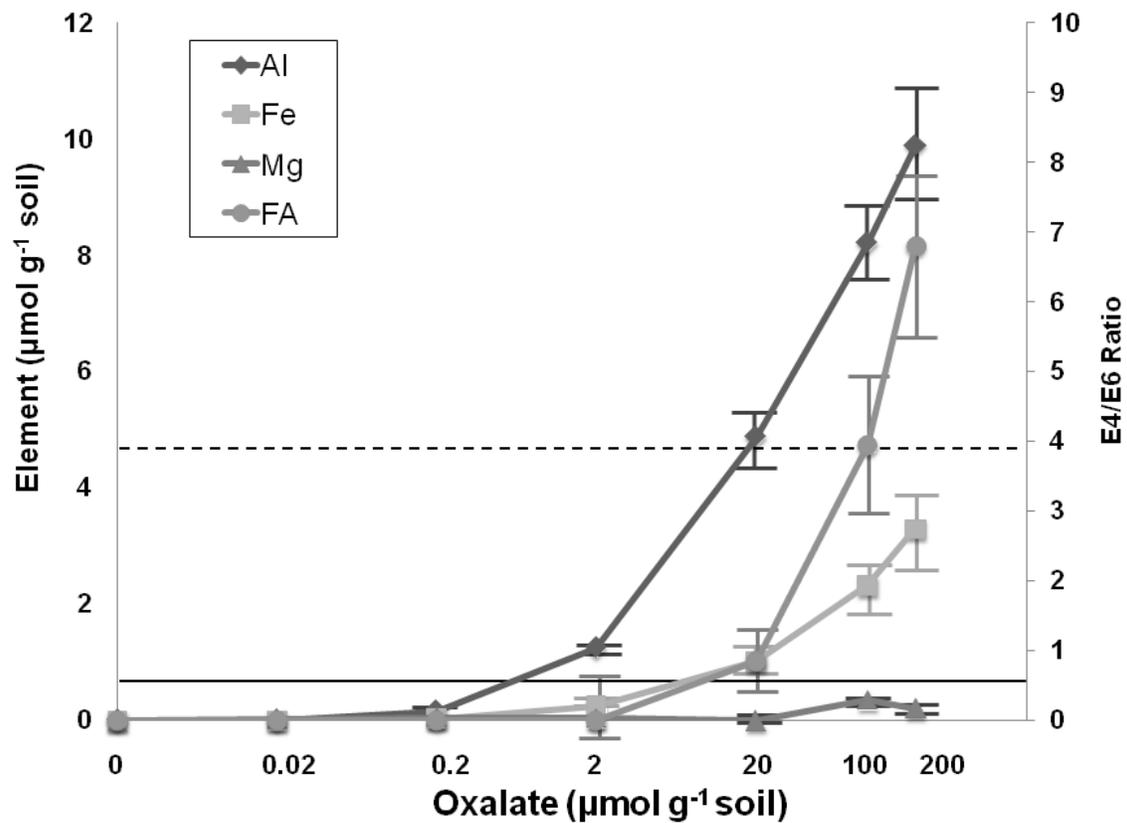


Figure 4-1 Effects of oxalate loading on metal and fulvic acid desorption in the bulk soil of a loblolly pine forest in coastal Virginia. Values represent mean and bars are std. error of the mean.  $\text{CuCl}_2$  extractable Al (dashed line).  $\text{CuCl}_2$  extractable Fe (solid line).

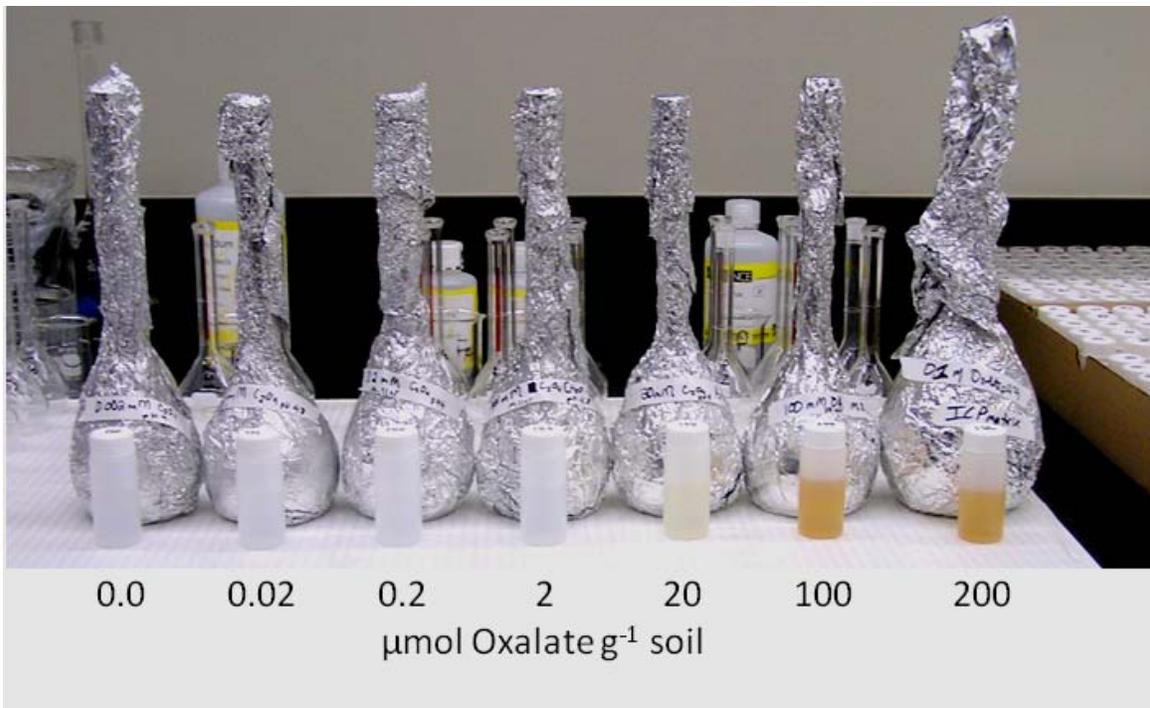


Figure 4-2 Effect of oxalate concentrations on desorbable fulvic acids in solution (photo: Bradley Miller).

The concentration of oxalate required ligand exchange versus dissolvable Fe pools in this study is similar to previous studies. Sato and Comerford (2006) delineated exchangeable versus desorbable concentrations of Fe at 1  $\mu\text{mol oxalate g}^{-1}$  soil. Their soil, a Kandiodult, was sampled to a depth of 30 cm and had 25.1  $\text{mg g}^{-1}$  organic C. The Paleaquults sampled in this study was sampled 5 cm deep and had almost three times the organic C 75  $\text{mg g}^{-1}$ . The additional SOC in this study likely increased the amount of exchangeable Fe and Al. Therefore, the soil in this study required high concentrations of oxalate to bind with the exchangeable metal cations before beginning the dissolution of the mineral surface. The concentration of exchangeable Fe (1 *N* ammonium acetate) in this study was 0.3  $\mu\text{mol g}^{-1}$  larger than exchangeable Fe in Sato and Comerford (2006).

### ***Long-term Fertilization Effects on Soil P Pools***

#### *Oxalate Exchangeable Phosphorus Pools*

I failed to reject the null hypothesis that there was a significant fertilization effect on the concentration of oxalate exchangeable P 27 years after P application. Oxalate exchangeable P pools in this study were determined based upon the release of Fe and Al in excess of exchangeable ( $\text{CuCl}_2$ ) concentrations, and desorption of FA into the soil solution (Fig. 4-3). Oxalate exchangeable P pools were defined as P desorbed with oxalate concentrations below 20  $\mu\text{mol g}^{-1}$  soil. The concentration of desorbed P remained below 0.001  $\mu\text{mol g}^{-1}$  (0.04  $\text{mg L}^{-1}$ ) in both the control and the fertilized plots with oxalate concentrations below 20  $\mu\text{mol g}^{-1}$  soil (Fig. 4-4). Phosphorus concentrations were similar ( $p = 0.163$ ) in the control (0.13  $\mu\text{mol g}^{-1}$ ) and fertilized (0.14  $\mu\text{mol g}^{-1}$ ) treatment at 2  $\mu\text{mol oxalate g}^{-1}$  soil. Any effects P fertilization may have had on ligand exchangeable P pools were gone after 27 years.

Similar to the results of this study, Gerke et al. (2000) testing a Fluvisol with citrate and oxalate found that P solubility below  $10 \mu\text{mol ligand g}^{-1}$  was negligible. Phosphorus pools increased approximately  $35 \mu\text{mol g}^{-1}$  soil by increase in g oxalate from 0 to  $30 \mu\text{mol g}^{-1}$  soil.

#### *Oxalate Dissolvable Phosphorus Pools*

There was a significant long-term fertilization effect ( $p=0.019$ ) on oxalate dissolvable P pools (Fig. 4-3). Oxalate dissolvable P pools in this study were determined based upon the release of Fe and Al in excess of exchangeable ( $\text{CuCl}_2$ ) concentrations, and the desorption of FA into the soil solution. Oxalate concentrations above  $20 \mu\text{mol g}^{-1}$  soil represent dissolvable P pools. The fertilization treatment increased the P concentration in the 20, 100, and  $200 \mu\text{mol oxalate g}^{-1}$  extractions by 76, 150, and 62%, respectively (Fig.4-4).

The concentration of oxalate desorbable P increased linearly with oxalate concentrations in excess of  $20 \mu\text{mol g}^{-1}$ . The highly weathered Kandiuult soil of Brazil had the oxalate dissolvable P estimated at concentrations above  $1 \mu\text{mol g}^{-1}$  (Sato and Comerford 2006). The rapid increase in soluble P above  $50 \mu\text{mol ligand g}^{-1}$  soil reported by Gerke et al. (2000) matches the pattern for ligand dissolvable P pools in this study and Sato and Comerford (2006).

The concentration of oxalate required to distinguish between ligand exchangeable versus dissolution P pools is soil and site specific. The mechanism for ligand dissolution involves the chelation of exchangeable metal cations, followed by the formation of inner-sphere complexes between the ligand and the mineral surface. Lastly, a critical concentration of the ligand is exceeded and the dissolution of the mineral surface begins. Because soil series and individual sites different in their physiochemical and biological properties, the ligand concentration required to distinguish the dissolution point will vary.

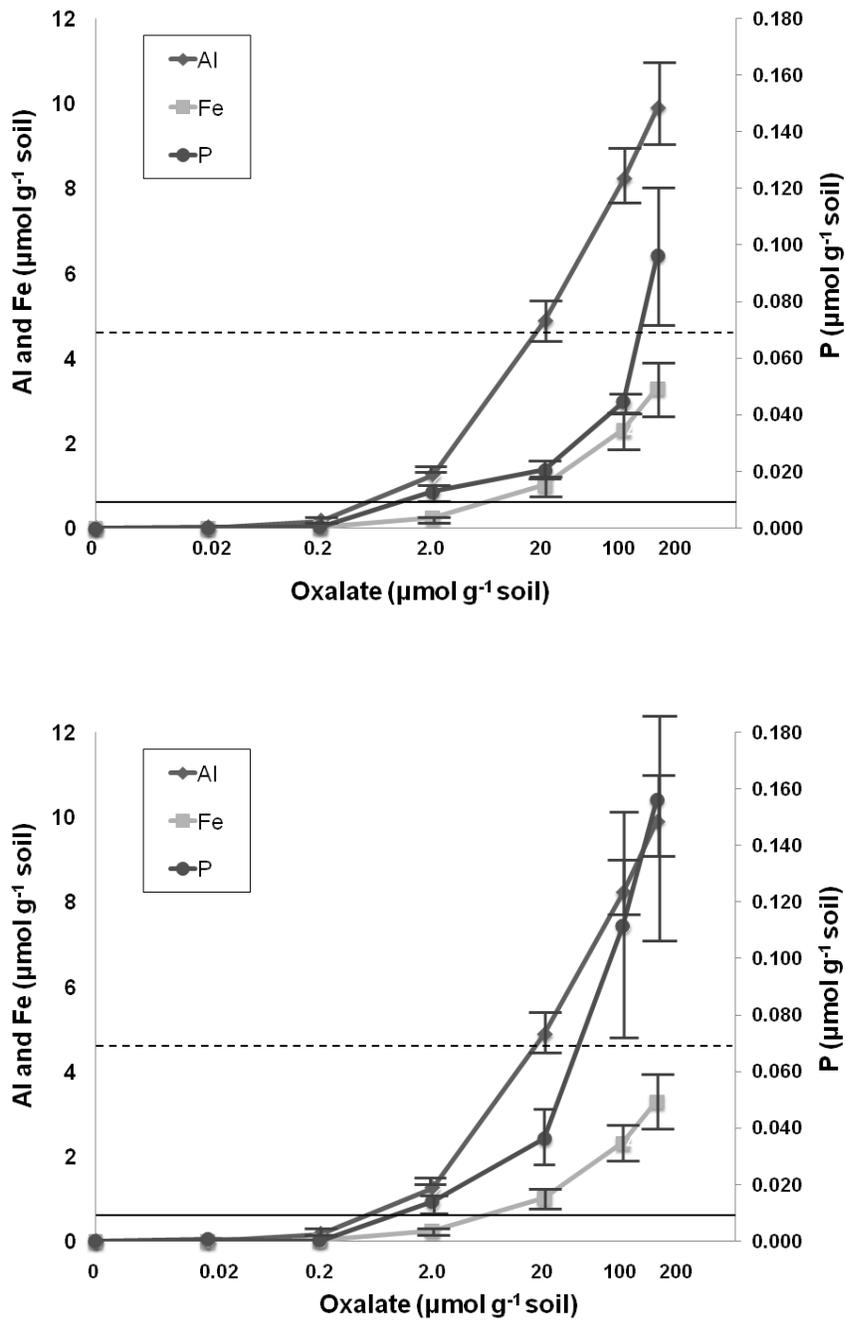


Figure 4-3 Phosphorus desorption in the bulk soil of the control (top) and fertilized (bottom) treatments in response to oxalate loading in a loblolly pine forest soil of coastal Virginia. Values represent mean and S.E.M.  $\text{CuCl}_2$  extractable Al (dashed line).  $\text{CuCl}_2$  extractable Fe (solid line).

