INCREASING THE NUTRIENT AVAILABILITY OF ORGANIC PHOSPHORUS THROUGH MANAGEMENT OF THE SOIL MICROBIOME: A STUDY OF LOCAL MICROORGANISM INOCULANT

by

ASPEN ROSE HATTABAUGH

(Under the Direction of Dorcas Franklin)

ABSTRACT

Crops are unable to use organic phosphorus as a nutrient source, as phosphorus must be in an ionic form for plant uptake. The conversion of organic to inorganic phosphorus is mediated by microbially-produced phosphatase enzymes. A treatment inoculated with locally harvested microbial communities (Local Effective Microorganisms) was applied to organic P-rich broiler litter, which was composted, and applied to test plots of Butterbean edamame in summer and Turkey hard red wheat in winter. Soil samples were taken pre-application of compost and again four weeks after application. Post-application samples showed significant differences of acid phosphatase activity and total phosphorus between plots amended with treated compost and plots with no amendments. Significant differences were also found for post-harvest levels of inorganic phosphorus and total phosphorus between LEM test plots and control plots.

INDEX WORDS: Nutrient cycling, organic phosphorus, inorganic phosphorus, sustainable agriculture, local effective microorganisms

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DEDICATION

My father came from a family of migrant farm laborers. He was the first in his family to complete college and he didn’t stop there. He worked his way through graduate school not once, but twice, to earn a Master’s degree and eventually, a Doctorate. He never used his upbringing as an excuse to do less or not to try something. He taught his children that you do, or you don’t, but try is a weasel word. As the first of his children to attend graduate school, I would like to dedicate this thesis to him and my mother.

My mother began teaching me to read and write early. Thanks to her tireless work to educate me before I ever attended school, I learned to love words. I so looked forward to going to “real” school, once I made it to elementary, I would take home extra books just for the feeling of a heavy backpack. Without her to be my first teacher, I would have never made it to here: submitting a thesis.

To both of you, thank you, from the bottom of my heart.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Purpose of Study

This thesis investigates a microbial treatment applied to composted broiler litter to enhance conversion rates of organic phosphorus into inorganic phosphorus, thereby creating a reliable source of plant-available phosphorus for agricultural uses while using a sustainable fertilizer source. Application and eventual incorporation of a fermented product, called Local Effective Microorganisms (LEM), into agricultural soil will hypothetically increase the microbial biodiversity and biomass, ideally increasing the percentage of the microbial community that is capable of catalyzing orthophosphate hydrolysis reactions. Specifically, it will increase the bacteria and fungi that produce extracellular phosphatases capable of hydrolyzing the inorganic phosphates from organic inositol phosphates. Phosphatases exist in both alkaline and acid forms. Phosphatases may also be categorized by the producing microbe: bacteria, yeasts, fungi, or plants. Within the scope of this research project, it may be most informative to determine the phosphatase-producers by source using whole genome sequencing to map the microbial community structure: bacteria and fungi are most likely to be the more prominent producers of phosphatases within the fermented local effective microorganism treatment (LEM). Chapter 2 details the soil phosphorus in a soybean-cereal rye crop system and the effects on crop productivity. Chapter 3 discusses soil phosphorus and crop productivity in a winter crop planted in plots with differing management history.