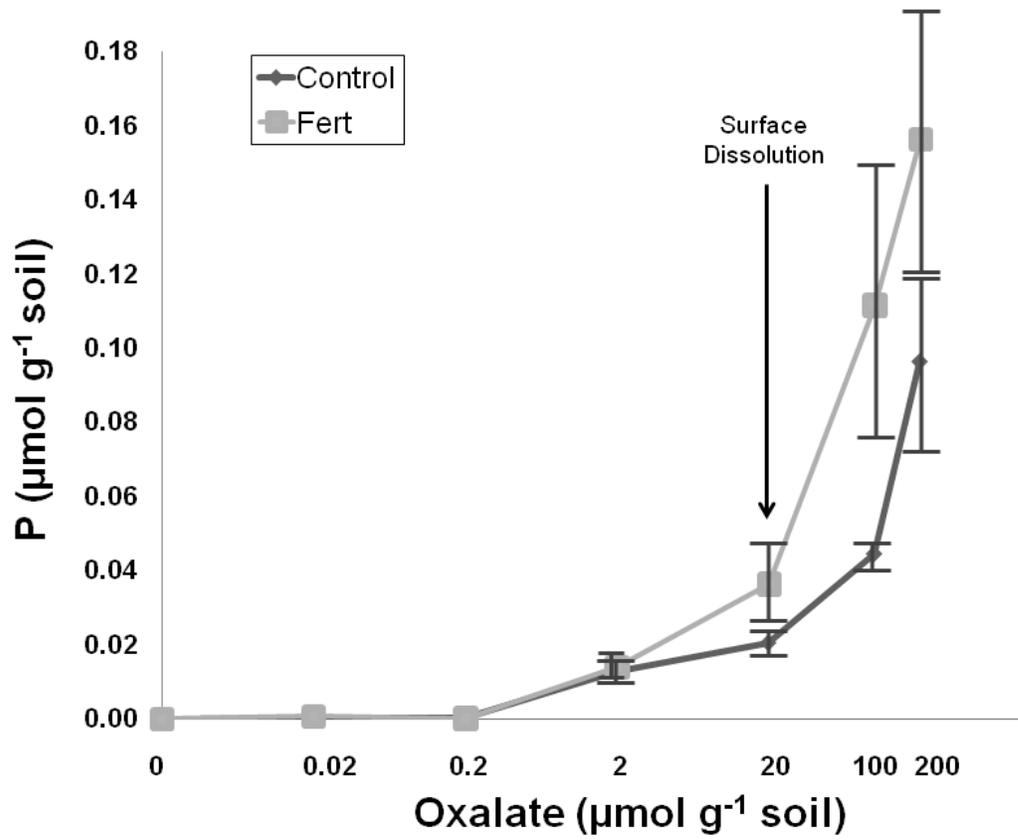


Figure 4-4 Phosphorus desorption in response to oxalate loading in a loblolly pine forest soil of coastal Virginia. Values represent mean and S.E.M..

Likewise, not all ligands equally mobilize metal cations in the soil (Gerke et al., 2000; Irving and Williams, 1953). Therefore, in the complex soil solution, the biologically available pools of P accessible via ligand exchange and dissolution reactions depend upon the concentration of all ligands capable of forming inner sphere complexes with the mineral surface in the rhizosphere. While this remains logistically impossible to measure, results from this pilot study does provide insight into the ECMR effects on soil P pools.

#### *ECM Rhizosphere Effect on P Pools*

There was a long-term effect of P fertilization and the ECM exploration type on BAP pools. Oxalate desorbable P pools extracted with 100  $\mu\text{mol}$  oxalate  $\text{g}^{-1}$  soil were significantly affected by fertilization ( $p=0.021$ ) and the ECMR ( $p=0.0153$ ). There was a significant ( $p = 0.086$ ) interaction effect (fertilizer x ECMR) on the concentration of P extracted with 100  $\mu\text{mol}$  oxalate  $\text{g}^{-1}$  soil (Fig. 4-5). Oxalate desorbable P in the short distance and dead ECMR were significantly smaller than P desorbed from the bulk soil and the contact ECMR.

The desorption of P in the short distance ECMR and dead ECMR were significantly different from the bulk soils in both the control and fertilized plots (Fig 4-5). The desorption of P in the bulk soil was more than double that in both the short distance and dead ECMR.

In contrast, desorbed P by the contact ECMR at 100  $\mu\text{mol}$  oxalate  $\text{g}^{-1}$  soil was larger than the bulk soil. This may be attributed to the influence of the contact ECMR on P pools. However, P was depleted from the rhizosphere of all three ECM exploration types in both the control and fertilized treatments at 20  $\mu\text{mol}$  oxalate  $\text{g}^{-1}$  soil (Fig. 4-6). This corresponds to all three ECMR removing 0.02 and 0.04  $\mu\text{mol}$  P  $\text{g}^{-1}$  soil from the bulk soils in the control and fertilized treatments respectively.

Total oxalate desorbable P was higher in the contact ECMR samples than the bulk soil (Fig. 4-7). The higher concentration of P from the contact ECMR samples extracted with 100  $\mu\text{mol}$  oxalate  $\text{g}^{-1}$  soil may be due to the contact ECMR increasing the rate of mineral dissolution and therefore desorption of more P. This may account for the discrepancy of between P desorption in the contact ECMR at 20 and 100  $\mu\text{mol}$  oxalate  $\text{g}^{-1}$  soil. Alternatively, the contact rhizosphere in the control plot may be enriched in P. Based upon the hypothetical classification of ECM based upon exploration types (Agerer 2001), the increase in desorbable P in the contact ECMR would be expected. Because the contact fungi can only absorb P from the rhizosphere soil, these fungi would either preferential grow in P enriched microsites, or produce exudates that could desorb more P from the mineral surface in the rhizosphere. For the purpose of generating the concentrations of BAP from the contact ECMR, the P desorbed with 20  $\mu\text{mol}$  oxalate  $\text{g}^{-1}$  soil will be used.

Biologically available P pools measured in the ECMR accounted for 0.63 and 3.02  $\text{kg P ha}^{-1}$  in the control and fertilized treatments (Table 4-7). The mechanisms of P desorption and sink for the BAP cannot be resolved in this study. The assumption is that the BAP was absorbed by the ECM fungi and some portion is made available to the tree. Similarly, the pool of BAP has been desorbed from the rhizosphere but the mechanism of desorption remains unclear. The BAP in the ECMR may have been exchanged from the mineral surface by some ligand exuded into the ECMR, or the BAP may have been organic P that was enzymatically cleaved and absorb by soil microbes. However, the results from these extractions do demonstrate an increase in BAP because of P fertilizer applied 27 years earlier.

#### *Total Oxalate Desorbable P*

There was a significant ( $p = 0.054$ ) treatment interaction effect (fertilizer x ECMR) on the concentration of total oxalate desorbable (TOD) P (Fig. 4-5). The TOD P concentration in the bulk soil of fertilizer treatment increased by  $0.15 \mu\text{mol g}^{-1}$  compared to the bulk soil of the control treatment ( $p = 0.025$ ). This increase corresponds to  $1.95 \text{ kg P ha}^{-1}$  and represents a portion of the P fertilizer applied 27 years earlier. However, the NaOH/EDTA extractable P was measured at  $33 \text{ kg ha}^{-1}$ . This means that the  $500 \mu\text{mol oxalate g}^{-1}$  soil did not release all the P sorbed in the soil. While oxalate is effective at lower pH values, for chelating poorly crystalline aluminosilicates materials, it is less effective at chelating Fe minerals compared to citrate (Jackson et al., 2005). The poorly crystalline fraction of this soil was dominated by CBD extractable Fe. The use of citrate instead of oxalate may improve P recovery from the soil as well as testing for total ligand desorbable P beyond  $1 \text{ mM g}^{-1}$  soil.

The ECMR treatments had significantly larger concentrations of TOD P than the bulk soil in the control plot (Fig 4-6). This increase suggests that the ECMR has modified the soil environment in some way that resulted in an increase in P pools extracted with  $500 \mu\text{mol oxalate g}^{-1}$  soil. It is possible the some portion of the dissolvable P has been made available because of the influence of the ECMR on the soil. Recent studies have also shown that ECM species may preferential occur in different soil horizons (O versus A) or microsites of differing physical characteristics such as windthrow mounds (Baier et al., 2006; Maestre et al., 2002; Tedersoo et al., 2008). However, I was unable to determine the exact cause of the increase in TOD P.

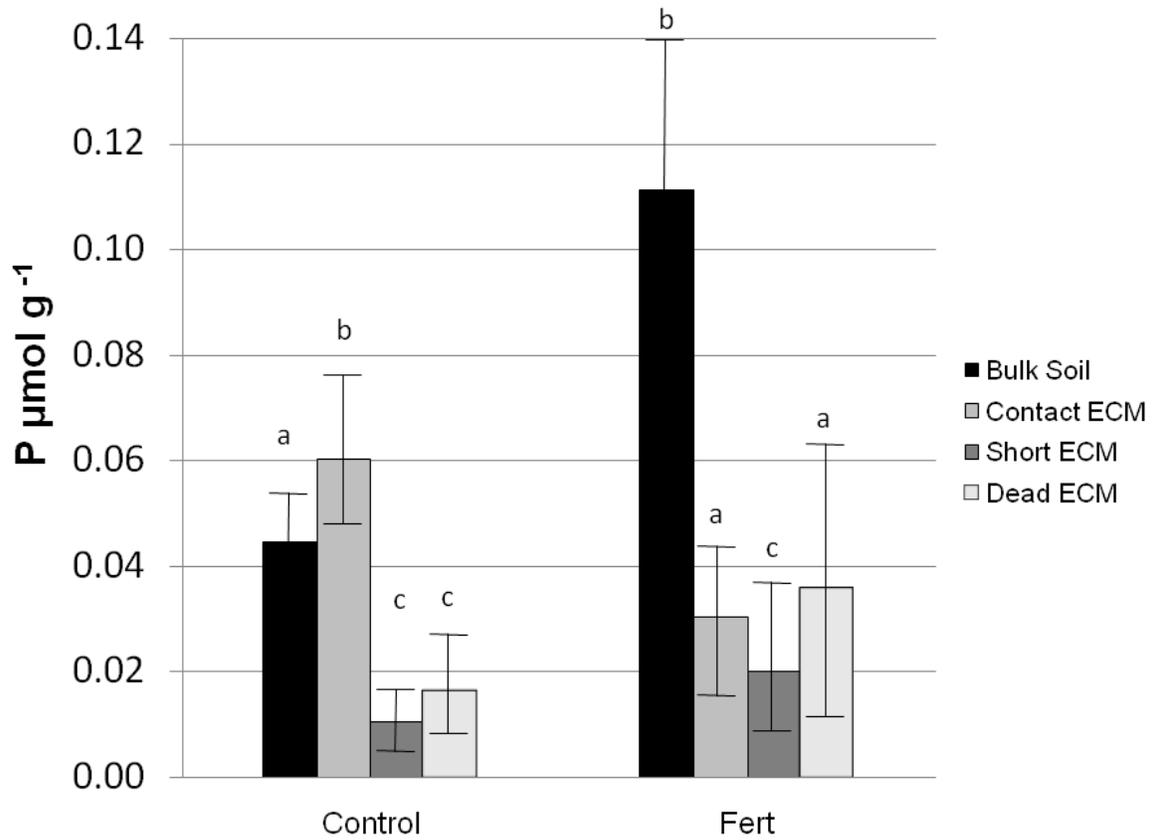


Figure 4-5 Fertilization x ECM rhizosphere interactions effects ( $p = 0.0869$ ) on desorbable P pools extracted with  $100 \mu\text{mol oxalate g}^{-1}$  soil. Samples were taken from a loblolly pine forest soil of coastal Virginia. Values represent mean and S.E.M. Bars with different letters are significantly different at  $p < 0.05$ .

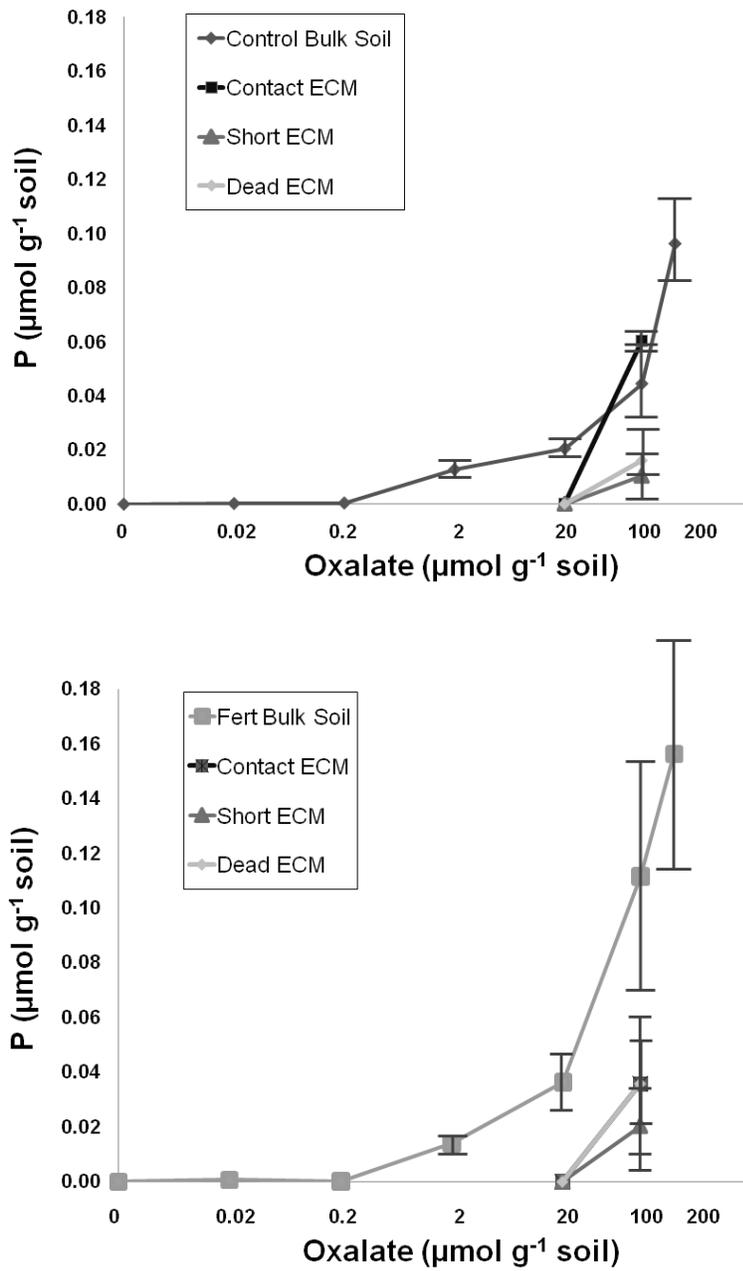


Figure 4-6 Phosphorus desorption in the ECM rhizosphere and bulk soil of the control (top) and fertilized (bottom) treatments from a loblolly pine forest soil of coastal Virginia. Values represent mean and S.E.M..

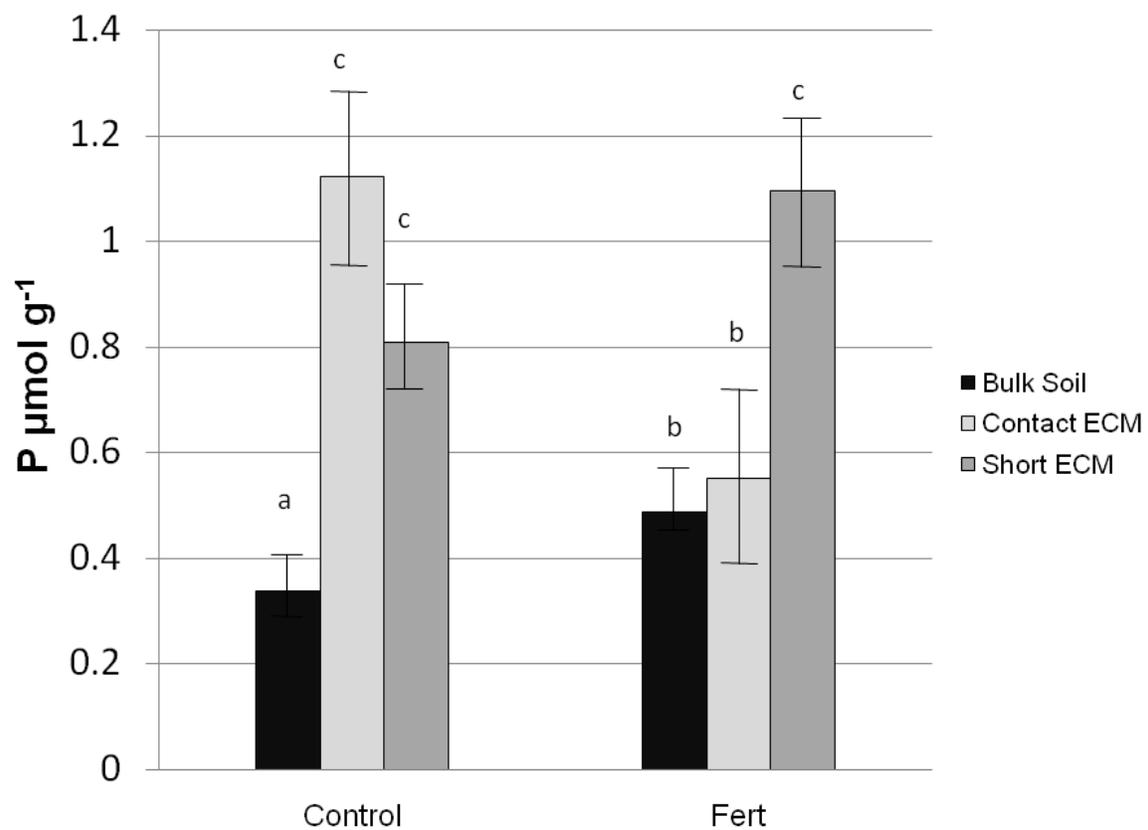


Figure 4-7 Total oxalate desorbable P concentration in the surface soil (0-5 cm) and ectomycorrhizal rhizosphere of a loblolly pine plantation of coastal Virginia. Values represent mean (S.E.M).

Table 4-7. Biologically available phosphorus pools in the ectomycorrhizal rhizosphere from a loblolly pine plantation of coastal VA.

Treatment	Control	Fertilized	Difference
P (mg kg <sup>-1</sup> )*			
Contact	0.62 <sup>‡</sup>	2.51	2.51
Short	1.06	2.82	1.77
Dead	0.88	2.35	1.47
Sum	1.94a	7.68b	5.75
P (kg ha <sup>-1</sup> )*			
Contact	0.27 <sup>‡</sup>	0.99	0.99
Short	0.46	1.11	0.65
Dead	0.38	0.92	0.54
Sum	0.63a	3.02b	2.40

\* Values represent the difference of phosphorus desorbed from the ECMR and bulk soil using 100  $\mu\text{mol}$  oxalate g<sup>-1</sup> soil. <sup>‡</sup> Concentration of phosphorus desorbed using 20  $\mu\text{mol}$  oxalate g<sup>-1</sup> soil. Values with different letters are significant at  $p < 0.05$ .

## Conclusions

After 27 years, there remains a long-term effect of P fertilization on soil P pools. The long-term fate of applied P is the increase in P associated with ligand dissolvable P pools (Hedley and McLaughlin) 2005. This study demonstrates that we can operationally define the differences in oxalate exchangeable versus dissolvable P pools. Soil and site specific properties will affect the concentration of ligand required to distinguish between ligand exchangeable and dissolvable P pools. These factors include exchangeable Fe and Al concentration, concentration of soil organic carbon, and mineralogy.

Results from this study indicate that the fertilizer P applied 27 years earlier is associated with the oxalate dissolvable P pools. Plants and microbes that continue to benefit from the P fertilizer treatment may be accessing the oxalate dissolvable P pools by exuding extracellular chemicals such as low-molecular-mass organic acids and phosphatases enzymes. The BAP had increased in the P fertilization treatment. Fertilization increased the BAP by  $2.4 \text{ kg P ha}^{-1}$ . This increase in BAP pools indicates that there continues to be a long-term effect of fertilization on soil biogeochemical pools regardless of being sequestered into the oxalate dissolvable P pools.

## Literature Cited

- Agerer, R. 1997. Descriptions of Ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger GmbH Schwabisch Gmund, Munich, Germany.
- Agerer, R. 2001. Exploration types of ectomycorrhizae - A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11:107-114.
- Agerer, R. 2006. Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress* 5:67-107.
- Arvieu, J.C., F. Leprince, and C. Plassard. 2003. Release of oxalate and protons by ectomycorrhizal fungi in response to P-deficiency and calcium carbonate in nutrient solution. *Annals of Forest Science* 60:815-821.
- Baier, R., J. Ingenhaag, H. Blaschke, A. Gottlein, and R. Agerer. 2006. Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (*Picea abies* [L.] Karst.) stand of the Bavarian Limestone Alps. *Mycorrhiza* 16:197-206.
- Bending, G.D., and D.J. Read. 1995. The structure and function of the vegetative mycelium of ectomycorrhizal plants V. Foraging behavior and translocation of nutrients from exploited litter. *New Phytologist* 130:401- 409.
- Bertsch, P.M., and P.R. Bloom. 1996. Aluminum. In D. L. Sparks, et al., eds. *Methods of Soil Analysis. Part 3: Chemical Methods*. Soil Science Society of America, Inc., and American Society of Agronomy, Inc., Madison, WI. p. 517-534.
- Borggaard, O. K. 1983. The influence of iron oxides on phosphate adsorption by soil. *European Journal of Soil Science* 34:333-341.
- Cade-Menun, B.J., and C.M. Preston. 1996. A comparison of soil extraction procedures for <sup>31</sup>P NMR spectroscopy. *Soil Science*. 161:770-785.
- Cajuste, L.J., R.J. Laird, B. Cajuste, Jr., and B.G. Cuevas. 1996. Citrate and oxalate influence on phosphate, aluminum, and iron in tropical soils. *Communications in Soil Science and Plant Analysis*. 27 (5/8):1377-1386.
- Casarin, V., C. Plassard, G. Souche, and J.-C. Arvieu. 2003. Quantification of oxalate ions and protons released by ectomycorrhizal fungi in rhizosphere soil. *Agronomie* 23:461-469.
- Chen, Y., N. Senesi, and M. Schnitzer. 1977. Information provided on humic substances by E4/E6 ratios. *Soil Science Society of America Journal* 41:352-8.
- Cromack Jr., K., P. Sollins, W.C. Graustein, K. Speidel, A.W. Todd, G. Spycher, C.Y. Li, and R.L. Todd. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. *Soil Biology and Biochemistry* 11:463-468.
- Durall, D.M., J.D. Marshall, M.D. Jones, R. Crawford, and J.M. Trappe. 1994. Morphological changes and photosynthate allocation in ageing *Hebeloma crustuliniforme* (Bull.) Quel. and *Laccaria bicolor* (Maire) Orton mycorrhizas of *Pinus ponderosa* Dougl. ex. Laws. *New Phytologist* 127:719-724.
- Essington, M.E. 2004. *Soil and Water Chemistry: An integrative approach*. CRC Press, New York.
- Fox, T.R., and N.B. Comerford. 1990. Low-molecular-weight organic acids in selected forest soils of the southeastern USA. *Soil Science Society of America Journal* 54:1139-1144.
- Fox, T.R., and N.B. Comerford. 1992. Influence of oxalate loading on phosphorus and aluminum solubility in spodosols. *Soil Science Society of America Journal* 56:290-294.

- Fox, T.R., N.B. Comerford, and W.W. McFee. 1990. Kinetics of phosphorus release from spodosols: effects of oxalate and formate. *Soil Science Society of America Journal* 54:1441-1447.
- Garcia-Marco, S., N. Martinez, F. Yunta, L. Hernandez-Apaolaza, and J.J. Lucena. 2006. Effectiveness of ethylenediamine-N(o-hydroxyphenylacetic)-N'(p-hydroxy-phenylacetic) acid (o,p-EDDHA) to supply iron to plants. *Plant and Soil* 279:31-40.
- Gerke, J., L. Beissner, and W. Romer. 2000. The quantitative effect of chemical phosphate mobilization by carboxylate anions on P uptake by a single root. I. The basic concept and determination of soil parameters. *Journal of Plant Nutrition and Soil Science* 163:207-212.
- Hedley, M.J., and M.J. McLaughlin. 2005. Reactions of phosphate fertilizers and by-products in soils. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. p. 181-252
- Illmer, P., and F. Schinner. 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biology and Biochemistry*. 24:389-395.
- Irving, H., and R.J.P. Williams. 1953. The stability of transition-metal complexes. *Journal of the American Chemical Society* 75:3192-3210.
- Jackson, M.L., C.H. Lim, and L.W. Zelazny. 2005. Oxides, Hydroxides, and Aluminosilicates, p. 101-150, *In* A. L. Page, et al., eds. *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods.*, 2nd. ed. American Society of Agronomy-Soil Science Society of America, Madison, WI.
- Johnson, S.E., and R.H. Loeppert. 2006. Role of organic acids in phosphate mobilization from iron oxide. *Soil Science Society of America Journal* 70:222-234.
- Lapeyrie, F., G.A. Chilvers, and C.A. Bhem. 1987. Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus* (Batsch. ex Fr.) Fr. *New Phytologist* 106:139-146.
- Maestre, F.T., S. Bautista, J. Cortina, G. Diaz, M. Honrubia, and R. Vallejo. 2002. Microsite and mycorrhizal inoculum effects on the establishment of *Quercus coccifera* in a semi-arid degraded steppe. *Ecological Engineering* 19:289-295.
- Sato, S., and N.B. Comerford. 2006. Organic anions and phosphorus desorption and bioavailability in a humid Brazilian Ultisol. *Soil Science* 171:695-705.
- Sims, J.T. 2000a. Soil test phosphorus: Mehlich 3, p. 17-19, *In* G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University.
- Sims, J.T. 2000b. A Phosphorus Sorption Index, p. 22-23, *In* G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University.
- Singh, K., E. Yoshimura, K. Kanazawa, P.C. Sudhakar, and S. Mori. 2001. Effect of organic acid ligands on nutrient mobilization from volcanic acid soils. *Indian Journal of Agricultural Sciences* 71:760-764.
- Smith, S.E., and D.J. Read. 1997. *Mycorrhizal Symbiosis*. 2nd ed. Academic Press, Inc., San Diego, CA.
- Sparks, D.L., A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnson, and M.E. Sumner. 1996. *Methods of Soil Analysis. Part 3: Chemical Methods* Soil Science Society of America, Inc., and American Society of Agronomy, Inc., Madison, WI.

- Sparling, G.P., K.N. Whale, and A.J. Ramsay. 1985. Quantifying the contribution from the soil microbial biomass to the extractable P levels of fresh and air-dried soils. *Australian Journal of Soil Research* 23:613-621.
- Stevenson, F.J. 1967. Organic acids in soils., p. 119-146, *In* A. D. McLaren and G. H. Peterson, eds. *Soil Biochemistry*. Marcel Dekker, New York.
- Tedersoo, L., T. Suvi, T. Jairus, and U. Koljalg. 2008. Forest microsite effects on community composition of ectomycorrhizal fungi on seedlings of *Picea abies* and *Betula pendula*. *Environmental Microbiology* 10:1189-1201.
- Turner, B.L. 2005. Storage-induced changes in phosphorus solubility of air-dried soils. *Soil Science Society of America Journal* 69:630-633.
- Turner, B.L., and P.M. Haygarth. 2003. Changes in Bicarbonate-extractable Inorganic and Organic Phosphorus by Drying Pasture Soils. *Soil Science Society of America Journal* 67:344-350.
- Turner, B.L., N. Mahieu, and L.M. Condron. 2003. Phosphorus-<sup>31</sup> Nuclear Magnetic Resonance Spectral Assignments of Phosphorus Compounds in Soil NaOH-EDTA Extracts. *Soil Science Society of America Journal* 67:497-510.
- Turner, B.L., B.J. Cade-Menun, L. Condron, and S. Newman. 2005. Extraction of soil organic phosphorus. *Talanta* 66:294-306.
- van Hees, P.A., U.S. Lundstrom, and R. Giesler. 2000. Low molecular weight organics and their Al complexes in soil solution - composition, distribution, and seasonal variation in three podzolized soils. *Geoderma* 94:173-200.
- van Hees, P.A.W., D.L. Godbold, G. Jentschke, and D.L. Jones. 2003. Impact of ectomycorrhizas on the concentration and biodegradation of simple organic acids in a forest soil. *European Journal of Soil Science* 54:697-706.
- van Hees, P.A.W., D.L. Jones, and D.L. Godbold. 2002. Biodegradation of low molecular weight organic acids in coniferous forest podzolic soils. *Soil Biology and Biochemistry*. 34:1261-1272.
- van Hees, P.A.W., D.L. Jones, G. Jentschke, and D.L. Godbold. 2004. Mobilization of aluminum, iron and silicon by *Picea abies* and ectomycorrhizas in a forest soil. *European Journal of Soil Science* 55:101-112.
- van Scholl, L., E. Hoffland, and N. van Breemen. 2006. Organic anion exudation by ectomycorrhizal fungi and *Pinus sylvestris* in response to nutrient deficiencies. *New Phytologist* 170:153-163.
- Villapando, R.R., and D.A. Graetz. 2001. Phosphorus Sorption and Desorption Properties of the Spodic Horizon from Selected Florida Spodosols. *Soil Science Society of America Journal* 65:331-339.
- Wallander, H. 2000. Uptake of P from apatite by *Pinus sylvestris* seedlings colonized by different ectomycorrhizal fungi. *Plant and Soil* :249-256.
- Wallander, H., L. Johansson, and J. Pallon. 2002. PIXE analysis to estimate the elemental composition of ectomycorrhizal rhizomorphs grown in contact with different minerals in forest soil. *FEMS Microbiology Ecology*. 39:147-156.
- Xu, R.K., A.Z. Zhao, and G.L. Ji. 2005. Effect of low molecular weight organic anions on adsorption of potassium by variable charge soils. *Communications in Soil Science and Plant Analysis* 36:1029-1039.

Zhang, Y.-J., N.D. Bryan, F.R. Livens, and M.N. Jones. 1996. Complexing of metal ions by humic substances., p. 194-206, *In* J. S. Gaffney, et al., eds. Humic and Fulvic Acids. ACS, Washington D.C.

## Chapter 5: SUMMARY AND CONCLUSION

Globally, forests cover 4 billion ha of land on the earth surface (30%), and productive plantation forests occupy 3 % or 120 million ha of the forested land (Kirilenko and Sedjo, 2007). The global demand for industrial wood products can be met by intensively managed plantation forests if growth rates of equal  $10 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$  and the percentage of land increased to 4% (Sedjo and Botkin, 1997).

There are 12 million ha of intensively managed pine plantations in the southeastern United States (Conner and Hartsell, 2002). Generally forested lands are located on less fertile soils. Therefore, fertilization with  $28\text{-}56 \text{ kg P ha}^{-1}$  at stand establishment has been a common silvicultural practice in these intensively managed pine forests (Albaugh et al., 2007; Fox et al., 2007). Phosphorus fertilization at establishment increases stand growth and can provide a good economic return on the investment (Fox et al., 2006; Fox et al., 2007). However, because of increasing fertilizer cost it is in the best interest of forest managers to delay and/or reduce the amount of P fertilizer when possible.

The goal of this dissertation was to determine if there are long-term effects of P fertilization on soil P pools in the southeastern United States. To quantify the changes in the soil P pools I extracted P using the Hedley fractionation procedure. I measured the Mehlich 3 extractable P concentrations. I quantified the P concentration in the O horizon. I analyzed organic P pools using liquid state  $^{31}\text{P}$ -NMR (nuclear magnetic resonance) spectroscopy. And I also performed a series of P extraction using oxalate to quantify ligand exchangeable versus dissolvable P pools in the bulk soil and the rhizosphere.

Results from the Hedley fractionation procedure (Chapter 3) indicated that there was a trend of increasing Hedley moderately labile P pools in response to fertilization in the poorly drained

sites. Fertilization effects were limited to the surface depths at all four sites. Hedley labile and moderately labile P pools were largest in the soil surface and decreased with depth. Mehlich-3 extractable P was largest in the 0-10 cm soil depth and decreased with depth. Fertilization significant ( $p < 0.05$ ) increased the Mehlich 3 extractable P in the GA and SC sites. There was a significant fertilizer treatment effect on Mehlich 3 extractable P at the VA ( $p = 0.065$ ) and AL ( $p=0.11$ ) sites. The decrease in Mehlich-3 and Hedley labile and moderately labile P pools with depth reflected the importance of maintaining the integrity of the soil surface for meeting the P demand of the following rotation.

I used liquid state  $^{31}\text{P}$  nuclear magnetic resonance (NMR) spectroscopy (Chapter 2) to characterize the pool of organic P in the surface soil of the VA site. The decision to analyze organic P pools was made based upon the trend of increasing organic P concentration in the Hedley fractionation procedure. After 27 years, P fertilization significantly ( $p = 0.035$ ) increased the P content of the forest floor by  $4.6 \text{ kg ha}^{-1}$ . There was a significant fertilizer effect ( $p = 0.047$ ) on the NaOH-EDTA extractable P in soil. The P content in the soil surface (0-10 cm) of the fertilized treatment plots increased by  $29 \text{ kg ha}^{-1}$ . The NaOH-EDTA extractable P in both treatments were dominated by inorganic orthophosphate.  $^{31}\text{P}$ -NMR spectroscopy revealed that orthophosphate monoesters were the only detectable organic P species in the control and fertilizer treatments. Results suggest that there is a trend towards increase organic P in the fertilized treatments. The increase in the P content of the O horizon may be limiting the accumulation of organic P in the mineral soil.

There was no significant effect of fertilization on oxalate exchangeable P concentrations ( $0.014 \mu\text{mol g}^{-1}$ ) in the bulk soil following fertilization. However, there was a significant increase ( $0.06 \mu\text{mol g}^{-1}$ , 60%) in oxalate dissolvable P pools in response to fertilization. The

ectomycorrhizal rhizosphere depleted a portion of P from these the dissolvable P pools.

Fertilization increased biologically available P pools by 396%. These results demonstrate that although the long-term effect of fertilization at this site is an increase in dissolvable P pools, the ectomycorrhizal rhizosphere has access to this dissolvable pool of P.

Mehlich-3 soil test were able to detect long-term changes in soil P pools when the soil sampled included the 0-10 cm soil depth. Similarly, the Mehlich 3 extractable P concentration increased 60% in response to fertilization when the surface soil sampled was 10 cm deep. The Mehlich 3 extractable P in the control plot was  $6.1 \text{ mg kg}^{-1}$  compared to  $9.8 \text{ mg kg}^{-1}$  in the fertilized plot.

After 27 years, there remains a long-term effect of P fertilization on soil P pools in the VA site. Fertilization immediately increases P on mineral exchange sites and in the soil solution. In some soils there may also be downward movement of the applied P fertilizer. Shortly after fertilization, the P in the soil solution decreases and P is sorbed to ligand exchange sites. The long-term fate of applied P is the increase is P associated with ligand dissolvable P pools in the soil.

Of the  $56 \text{ kg P ha}^{-1}$  originally applied I have accounted for  $33.6 \text{ kg P}$  (60%) in the soil and O horizon. Results from a variety of studies put the P content of fertilized pine stands at  $21\text{-}34 \text{ kg P ha}^{-1}$  (Albaugh et al., 2008; Wells and Jorgensen, 1975; Will et al., 2006). Based upon the increased growth of the VA fertilized pine stand at age 16 the stand has taken up  $6 \text{ kg P ha}^{-1}$ . I have accounted for  $39.6 \text{ kg P ha}^{-1}$  or 70.7 % of the fertilizer P applied in 1980.

Many sites in the southeastern United States and abroad have also shown long-term P fertilization effects on stand growth (Albaugh et al., 2004; Ballard, 1978; Comerford et al., 2002; Turner et al., 2002). One study of P fertilization applied 50 years earlier showed that the stand

volume increased during the second rotation and soil P pools remained significantly higher than the control plots. This suggested that fertilization effectively increased the site index (Turner et al., 2002).

Comerford et al. (2002) argue that in a Georgia Coastal Plains loblolly pine plantation operationally fertilized with concentrated super phosphate at  $50 \text{ kg P ha}^{-1}$  29 years earlier had enough P to meet the demand of the following rotation. Similar to results in this study, Comerford et al. (2002) reported a significant increase in  $P_o$  in the O horizon. Comerford et al. (2002) estimated that a one-year old southern pine stand need upwards of  $2.5 \text{ kg P ha}^{-1}$ . Results from this study show that the biologically available pools of P in the fertilized treatment would meet this demand  $3.02 \text{ kg P ha}^{-1}$ . Additionally, Mehlich 3 extractable P is  $9.8 \text{ mg kg}^{-1}$  in the fertilized plots, higher than the P critical level of  $6 \text{ mg kg}^{-1}$  (Fox et al., 2007). Based upon these results, I can say there has been a significant and long-term increase in soil P pools. Also, the results suggest the next pine rotation may not need P fertilization at establishment to meet the stand demand during the first one – three years of growth.

## Literature Cited

- Albaugh, T.J., H.L. Allen, and T.R. Fox. 2007. Historical Patterns of Forest Fertilization in the Southeastern United States from 1969 to 2004. *Southern Journal of Applied Forestry* 31:129-137.
- Albaugh, T.J., H.L. Allen, and T.R. Fox. 2008. Nutrient use and uptake in *Pinus taeda*. *Tree Physiology* 28:1083-98.
- Albaugh, T.J., H.L. Allen, P.M. Dougherty, and K.H. Johnsen. 2004. Long term growth responses of loblolly pine to optimal nutrient and water resource availability. *Forest Ecology and Management* 192:3-19.
- Ballard, R. 1978. Effect of first rotation phosphorus applications on fertilizer requirements of second rotation pine. *New Zealand Journal of Forestry Science*. 8:135-145.
- Comerford, N.B., M. McLeod, and M. Skinner. 2002. Phosphorus form and bioavailability in the pine rotation following fertilization, P fertilization influences P form and potential bioavailability to pine in the subsequent rotation. *Forest Ecology and Management* 169:203-211.
- Conner, R.C., and A.J. Hartsell. 2002. Forest Area and Conditions. In D. N. Wear and J. G. Greis, (eds.) *Southern Forest Resource Assessment*. Southern Research Station, Asheville, NC. p. 357-402.
- Fox, T.R., H.L. Allen, T.J. Albaugh, R. Rubilar, and C.A. Carlson. 2006. Forest Fertilization in Southern Pine Plantations. *Better Crops* 90:12-15.
- Fox, T.R., H.L. Allen, T.J. Albaugh, R. Rubilar, and C.A. Carlson. 2007. Tree Nutrition and Forest Fertilization of Pine Plantations in the Southern United States. *Southern Journal of Applied Forestry* 31:5-11.
- Kirilenko, A.P., and R.A. Sedjo. 2007. Climate Change and Food Security Special Feature: Climate change impacts on forestry. *PNAS* 104:19697-19702.
- Sedjo, R.A., and D. Botkin. 1997. Using forest plantations to spare natural forests. *Environment* 39:14-30.
- Turner, J., M.J. Lambert, and F.R. Humphreys. 2002. Continuing growth response to phosphate fertilizers by a *Pinus radiata* plantation over fifty years. *Forest Science*. 48:556-568.
- Wells, C.G., and J.R. Jorgensen. 1975. Nutrient cycling in loblolly pine plantations. *Les Presses de L'Université, Laval, Quebec*. p. 137-158.
- Will, R.E., D. Markewitz, R.L. Hendrick, D.F. Meason, T.R. Crocker, and B.E. Borders. 2006. Nitrogen and phosphorus dynamics for 13-year-old loblolly pine stands receiving complete competition control and annual N fertilizer. *Forest Ecology and Management* 227:155-168.

## Dissertation References

- Agerer, R. 1997. Descriptions of Ectomycorrhizae. Einhorn-Verlag Eduard Dietenberger GmbH Schwabisch Gmund,, Munich, Germany.
- Agerer, R. 2001. Exploration types of ectomycorrhizae - A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza* 11:107-114.
- Agerer, R. 2006. Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress* 5:67-107.
- Albaugh, T.J., H.L. Allen, and T.R. Fox. 2007. Historical Patterns of Forest Fertilization in the Southeastern United States from 1969 to 2004. *Southern Journal of Applied Forestry* 31:129-137.
- Albaugh, T.J., H.L. Allen, and T.R. Fox. 2008. Nutrient use and uptake in *Pinus taeda*. *Tree Physiology* 28:1083-98.
- Albaugh, T.J., H.L. Allen, P.M. Dougherty, and K.H. Johnsen. 2004. Long term growth responses of loblolly pine to optimal nutrient and water resource availability. *Forest Ecology and Management* 192:3-19.
- Albaugh, T.J., H.L. Allen, P.M. Dougherty, L.W. Kress, and J.S. King. 1998. Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions. *Forest Science* 44.
- Allen, B.L., and A.P. Mallarino. 2006. Relationships between extractable soil phosphorus and phosphorus saturation after long-term fertilizer or manure application. *Soil Science Society of America Journal* 70:454-463.
- Allen, H.L. 1996. Effects of site preparation and early fertilization and weed control on 14-yr loblolly pine growth. College of Forest Resources, North Carolina State University, Raleigh, NC.
- Allen, H.L., P.M. Dougherty, and R.G. Campbell. 1990. Manipulation of water and nutrients -- Practice and opportunity in Southern U.S. pine forests. *Forest Ecology and Management* 30:437-453.
- Allen, H.L., D.L. Kelting, K.B. Piatek, and S.B. Jeffries. 2000. Long-term forest site productivity in third rotation loblolly pine plantations with contrasting silvicultural treatments. Paper presented at the Long-term Soil Productivity Conference. USFS, Alexandria, LA.
- Alvarez, M., R. Godoy, W. Heyser, and S. Hartel. 2004. Surface-bound phosphatase activity in living hyphae of ectomycorrhizal fungi of *Nothofagus obliqua*. *Mycologia* 96:479-487.
- Arvieu, J.C., F. Leprince, and C. Plassard. 2003. Release of oxalate and protons by ectomycorrhizal fungi in response to P-deficiency and calcium carbonate in nutrient solution. *Annals of Forest Science* 60:815-821.
- Baier, R., J. Ingenhaag, H. Blaschke, A. Gottlein, and R. Agerer. 2006. Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (*Picea abies* [L.] Karst.) stand of the Bavarian Limestone Alps. *Mycorrhiza* 16:197-206.
- Ballard, R. 1978. Effect of first rotation phosphorus applications on fertilizer requirements of second rotation pine. *New Zealand Journal of Forestry Science*. 8:135-145.
- Ballard, R. 1980. Phosphorus nutrition and fertilization of forest trees. In: *Role of phosphorus in agriculture*. F. E. Khasawneh, E. C. Sample, E. J. Kamprath.:763-804.

- Bardgett, R.D., and K.F. Chan. 1999. Experimental evidence that soil fauna enhance nutrient mineralization and plant nutrient uptake in montane grassland ecosystems. *Soil Biology and Biochemistry* 31:1007-1014.
- Beauchemin, S., R.R. Simard, and D. Cluis. 1996. Phosphorus sorption-desorption kinetics of soil under contrasting land uses. *Journal of environmental quality*. 25:1317-1325.
- Beck, M.A., and P.A. Sanchez. 1994. Soil phosphorus fraction dynamics during 18 years of cultivation on a typic paleudult. *Soil Science Society of America Journal* 58:1424-1431.
- Bending, G.D., and D.J. Read. 1995a. The structure and function of the vegetative mycelium of ectomycorrhizal plants V. Foraging behavior and translocation of nutrients from exploited litter. *New Phytologist* 130:401-409.
- Bending, G.D., and D.J. Read. 1995b. The structure and function of the vegetative mycelium of ectomycorrhizal plants VI. Activities of nutrient mobilizing enzymes in birch litter colonized by *Paxillus involutus* (Fr.) Fr. *New Phytologist* 130:411-417.
- Bertsch, P.M., and P.R. Bloom. 1996. Aluminum, p. 517-534, In D. L. Sparks, et al., eds. *Methods of Soil Analysis. Part 3: Chemical Methods*. Soil Science Society of America, Inc., and American Society of Agronomy, Inc., Madison, WI.
- Bowman, R.A., and C.V. Cole. 1978. An exploratory method for fractionation of organic phosphorus from grassland soils. *Soil Science*. 125:95-101.
- Bowman, R.A., J.B. Rodriguez, and J.R. Self. 1998. Comparison of methods to estimate occluded and resistant soil phosphorus. *Soil Science Society of America Journal* 62:338-342.
- Brady, N.C., and R.R. Weil. 2002. *The nature and properties of soils*. 13th ed. Prentice Hall, Upper Saddle River.
- Borggaard, O. K. 1983. The influence of iron oxides on phosphate adsorption by soil. *European Journal of Soil Science* 34:333-341.
- Bücking, H., and W. Heyser. 1999. Elemental composition and function of polyphosphates in ectomycorrhizal fungi – an X-ray microanalytical study. *Mycological Research* 103:31-39.
- Cade-Menun, B.J. 2005. Characterizing phosphorus in environmental and agricultural samples by <sup>31</sup>P nuclear magnetic resonance spectroscopy. *Talanta* 66:359-371.
- Cade-Menun, B.J., and C.M. Preston. 1996. A comparison of soil extraction procedures for <sup>31</sup>P NMR spectroscopy. *Soil Science*. 161:770-785.
- Cade-Menun, B.J., and L.M. Lavkulich. 1997. A comparison of methods to determine total, organic, and available phosphorus in forest soils. *Communications in Soil Science and Plant Analysis*. 28:651-663.
- Cade-Menun, B.J., S.M. Berch, C.M. Preston, and L.M. Lavkulich. 2000. Phosphorus forms and related soil chemistry of podzolic soils on northern Vancouver Island. I. A comparison of two forest types. *Canadian Journal of Forest Research* . 30:1714-1725.
- Cajuste, L.J., R.J. Laird, B. Cajuste, Jr., and B.G. Cuevas. 1996. Citrate and oxalate influence on phosphate, aluminum, and iron in tropical soils. *Communications in Soil Science and Plant Analysis*. 27:1377-1386.
- Casarin, V., C. Plassard, G. Souche, and J.-C. Arvieu. 2003. Quantification of oxalate ions and protons released by ectomycorrhizal fungi in rhizosphere soil. *Agronomie* 23:461-469.
- Chamberlain, P.M., N.P. McNamara, J. Chaplow, A.W. Stott, and H.I.J. Black. 2006. Translocation of surface litter carbon into soil by Collembola. *Soil Biology and Biochemistry* 38:2655-2664.

- Chang, S.C., and M.L. Jackson. 1957. Fractionation of soil phosphorus. *Soil Science* 84:133-144.
- Chen, C.R., L.M. Condon, M.R. Davis, and R.R. Sherlock. 2000. Effects of afforestation on phosphorus dynamics and biological properties in a New Zealand grassland soil. *Plant and Soil*. 220:151-163.
- Chen, C.R., L.M. Condon, M.R. Davis, and R.R. Sherlock. 2002. Phosphorus dynamics in the rhizosphere of perennial ryegrass (*Lolium perenne* L.) and radiata pine (*Pinus radiata* D. Don.). *Soil Biology Biochemistry* 34:487-499.
- Chen, C.R., L.M. Condon, B.L. Turner, N. Mahieu, M.R. Davis, Z.H. Xu, and R.R. Sherlock. 2004. Mineralisation of soil orthophosphate monoesters under pine seedlings and ryegrass. *Australian Journal of Soil Research* 42:189-196.
- Chen, Y., N. Senesi, and M. Schnitzer. 1977. Information provided on humic substances by E4/E6 ratios. *Soil Science Society of America Journal* 41:352-8.
- Chilimba, A.D.C., S.K. Mughogho, and J. Wendt. 1999. Mehlich 3 or modified Olsen for soil testing in Malawi. *Communications in Soil Science and Plant Analysis*. 30:1231-1250.
- Colpaert, J.V., and K.K. vanTichelen. 1996. Decomposition, nitrogen and phosphorus mineralization from beech leaf litter colonized by ectomycorrhizal or litter-decomposing basidiomycetes. *New Phytologist* 134:123-132.
- Colpaert, J.V., A. VanLaere, K.K. VanTichelen, and J.A. VanAssche. 1997. The use of inositol hexaphosphate as a phosphorus source by mycorrhizal and non-mycorrhizal Scots Pine (*Pinus sylvestris*). *Functional Ecology* 11:407-415.
- Comerford, N.B., M. McLeod, and M. Skinner. 2002a. Phosphorus form and bioavailability in the pine rotation following fertilization, P fertilization influences P form and potential bioavailability to pine in the subsequent rotation. *Forest Ecology and Management* 169:203-211.
- Comerford, N.B., M. McLeod, and M. Skinner. 2002b. Phosphorus form and bioavailability in the pine rotation following fertilization - P fertilization influences P form and potential bioavailability to pine in the subsequent rotation. *Forest Ecology and Management* 169:203-211.
- Condon, L., E. Frossard, R.H. Newman, P. Tekely, and J.L. Morel. 1997. Use of <sup>31</sup>P NMR in the study of soils and the environment. In M. A. Nannny, et al., eds. *Nuclear Magnetic Resonance Spectroscopy in Environmental Chemistry*. Oxford University Press Inc., New York. p. 247-271.
- Condon, L.M., B.L. Turner, and B.J. Cade-Menun. 2005. Chemistry and dynamics of soil organic phosphorus. *American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc.* p. 87-121.
- Condon, L.M., M.R. Davis, R.H. Newman, and I.S. Cornforth. 1996. Influence of conifers on the forms of phosphorus in selected New Zealand grassland soils. *Biology and Fertility of Soils* 21:37-42.
- Condon, L.M., E. Frossard, H. Tiessen, R.H. Newman, and J.W.B. Stewart. 1990. Chemical nature of organic phosphorus in cultivated and uncultivated soils under different environmental conditions. *Journal of Soil Science* 41:41-50.
- Conn, C., and J. Dighton. 2000. Litter quality influences on decomposition, ectomycorrhizal community structure and mycorrhizal root surface acid phosphatase activity. *Soil Biology Biochemistry* 32:489-496.

- Conner, R.C., and A.J. Hartsell. 2002. Forest Area and Conditions, pp. 357-402, In D. N. Wear and J. G. Greis, (eds.) Southern Forest Resource Assessment. Southern Research Station, Asheville, NC.
- Cooperative, F.N. 1991. Effects of site preparation, fertilization, and weed control on the growth and nutrition of old loblolly pine. Raleigh, NC. North Carolina State and Virginia Tech.
- Cooperative, F.N. 1996. Effects of site preparation and early fertilization and weed control on 14-year old loblolly pine growth. Raleigh, NC. North Carolina State and Virginia Tech.
- Cox, F.R., and J.S. Barnes. 2002. Peanut, corn, and cotton critical levels for phosphorus and potassium on Goldsboro soil. *Communications in Soil Science and Plant Analysis*. 33:1173-1186.
- Cromack Jr., K., P. Sollins, W.C. Graustein, K. Speidel, A.W. Todd, G. Spycher, C.Y. Li, and R.L. Todd. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. *Soil Biology and Biochemistry* 11:463-468.
- Cross, A.F., and W.H. Schlesinger. 1995. A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64:197-214.
- Dai, K.H., M.B. David, G.F. Vance, and A.J. Krzyszowska. 1996. Characterization of phosphorus in a spruce-fir spodosol by phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Science Society of America Journal* 60:1943-1950.
- Dalla-Tea, F., and E.J. Jokela. 1991. Needlefall, canopy light interception and productivity of young intensively managed slash and loblolly pine stands. *Forest Science* 37:1298-1313.
- Delgado, A., and J. Torrent. 2000. Phosphorus forms and desorption patterns in heavily fertilized calcareous and limed acid soils. *Soil Science Society of America Journal* 64:2031-2037.
- Drummond, M.A. 2008. Souther Coastal Plain, In T. R. L. W. Acevedo, ed. Status and trends of eastern United States Land Cover. USGS.
- Durall, D.M., J.D. Marshall, M.D. Jones, R. Crawford, and J.M. Trappe. 1994. Morphological changes and photosynthate allocation in ageing *Hebeloma crustuliniforme* (Bull.) Quel. and *Laccaria bicolor* (Maire) Orton mycorrhizas of *Pinus ponderosa* Dougl. ex. Laws. *New Phytologist* 127:719-724.
- Eick, M.J., J.D. Peak, and W.D. Brady. 1999. The Effect of Oxyanions on the Oxalate-Promoted Dissolution of Goethite. *Soil Science Society of America Journal* 63:1133-1141.
- Essington, M.E. 2004. *Soil and Water Chemistry: An integrative approach*. CRC Press, New York.
- Field, J.P., K.W. Farrish, and E.A. Carter. 2003. Soil and nutrient losses following site preparation burning in a harvested loblolly pine site. *Transactions of the ASAE* 46:1697-1703.
- Fife, D.N., and E.K.S. Nambiar. 1999. Response to phosphorus application of second rotation radiata pine on podsolised sands from planting to first thinning: implications for management. *Australian Forestry*. 62:109-119.
- Fox, T.R., and N.B. Comerford. 1990a. Influence of oxalate loading on phosphorus and aluminum solubility in spodosols. *Soil Science Society of America Journal* 56:290-294.
- Fox, T.R., and N.B. Comerford. 1990b. Low-molecular-weight organic acids in selected forest soils of the southeastern USA. *Soil Science Society of America Journal* 54:1139-1144.
- Fox, T.R., and N.B. Comerford. 1992b. Rhizosphere phosphatase activity and phosphatase hydrolysable organic phosphorus in two forested spodosols. *Soil Biology Biochemistry* 24:579-583.

- Fox, T.R., L.A. Morris, and R.A. Maimone. 1988. The impact of windrowing on the productivity of a rotation age loblolly pine plantation, In USDA, (ed.). Forest Service General Technical Report, SO-74.
- Fox, T.R., N.B. Comerford, and W.W. Mcfee. 1990. Kinetics of Phosphorus Release from Spodosols - Effects of Oxalate and Formate. *Soil Science Society of America Journal* 54:1441-1447.
- Fox, T.R., E.J. Jokela, and H.L. Allen. 2007a. The development of pine plantation silviculture in the southern United States. *Journal of Forestry* 105:337-347.
- Fox, T.R., H.L. Allen, T.J. Albaugh, R. Rubilar, and C.A. Carlson. 2006. Forest Fertilization in Southern Pine Plantations. *Better Crops* 90:12-15.
- Fox, T.R., H.L. Allen, T.J. Albaugh, R. Rubilar, and C.A. Carlson. 2007b. Tree Nutrition and Forest Fertilization of Pine Plantations in the Southern United States. *Southern Journal of Applied Forestry* 31:5-11.
- Garcia-Marco, S., N. Martinez, F. Yunta, L. Hernandez-Apaolaza, and J.J. Lucena. 2006. Effectiveness of ethylenediamine-N(o-hydroxyphenylacetic)-N'(p-hydroxy-phenylacetic) acid (o,p-EDDHA) to supply iron to plants. *Plant and Soil* 279:31-40.
- Geelhoed, J.S., G.R. Findenegg, and W.H. VanRiemsdijk. 1997. Availability to plants of phosphate adsorbed on goethite: experiment and simulation. *European Journal of Soil Science* 48:473-481.
- Gentle, S.W., F.R. Humphreys, and M.J. Lambert. 1986. Continuing response of *Pinus radiata* to phosphate fertilizers over two rotations. *Forest Science* 32:822-829.
- George, T.S., B.L. Turner, P.J. Gregory, B.J. Cade-Menun, and A.E. Richardson. 2006. Depletion of organic phosphorus from Oxisols in relation to phosphatase activities in the rhizosphere. *European Journal of Soil Science* 57:47-57.
- Gerke, J., L. Beissner, and W. Romer. 2000. The quantitative effect of chemical phosphate mobilization by carboxylate anions on P uptake by a single root. I. The basic concept and determination of soil parameters. *Journal of Plant Nutrition and Soil Science* 163:207-212.
- Gil-Sotres, F., W. Zech, and H.G. Alt. 1990. Characterization of phosphorus fractions in surface horizons of soils from Galicia (N.W. Spain) by  $^{31}\text{P}$  NMR spectroscopy. *Soil Biology and Biochemistry* 22:75-79.
- Gressel, N., J.G. McColl, C.M. Preston, R.H. Newman, and R.F. Powers. 1996. Linkages between phosphorus transformations and carbon decomposition in a forest soil. *Biogeochemistry* 33:97-123.
- Harding, R.B., and E.J. Jokela. 1994. Long-term effects of forest fertilization on site organic matter nutrients. *Soil Science Society of America Journal* 58:216-221.
- Hawkes, G.E., D.S. Powlson, E.W. Randall, and K.R. Tate. 1984. A  $^{31}\text{P}$  nuclear magnetic resonance study of the phosphorus species in alkali extracts of soils from long-term field experiments. *Journal of Soil Science* 35:34-45.
- He, Z., T.S. Griffin, and C.W. Honeycutt. 2004. Enzymatic hydrolysis of organic phosphorus in swine manure and soil. *Journal of environmental quality* 33:367-372.
- Hedley, M.J., and M.J. McLaughlin. 2005. Reactions of phosphate fertilizers and by-products in soils. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc. p. 181-252

- Hedley, M.J., J.W.B. Stewart, and B.S. Chauhan. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Journal - Soil Science Society of America Journal* 46:970-976.
- Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil* 237:173-195.
- Illmer, P., and F. Schinner. 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biology and Biochemistry*. 24:389-395.
- Indiati, R., C. Izza, A. Figliolia, B. Felici, E. Coppola, C. Buondonno, and A. Buondonno. 1997. Comparing test for soil fertility. III. Evaluation of phosphate availability in Alfisols by Olsen, Mehlich 3, electro-ultrafiltration, and the paper-strip methodology procedures. *Communications in Soil Science and Plant Analysis*. 28:997-1009.
- Irving, H., and R.J.P. Williams. 1953. The stability of transition-metal complexes. *Journal of the American Chemical Society* 75:3192-3210.
- Jackson, M.L., C.H. Lim, and L.W. Zelazny. 2005. Oxides, Hydroxides, and Aluminosilicates, p. 101-150. In A. L. Page, et al., eds. *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods*. , 2nd. ed. American Society of Agronomy-Soil Science Society of America, Madison, WI.
- Jakobsen, I., M.E. Leggett, and A.E. Richardson. 2005. Rhizosphere microorganisms and plant phosphorus uptake American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc.
- Johnson, A.H., J. Frizano, and D.R. Vann. 2003. Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. *Oecologia* 135:487-499.
- Johnson, S.E., and R.H. Loeppert. 2006. Role of organic acids in phosphate mobilization from iron oxide. *Soil Science Society of America Journal* 70:222-234.
- Kelly, J.M., and J.K. Kelly. 2001. Phosphorus and potassium uptake kinetics in red maple seedlings. *Forest Science*. 47:397-402.
- Kelting, D.L. 2000. Loblolly pine growth responses to early fertilization. College of Natural Resources, North Carolina State University, Raleigh, NC.
- Kirilenko, A.P., and R.A. Sedjo. 2007. Climate Change and Food Security Special Feature: Climate change impacts on forestry. *PNAS* 104:19697-19702.
- Kitayama, K., N. Majalap-Lee, and S. Aiba. 2000. Soil phosphorus fractionation and phosphorus-use efficiencies of tropical rainforests along altitudinal gradients of Mount Kinabalu, Borneo. *Oecologia*. 123 (3):342-349.
- Kyle, K.H., L.J. Andrews, T.R. Fox, W.M. Aust, J.A. Burger, and G.H. Hansen. 2005. Long-term effects of drainage, bedding, and fertilization on growth of loblolly pine (*Pinus taeda* L.) in the Coastal Plain of Virginia. *Southern Journal of Applied Forestry* 29:205-214.
- Lapeyrie, F., G.A. Chilvers, and C.A. Bhem. 1987. Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus* (Batsch. ex Fr.) Fr. *New Phytologist* 106:139-146.
- Lawrence, D., and W.H. Schlesinger. 2001. Changes in soil phosphorus during 200 years of shifting cultivation in Indonesia. *Ecology* 82:2769-2780.
- Lindo, Z., and S. Visser. 2003. Microbial biomass, nitrogen and phosphorus mineralization, and mesofauna in boreal conifer and deciduous forest floors following partial and clear-cut harvesting. *Canadian Journal of Forest Research* 33:1610-1619.

- Liu, Q., P. Loganathan, M.J. Hedley, and M.F. Skinner. 2004. The mobilisation and fate of soil and rock phosphate in the rhizosphere of ectomycorrhizal *Pinus radiata* seedlings in an Allophanic soil. *Plant and Soil* 264:219-229.
- Lockaby, B.G., J.H. Miller, and R.G. Clawson. 1995. Influences of community composition on biogeochemistry of loblolly pine (*Pinus taeda*) systems. *American Midland Naturalist* 134:176-184.
- Maestre, F.T., S. Bautista, J. Cortina, G. Diaz, M. Honrubia, and R. Vallejo. 2002. Microsite and mycorrhizal inoculum effects on the establishment of *Quercus coccifera* in a semi-arid degraded steppe. *Ecological Engineering* 19:289-295.
- Magid, J., H. Tiessen, and L.M. Condron. 1996. Dynamics of organic phosphorus in soils under natural and agricultural ecosystems. In G. Piccolo, ed. *Humic substances in terrestrial ecosystems*. Elsevier, Amsterdam. p. 429-466.
- Moody, P.W., D.G. Edwards, and L.C. Bell. 1995. Effect of banded fertilizers on soil solution composition and short-term root-growth .2. Mono-ammonium and di-ammonium phosphates. *Australian Journal of Soil Research* 33:689-707.
- Muljadi, D., A.M. Posner, and J.P. Quirk. 1966a. The mechanism of phosphate adsorption by kaolinite, gibbsite, and pseudoboehmite. Part 1. The isotherm and the affect of pH on adsorption. *Journal of Soil Science* 17:219-229.
- Muljadi, D., A.M. Posner, and J.P. Quirk. 1966b. The mechanism of phosphate adsorption by kaolinite, gibbsite, and pseudoboehmite. Part 2. The location of the adsorption sites. . *Journal of Soil Science* 17:230-237.
- Muljadi, D., A.M. Posner, and J.P. Quirk. 1966c. The mechanism of phosphate adsorption by kaolinite, gibbsite, and pseudoboehmite. Part 3. The effect of temperature on the adsorption. . *Journal of Soil Science* 17:238-247.
- Murphy, J.F., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* 27:31-36.
- Newman, R.H., and K.R. Tate. 1980. Soil characterized by <sup>31</sup>P nuclear magnetic resonance. *Communications in Soil Science and Plant Analysis* 11:835-842.
- Parfitt, R., R.J. Atkinson, and R.C. Smart. 1975. The mechanism of phosphate fixation on iron oxides. *Soil Science Society of America Journal* 39:837-841.
- Parsons, K.J., V.D. Zheljzkov, J. MacLeod, and C.D. Caldwell. 2007. Soil and Tissue Phosphorus, Potassium, Calcium, and Sulfur as Affected by Dairy Manure Application in a No-Till Corn, Wheat, and Soybean Rotation. *Agronomy Journal* 99:1306-1316.
- Penn, C.J., G.L. Mullins, and L.W. Zelazny. 2005. Mineralogy in relation to phosphorus sorption and dissolved phosphorus losses in runoff. *Soil Science Society of America Journal* 69:1532-1540.
- Phillips, R.P., and T.J. Fahey. 2006. Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects. *Ecology* 87:1302-1313.
- Piatek, K.B., and H.L. Allen. 2001. Are forest floors in mid-rotation stands of loblolly pine (*Pinus taeda*) a sink for nitrogen and phosphorus. *Canadian Journal of Forest Research* 31:1164-1174.
- Pierzynski, G., R.W. McDowell, and J.T. Sims. 2005a. Chemistry, cycling, and potential movement of inorganic phosphorus in soils, p. 53-86, In J. T. Sims and A. N. Sharpley, eds. *Phosphorus: Agriculture and the Environment* American Society of Agronomy, Madison, WI.

- Pierzynski, G.M. 2000. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters* North Carolina State University.
- Pierzynski, G.M., R.W. McDowell, and J.T. Sims. 2005b. Chemistry, cycling, and potential movement of inorganic phosphorus in soils American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc.
- Polglase, P.J., E.J. Jokela, and N.B. Comerford. 1992a. Phosphorus, nitrogen, and carbon fractions in litter and soil of southern pine plantations. *Soil Science Society of America Journal* 56:566-573.
- Polglase, P.J., N.B. Comerford, and E.J. Jokela. 1992b. Mineralization of nitrogen and phosphorus from soil organic matter in southern pine plantations. *Soil Science Society of America Journal* 56:921-927.
- Polglase, P.J., E.J. Jokela, and N.B. Comerford. 1992c. Nitrogen and phosphorus release from decomposing needles of southern pine plantations. *Soil Science Society of America Journal* 56:914-920.
- Polglase, P.J., N.B. Comerford, and E.J. Jokela. 1992d. Mineralization of Nitrogen and Phosphorus from Soil Organic-Matter in Southern Pine Plantations. *Soil Science Society of America Journal* 56:921-927.
- Pritchett, W.-L. 1979. *Properties and management of forest soils* Wiley, New York
- Pritchett, W.L., and N.B. Comerford. 1982. Long-term response to phosphorus fertilization on selected southeastern coastal plain soils. *Soil Science Society of America Journal* 46:640 – 644.
- Pritchett, W.L., Llewellyn, W.R., Swinford, K.R. 1961. Response of slash pine to colloidal phosphate fertilization. *Soil Science Society of America Journal* 25:397-400.
- Quin, L.D., and G.S. Quin. 2001. Screening for carbon- bound phosphorus in marine animals by high resolution <sup>31</sup>P-NMR spectroscopy: Coastal and hydrothermal vent invertebrates. *Comparative Biochemistry and Physiology* 128:173-185.
- Raghothama, K.G. 1999. Phosphate acquisition. *Annual Review of Plant Physiology and Plant Molecular Biology* 50:665-693.
- Raghothama, K.G. 2005. Phosphorus and plant nutrition: An overview. American Society of Agronomy, Inc., Crop Science Society of America, Inc., Soil Science Society of America, Inc., Madison.
- Rasmussen, N., D.C. Lloyd, R.G. Ratcliffe, P.E. Hansen, and I. Jakobsen. 2000. <sup>31</sup>P NMR for the study of P metabolism and translocation in arbuscular mycorrhizal fungi. *Plant and Soil* 226:245-253.
- Richter, D.D., H.L. Allen, J. Li, D. Markewitz, and J. Raikes. 2006. Bioavailability of slowly cycling soil phosphorus: major restructuring of soil P fractions over four decades in an aggrading forest. *Oecologia* 150:259-271.
- Saggar, S., R.L. Parfitt, G. Salt, and M.F. Skinner. 1998. Carbon and phosphorus transformations during decomposition of pine forest floor with different phosphorus status. *Biology and Fertility of Soils* 27:197-204.
- Sample, E.C., R.J. Soper, and G.J. Racz. 1980. Reactions of phosphate fertilizers in soils. Role of phosphorus in agriculture, editors F. E. Khasawneh, E. C. Sample, E. J. Kamprath.:263-310.
- Sato, S., and N.B. Comerford. 2006. Organic anions and phosphorus desorption and bioavailability in a humid Brazilian Ultisol. *Soil Science* 171:695-705.

- Sedjo, R.A., and D. Botkin. 1997. Using forest plantations to spare natural forests. *Environment* 39:14-30.
- Sims, J.T. 2000a. Soil test phosphorus: Mehlich 1, p. 15-16, In G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University.
- Sims, J.T. 2000b. A Phosphorus Sorption Index, p. 22-23, In G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University.
- Sims, J.T. 2000c. Soil test phosphorus: Mehlich 3, p. 17-19, In G. M. Pierzynski, ed. *Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters*. North Carolina State University.
- Singh, K., E. Yoshimura, K. Kanazawa, P.C. Sudhakar, and S. Mori. 2001. Effect of organic acid ligands on nutrient mobilization from volcanic acid soils. *Indian Journal of Agricultural Sciences* 71:760-764.
- Smeck, N.E. 1985. Phosphorus dynamics in soils and landscapes. *Geoderma* 36:185-199.
- Smith, S.E., and D.J. Read. 1997. *Mycorrhizal Symbiosis*. 2nd ed. Academic Press, Inc., San Diego, CA.
- Solomon, D., J. Lehmann, T. Mamo, F. Fritzsche, and W. Zech. 2002. Phosphorus forms and dynamics as influenced by land use changes in the sub-humid Ethiopian highlands. *Geoderma* 105:21-48.
- Sommers, L.E., and D.W. Nelson. 1972. Determination of total phosphorus in soils: a rapid perchloric acid digestion procedure. *Proceeding of Soil Science Society of America*, no 3:902-904.
- Sparks, D.L., A.L. Page, P.A. Helmke, R.H. Loeppert, P.N. Soltanpour, M.A. Tabatabai, C.T. Johnson, and M.E. Sumner. 1996. *Methods of Soil Analysis. Part 3: Chemical Methods* Soil Science Society of America, Inc., and American Society of Agronomy, Inc., Madison, WI.
- Sparling, G.P., K.N. Whale, and A.J. Ramsay. 1985. Quantifying the contribution from the soil microbial biomass to the extractable P levels of fresh and air-dried soils. *Australian Journal of Soil Research* 23:613-621.
- Staaf, H., and B. Berg. 1982. Accumulation and release of plant nutrients in decomposing scots pine needle litter. Long-term decomposition in a Scots Pine forest. II. *Canadian Journal of Botany* 60:1561-1568.
- Stevenson, F.J. 1967. Organic acids in soils., p. 119-146, In A. D. McLaren and G. H. Peterson, eds. *Soil Biochemistry*. Marcel Dekker, New York.
- Sui, Y., M.L. Thompson, and C. Shang. 1999. Fractionation of phosphorus in a Mollisol amended with biosolids. *Soil Science Society of America Journal* 63:1174-1180.
- Tate, K.R., and R.H. Newman. 1982. Phosphorus fractions of a clinosequence of soils in New Zealand tussock grassland. *Soil Biology and Biochemistry*. 14 (3):191-196.
- Tedersoo, L., T. Suvi, T. Jairus, and U. Koljalg. 2008. Forest microsite effects on community composition of ectomycorrhizal fungi on seedlings of *Picea abies* and *Betula pendula*. *Environmental Microbiology* 10:1189-1201.
- Tew, D.T., L.A. Morris, H.L. Allen, and C.G. Wells. 1986. Estimates of nutrient removal, displacement and loss resulting from harvest and site preparation of a *Pinus taeda* plantation in the piedmont of North Carolina. *Forest Ecology and Management* 15:257-267.

- Tiessen, H., and J.O. Moir. 1993. Characterization of available P by sequential extraction., p. 75-86, In M. R. Carter, ed. *Soil Sampling and Methods of Analysis*. Lewis Publishers.
- Tiessen, H., J.W.B. Stewart, and C.V. Cole. 1984a. Pathways of phosphorus transformations in soils of differing pedogenesis. *Soil Science Society of America Journal* , vol 48:853-858.
- Tiessen, H., J. Stewart, and C.V. Cole. 1984b. Pathways of phosphorus transformations in soils of differing pedogenesis. *Soil Science Society of America Journal* 48:853-858.
- Tucker, M.R. 1992. Determination of phosphorus by Mehlich 3 extractant. , p. 9-12, In S. J. Donohue, ed. *Reference soil and media diagnostic procedure for the southern region of the United States*. Va. Agric. Exp. Station, Blacksburg, VA.
- Turner, B.L. 2005. Storage-induced changes in phosphorus solubility of air-dried soils. *Soil Science Society of America Journal* 69:630-633.
- Turner, B.L., and P.M. Haygarth. 2003. Changes in Bicarbonate-extractable Inorganic and Organic Phosphorus by Drying Pasture Soils. *Soil Science Society of America Journal* 67:344-350.
- Turner, B.L., and A.E. Richardson. 2004. Identification of scyllo-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. *Soil Science Society of America Journal* 68:802-808.
- Turner, B.L., N. Mahieu, and L.M. Condon. 2003a. Phosphorus-31 Nuclear Magnetic Resonance Spectral Assignments of Phosphorus Compounds in Soil NaOH-EDTA Extracts. *Soil Science Society of America Journal* 67:497-510.
- Turner, B.L., N. Mahieu, and L.M. Condon. 2003b. Quantification of myo-inositol hexakisphosphate in alkaline soil extracts by solution <sup>31</sup>P NMR spectroscopy and spectral deconvolution. *Soil Science* 168:469-478.
- Turner, B.L., B.J. Cade-Menun, and D.T. Westermann. 2003c. Organic phosphorus composition and potential bioavailability in semi-arid arable soils of the western United States. *Soil Science Society of America Journal* 67:1168-1179.
- Turner, B.L., M.J. Paphazy, P.M. Haygarth, and I.D. McKelvie. 2002a. Inositol phosphates in the environment. *Philosophical Transactions of the Royal Society of London, B* 357:449-469.
- Turner, B.L., N. Mahieu, L.M. Condon, and C.R. Chen. 2005a. Quantification and bioavailability of scyllo-inositol hexakisphosphate in pasture soils. *Soil Biology and Biochemistry*. v. 37, no. 11:2155-2158.
- Turner, B.L., N. Mahieu, L.M. Condon, and C.R. Chen. 2005b. Quantification and bioavailability of scyllo-inositol hexakisphosphate in pasture soils. *Soil Biology and Biochemistry* 37:2155-2158.
- Turner, B.L., B.J. Cade-Menun, L. Condon, and S. Newman. 2005c. Extraction of soil organic phosphorus. *Talanta* 66:294-306.
- Turner, B.L., L. Condon, S. Richardson, D.A. Peltzer, and V. Allison. 2006. Transformations of soil organic phosphorus during ecosystem development. *ASA-CSSA-Soil Science Society of America Journal* 2006 International Annual Meetings, Indianapolis, IN.
- Turner, J., and M.J. Lambert. 1988. Long-term effects of phosphorus fertilization on forests., p. 125-133, In B. B. and W. C. H., eds. *Forest site evaluation and long-term productivity*. University of Washington Press, Seattle, WA.
- Turner, J., M.J. Lambert, and F.R. Humphreys. 2002b. Continuing growth response to phosphate fertilizers by a *Pinus radiata* plantation over fifty years. *Forest Science*. 48:556-568.

- van Hees, P.A., U.S. Lundstrom, and R. Giesler. 2000. Low molecular weight organics and their Al complexes in soil solution - composition, distribution, and seasonal variation in three podzolized soils. *Geoderma* 94:173-200.
- van Hees, P.A.W., D.L. Jones, and D.L. Godbold. 2002. Biodegradation of low molecular weight organic acids in coniferous forest podzolic soils. *Soil Biology and Biochemistry*. 34:1261-1272.
- van Hees, P.A.W., D.L. Godbold, G. Jentschke, and D.L. Jones. 2003. Impact of ectomycorrhizas on the concentration and biodegradation of simple organic acids in a forest soil. *European Journal of Soil Science* 54:697-706.
- van Hees, P.A.W., D.L. Jones, G. Jentschke, and D.L. Godbold. 2004. Mobilization of aluminum, iron and silicon by *Picea abies* and ectomycorrhizas in a forest soil. *European Journal of Soil Science* 55:101-112.
- van Scholl, L., E. Hoffland, and N. van Breemen. 2006. Organic anion exudation by ectomycorrhizal fungi and *Pinus sylvestris* in response to nutrient deficiencies. *New Phytologist* 170:153-163.
- Villapando, R.R., and D.A. Graetz. 2001. Phosphorus Sorption and Desorption Properties of the Spodic Horizon from Selected Florida Spodosols. *Soil Science Society of America Journal* 65:331-339.
- Walker, L.C. 1960. Fertilizing southern pine: A roundup of observations and concepts, p. 86-95, In P. D. Burns, ed. *Southern forest soils*. Louisiana State Univ., Baton Rouge.
- Walker, T.W., and J.K. Syers. 1976. The fate of phosphorus during pedogenesis. *Geoderma* 15:1-19.
- Wallander, H. 2000. Uptake of P from apatite by *Pinus sylvestris* seedlings colonized by different ectomycorrhizal fungi. *Plant and Soil*:249-256.
- Wallander, H., L. Johansson, and J. Pallon. 2002. PIXE analysis to estimate the elemental composition of ectomycorrhizal rhizomorphs grown in contact with different minerals in forest soil. *FEMS Microbiology Ecology*. 39:147-156.
- Wells, C.G., and J.R. Jorgensen, (eds.) 1975. Nutrient cycling in loblolly pine plantations, pp. 137-158. Les Presses de L'Université, Laval, Quebec.
- Wells, C.G., D.M. Crutchfield, N.M. Berennyi, and C.B. Davey. 1973. Soil and foliar guidelines for phosphorus fertilization of loblolly pine. USDA For Serv Res SE US Dep Agric For Serv Southeast For Exp Stn 110.
- Wells, C.G., J.R. Craig, M.B. Kane, and H.L. Allen. 1986. Foliar and soil tests for the prediction of phosphorus response in loblolly pine. *Soil Science Society of America Journal* 50:1330-1335.
- Will, R.E., D. Markewitz, R.L. Hendrick, D.F. Meason, T.R. Crocker, and B.E. Borders. 2006. Nitrogen and phosphorus dynamics for 13-year-old loblolly pine stands receiving complete competition control and annual N fertilizer. *Forest Ecology and Management* 227:155-168.
- Wright, R.B., B.G. Lockaby, and M.R. Walbridge. 2001. Phosphorus availability in an artificially flooded southeastern floodplain forest soil. *Soil Science Society of America Journal* 65:1293-1302.
- Xu, R.K., A.Z. Zhao, and G.L. Ji. 2005. Effect of low molecular weight organic anions on adsorption of potassium by variable charge soils. *Communications in Soil Science and Plant Analysis* 36:1029-1039.

- Yuan, G., and L.M. Lavkulich. 1994. Phosphate sorption in relation to extractable iron and aluminum in spodosols. *Soil Science Society of America Journal* 58:343-346.
- Zhang, T.Q., A.F. Mackenzie, and F. Sauriol. 1999. Nature of soil organic phosphorus as affected by long-term fertilization under continuous corn (*Zea mays* L.): a  $^{31}\text{P}$  NMR study. *Soil Science* 164:662-670.
- Zhang, T.Q., A.F. MacKenzie, B.C. Liang, and C.F. Drury. 2004. Soil Test Phosphorus and Phosphorus Fractions with Long-Term Phosphorus Addition and Depletion. *Soil Science Society of America Journal* 68:519-528.
- Zhang, Y.-J., N.D. Bryan, F.R. Livens, and M.N. Jones. 1996. Complexing of metal ions by humic substances. In J. S. Gaffney, et al., eds. *Humic and Fulvic Acids*. ACS, Washington D.C. p. 194-206.

## APPENDICES

Appendix 1-1. Results of the Hedley sequential phosphorus extractions (mg/kg) from Wahee soil supporting a loblolly pine plantation in the Coastal Plains of South Carolina.

Depth (cm)	0-10		10-20		20-40		40-60		60-100		Sum of Depths	
Treatment	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum
DiWater Pi	0.85 (0.05)	0.78 (0.05)	0.2 (0.05)	0.31 (0.05)	0.26 (0.05)	0.06 (0.06)	0.16 (0.05)	0.3 (0.05)	0.05 (0.05)	0.06 (0.05)	1.51	1.50
Bicarb Pi	0.15 (0.01)	0 (0.01)	0 (0.01)	0.15 (0.01)	0.02 (0.02)	0 (0.01)	0.03 (0.01)	0.144 (0.1)	0.01 (0.01)	0.053 (0.01)	0.21	0.35
Bicarb Po	2.27 (0.07)	2.95 (0.07)	0.56 (0.07)	0.74 (0.07)	0.03 (0.07)	0.1 (0.08)	0 (0.07)	1.28 (0.2)	0 (0.07)	1.87 (0.07)	2.87	6.94
NaOH Pi	0.39 (0.14)	0.43 (0.14)	0.51 (0.14)	0.66 (0.14)	1.61 (0.14)	3.03 (0.16)	0.88 (0.14)	1.37 (0.14)	0.58 (0.14)	0.76 (0.14)	3.97	6.24
NaOH Po	10.68 (0.09)	11.09 (0.11)	4.55 (0.11)	6.63 (0.11)	1.83 (0.11)	1.34 (0.14)	1.54 (0.11)	1 (0.11)	0.7 (0.11)	0.78 (0.11)	19.29	20.84
1M HCL Pi	0.62 (0.15)	0.49 (0.15)	0.28 (0.15)	0.21 (0.15)	0.84 (0.15)	0.98 (0.16)	0.47 (0.15)	0.41 (0.15)	0.21 (0.15)	0.72 (0.15)	2.43	2.81
Conc. HCl Pi	1.17 (0.2)	0.76 (0.19)	0.91 (0.19)	0.53 (0.19)	0.61 (0.19)	0.09 (0.22)	1.89 (0.19)	0.82 (0.19)	0.1 (0.19)	0.5 (0.19)	4.69	2.70
Conc. HCl Po	2.75 (0.18)	2.6 (0.16)	2.66 (0.17)	2.56 (0.16)	0.21 (0.16)	0.16 (0.2)	0.32 (0.16)	0.01 (0.16)	0.02 (0.16)	0.01 (0.16)	5.96	5.34
H2SO4 Pi	2.54 (0.3)	3.57 (0.29)	2.59 (0.3)	1.75 (0.29)	3.14 (0.29)	1.95 (0.35)	3.53 (0.29)	1.6 (0.29)	4.19 (0.29)	5.13 (0.29)	15.99	14.00
Tot Hed Ext P	21.41 (0.14)	22.67 (0.13)	12.26 (0.13)	13.54 (0.13)	2.6 (0.13)	7.69 (0.16)	8.82 (0.13)	12.59 (0.13)	5.86 (0.13)	15.65 (0.13)	50.94	72.14

Values are least squared means (n=3). Values in parentheses are standard error of the means. Diwater = deionized water. Bicarb = sodium bicarbonate. NaOH = sodium hydroxide. 1M HCL = One molar hydrochloric acid. Conc. HCL = concentrated hydrochloric acid. H2SO4 = concentrated sulfuric acid. Hed Tot Ext = Hedley total extractable. Pi = inorganic phosphorus. Po=organic phosphorus.

Appendix 1-2. Results of the Hedley sequential phosphorus extractions (mg/kg) from Grantham soil supporting a loblolly pine plantation in the Coastal Plains of Virginia.

Depth (cm)	0-10		10-20		20-40		40-60		60-100		Sum of Depths	
Treatment	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum
DiWater Pi	1.66 (0.14)	2.91 (0.14)	0.7 (0.14)	1.26 (0.14)	0.27 (0.14)	0.29 (0.14)	0.39 (0.14)	0.3 (0.14)	0.46 (0.14)	0.43 (0.14)	3.48	5.19
Bicarb Pi	1.45 (0.02)	1.01 (0.02)	0.18 (0.02)	0.23 (0.02)	0.04 (0.02)	0.06 (0.02)	0 (0.02)	0.05 (0.02)	0 (0.02)	0 (0.02)	1.67	1.35
Bicarb Po	2 (0.19)	1.02 (0.2)	1.15 (0.19)	0.67 (0.19)	0.17 (0.19)	0.35 (0.19)	1.35 (0.19)	0.56 (0.19)	0.02 (0.19)	0.32 (0.19)	4.69	2.92
NaOH Pi	2.49 (0.18)	2.69 (0.18)	1.54 (0.18)	1.19 (0.17)	2.38 (0.17)	3.36 (0.17)	1.45 (0.17)	1.92 (0.21)	1.33 (0.18)	1.43 (0.17)	9.19	10.59
NaOH Po	4.79 (0.27)	8.42 (0.28)	4.7 (0.26)	6.19 (0.26)	2.06 (0.26)	2.75 (0.27)	1.6 (0.26)	0.55 (0.32)	1.82 (0.26)	1.68 (0.26)	14.97	19.59
1M HCL Pi	0.95 (0.17)	1.36 (0.17)	0.29 (0.17)	0.41 (0.17)	0.17 (0.17)	0.52 (0.17)	0.04 (0.17)	0.43 (0.19)	0.36 (0.17)	0.46 (0.17)	1.81	3.18
Conc. HCl Pi	1.16 (0.22)	1.32 (0.22)	2.85 (0.22)	1.73 (0.22)	2.83 (0.22)	2.71 (0.22)	3.38 (0.22)	2.01 (0.22)	3.41 (0.22)	2.95 (0.22)	13.63	10.72
Conc. HCl Po	0.02 (0.06)	0.01 (0.06)	0 (0.06)	0.02 (0.06)	0 (0.06)	0.19 (0.06)	0 (0.06)	0 (0.06)	0.03 (0.06)	0 (0.06)	0.05	0.22
H2SO4 Pi	7.8 (0.13)	4.36 (0.13)	8.15 (0.13)	5.85 (0.13)	4.48 (0.13)	3.53 (0.13)	4.62 (0.13)	3.98 (0.15)	6.73 (0.13)	6.44 (0.13)	31.78	24.16
Tot Hedt Ext P	22.33 (0.13)	34.61 (0.14)	31.06 (0.13)	29.05 (0.13)	23.9 (0.13)	25.25 (0.13)	24.33 (0.13)	21.31 (0.16)	25.66 (0.13)	13.7 (0.13)	127.28	123.92

Values are least squared means (n=3). Values in parentheses are standard error of the means. Diwater = deionized water. Bicarb = sodium bicarbonate. NaOH = sodium hydroxide. 1M HCL = One molar hydrochloric acid. Conc. HCL = concentrated hydrochloric acid. H2SO4 = concentrated sulfuric acid. Hed Tot Ext = Hedley total extractable. Pi = inorganic phosphorus. Po=organic phosphorus.

**Appendix 1-3. Results of the Hedley sequential phosphorus extractions (mg/kg) from Dothan soil supporting a loblolly pine plantation in the Coastal Plains of Alabama.**

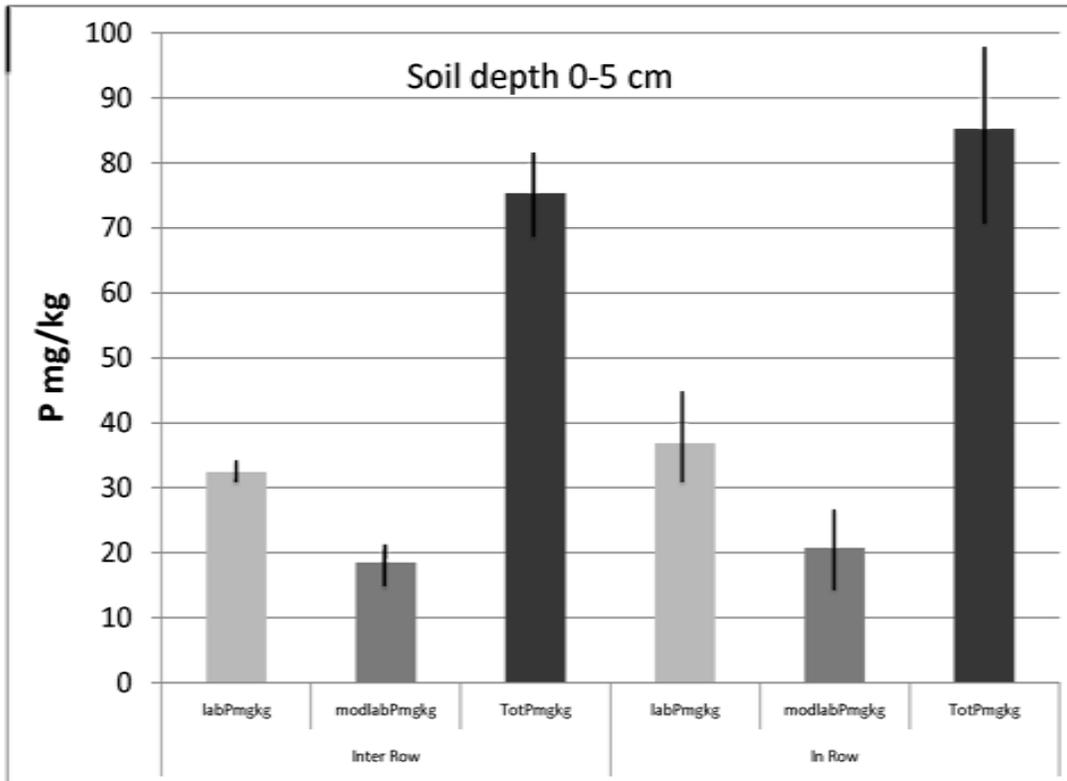
Depth (cm)	0-10		10-20		20-40		Sum of Depths	
Treatment	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum
DiWater Pi	0.43 (0.09)	0.5 (0.09)	0.12 (0.09)	0.36 (0.09)	0 (0.09)	0.14 (0.09)	0.64	0.95
Bicarb Pi	0.01 (0)	0 (0)	0 (0)	0.03 (0)	0.02 (0)	0.15 (0)	0.01	0.04
Bicarb Po	1.03 (0.11)	0.86 (0.11)	0.79 (0.11)	0.68 (0.11)	0.6 (0.11)	0.86 (0.11)	1.92	1.65
NaOH Pi	0.17 (0.21)	0.52 (0.21)	0.42 (0.21)	0.36 (0.21)	1.77 (0.21)	1.48 (0.21)	0.80	1.10
NaOH Po	6.73 (0.17)	6.25 (0.16)	4.86 (0.16)	6.08 (0.17)	1.12 (0.16)	0.93 (0.16)	11.74	12.50
1M HCL Pi	0.34 (0.06)	0.54 (0.06)	0.31 (0.06)	0.39 (0.06)	0.26 (0.06)	0.07 (0.06)	0.71	0.99
Conc. HCL Pi	1.11 (0.1)	0.95 (0.1)	1.04 (0.1)	1.04 (0.1)	0.2 (0.1)	0.38 (0.1)	2.25	2.09
Conc. HCL Po	0.45 (0.11)	0.4 (0.11)	0.72 (0.11)	0.44 (0.11)	0.16 (0.11)	0.09 (0.11)	1.29	0.96
H2SO4 Pi	1.55 (0.1)	1.38 (0.1)	1.96 (0.1)	1.85 (0.1)	1.21 (0.1)	1.9 (0.1)	3.61	3.33
Tot Hed Ext P	11.81 (0.18)	11.42 (0.17)	10.21 (0.17)	11.24 (0.18)	5.33 (0.17)	5.99 (0.17)	22.03	22.66

Values are least squared means (n=3). Values in parentheses are standard error of the means. Diwater = deionized water. Bicarb = sodium bicarbonate. NaOH = sodium hydroxide. 1M HCL = One molar hydrochloric acid. Conc. HCL = concentrated hydrochloric acid. H2SO4 = concentrated sulfuric acid. Hed Tot Ext = Hedley total extractable. Pi = inorganic phosphorus. Po=organic phosphorus.

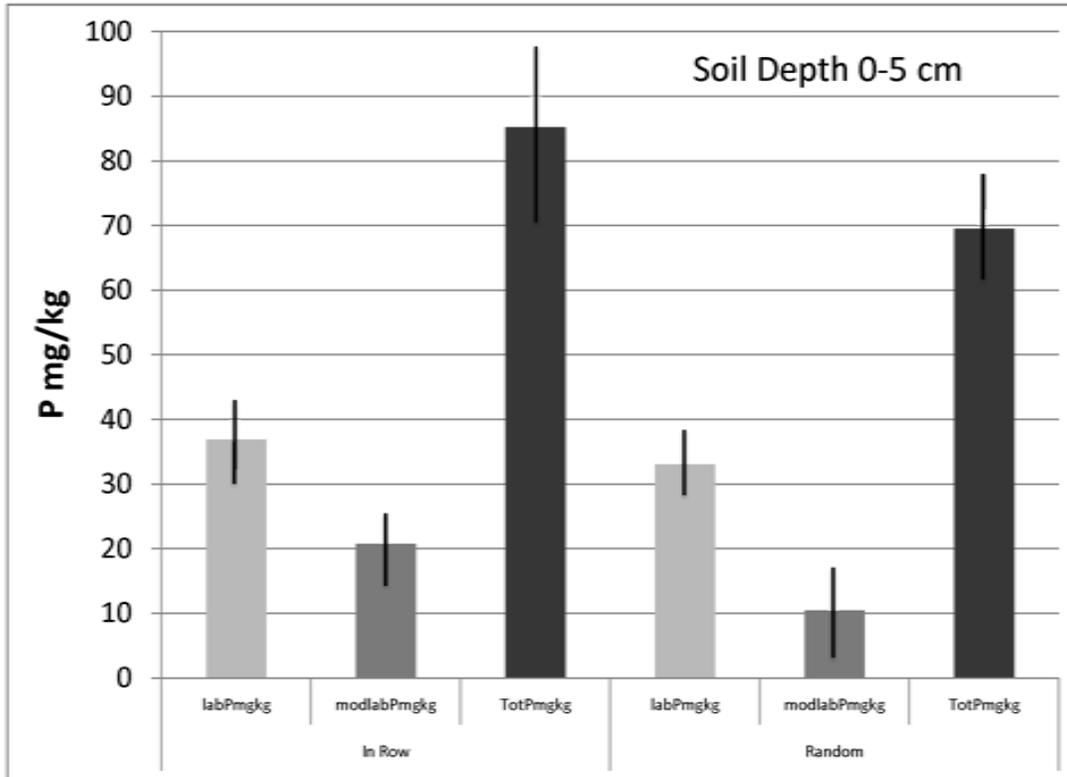
Appendix 1-4. Results of the Hedley sequential phosphorus extractions (mg/kg) from a Wagram soil supporting a loblolly pine plantation in the Coastal Plains of Georgia.

Depth (cm)	0-10		10-20		20-40		40-60		60-100		Sum of Depths	
Treatment	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum	Control	Maximum
DiWater Pi	0.27 (0.04)	0.25 (0.04)	0.11 (0.04)	0.24 (0.04)	0.09 (0.04)	0 (0.04)	0.05 (0.04)	0.02 (0.04)	0.13 (0.04)	0.11 (0.04)	0.67	0.62
Bicarb Pi	0.05 (0)	0 (0)	0 (0)	0 (0)	0.15 (0)	0.1 (0)	0.08 (0)	0.03 (0)	0.11 (0)	0 (0)	0.00	0.13
Bicarb Po	0.47 (0.07)	1.18 (0.07)	0.4 (0.07)	0.25 (0.07)	0.15 (0.07)	0.25 (0.07)	0.09 (0.07)	0.11 (0.07)	0.14 (0.07)	0.02 (0.07)	1.24	1.80
NaOH Pi	0.01 (0.12)	0.01 (0.12)	0 (0.12)	0 (0.12)	1.22 (0.12)	2.55 (0.12)	1.69 (0.12)	1.23 (0.12)	0.86 (0.12)	1.29 (0.12)	3.78	5.08
NaOH Po	1.4 (0.13)	0.59 (0.13)	1.02 (0.13)	0.87 (0.13)	1.99 (0.13)	1.79 (0.13)	1.12 (0.13)	1.28 (0.14)	0.63 (0.13)	0.9 (0.13)	6.15	5.44
1M HCL Pi	0.25 (0.06)	0.22 (0.06)	0.17 (0.06)	0.08 (0.06)	0.27 (0.06)	0.24 (0.06)	0.13 (0.06)	0.22 (0.07)	0.14 (0.06)	0.33 (0.06)	0.94	1.10
Conc. HCL Pi	1.27 (0.16)	1.17 (0.16)	1.53 (0.16)	1.28 (0.16)	0.42 (0.16)	0.56 (0.16)	0.57 (0.16)	0.95 (0.17)	1.18 (0.16)	1.77 (0.16)	4.97	5.74
Conc. HCL Po	0.06 (0.06)	0 (0.06)	0 (0.06)	0.14 (0.06)	0.04 (0.06)	0.08 (0.06)	0.05 (0.06)	0.13 (0.06)	0.02 (0.06)	0.07 (0.06)	0.16	0.41
H2SO4 Pi	8.77 (0.2)	9.27 (0.19)	11 (0.19)	11.14 (0.19)	5.42 (0.19)	5.11 (0.19)	2.58 (0.19)	3.46 (0.2)	3.82 (0.19)	5.15 (0.19)	31.59	34.14
Tot Hed Ext P	12.56 (0.1)	12.68 (0.09)	14.22 (0.09)	14.01 (0.09)	9.74 (0.09)	10.71 (0.09)	6.36 (0.09)	7.43 (0.1)	7.03 (0.09)	9.64 (0.09)	49.91	54.48

Values are least squared means (n=3). Values in parentheses are standard error of the means. Diwater = deionized water. Bicarb = sodium bicarbonate. NaOH = sodium hydroxide. 1M HCL = One molar hydrochloric acid. Conc. HCL = concentrated hydrochloric acid. H2SO4 = concentrated sulfuric acid. Hed Tot Ext = Hedley total extractable. Pi = inorganic phosphorus. Po=organic phosphorus.



Appendix 1-5 Results of soil sampling procedure (in row versus between rows) in the fertilizer treatment on Hedley phosphorus pools. Phosphorus fertilizer was placed in the treatment row in 1980.



Appendix 1-6 Results of soil sampling procedure (in row versus randomly in fertilizer treatment) in the fertilizer treatment on Hedley phosphorus pools. Phosphorus fertilizer was placed in the treatment row in 1980.