Literature Review

Phosphorus and its Uses in Agricultural Systems

Phosphorus is the second most growth-limiting nutrient in agricultural systems, but one of the least available nutrients found in soil. Much of the P required for crop production is acquired through fertilizer applications. Many soil types contain high levels of the mineral parent of phosphorus, apatite; however, a soils mineral phosphorus content has little to do with the availability of phosphorus as a nutrient source (Havlin, *et al*., 2013). Due to low P availability in soils, the use of inorganic fertilizers has risen dramatically since the 1940's, a result of the "Green Revolution." Rock phosphate for P-fertilizer is mined from multiple sources around the world, however, reserves of minable rock phosphate are quite low. Cordell *et al.* (2009) have predicted a 50-100 year lifespan of world rock phosphate reserves, with peak production occurring around 2030. The United States Geological Survey projects a world reserve of rock phosphate at approximately 300 billion tons (USGS, 2015). The Food and Agriculture Organization and USGS both foresee rising phosphorus fertilizer consumption in light of an exponentially increasing world population. With an estimated 2.25 billion increase in the world's population over the next forty years (FAO, 2012), world phosphorus consumption is expected to rise from 42.2 Megatons (Mt) in 2014 to 45.9 Mt in 2018 (USGS, 2015) with phosphorus consumption continuing to rise in conjunction with expanding population numbers. Considering the limitations and unsustainability of current inorganic phosphorus production, it becomes necessary to find a consistently available, readily accessible, and affordable source for fertilizer phosphorus.

Phosphorus in Soils

Phosphorus is present in soils due to the weathering of the mineral apatite (Essington, 2015) and the degradation of organisms and plant residues (Sylvia, *et al*, 2009). The weathering of apatite produces orthophosphate anion, the plant-available form of phosphorus. Two main forms of orthophosphate dominate in soil solution, depending upon soil pH: $H_2PO_4^-$ dominates at acidic pH (below pH 7.2) and HPO_4^{2-}, which dominates at alkaline pH (above pH 7.2) (Havlin, *et al* 2013). Soil phosphorus primarily exists as a solid phase, with organic and inorganic phosphorus adsorbed or precipitated to inorganic soil colloids or humic surfaces (Gerke, 2015), rather than existing solely in soil solution. As the majority of phosphorus moves to plant roots through diffusion, it is important for phosphorus in the solid phase to remain labile and able to desorb from soil exchange sites to replenish solution-phosphorus that has become very low in concentration due to diffusion to crop roots.

Inorganic phosphorus anions may sorb to a soil surface, but are considered labile in soil solution and able to be taken up by plants and other organisms. Organic phosphorus is less labile and may accumulate in soils because organic phosphorus exhibits strong adsorptive capacities to many clays, including calcite, kaolinite, montmorillonite, and goethite, as well as aluminum hydroxides, especially in low-pH soils (Menezes-Blackburn, *et al*. 2012). In soils, phytate molecules may display increasing inhibition of phosphate group hydrolysis from the carbon ring through high-affinity interactions with cations ($Fe^{3+} > Al^{3+} > Ca^{2+}$), forming pH-dependent, recalcitrant precipitates (Dao, 2003). Phytate can also chelate divalent base cations, particularly calcium, iron, magnesium, manganese, and zinc, which not only make the base cations unavailable as nutrients (this property of phytate has led to its labeling as an anti-nutrient), but also protects the phytate molecule from hydrolytic release and dephosphorylation of phosphate groups (Dao, 2003). Gibson et al. (2010) determined an increase in bioavailability for iron, zinc, and calcium chelated in phytic acid in the presence of phosphatases. Increasing the biodiversity and biomass of phytase-producing microbes would not only increase available phosphorus, but would increase the availability of the divalent cation nutrients sequestered within phytate under varying soil and climactic conditions. In the soil, this would lead to increased plant nutrient availability as the now-available ionic forms of iron, calcium, zinc, and phosphorus are in soil solution and able to be sorbed to soil exchange sites. When there is a lower amount of phosphorus present in solution, organic phosphorus will sorb to iron and/or aluminum oxides in a fast reaction. Sorbed phosphorus remains relatively labile, and may easily dissociate back into solution from a sorption site to replenish solution-P that has been depleted by plant uptake. When solution phosphorus is high, it will slowly precipitate out into iron and aluminum phosphates in acidic soils and calcium or magnesium phosphates in alkaline soils. Precipitated phosphorus is much more stable, and therefore, less labile. Gerke (2015) states that the P-fixation to the soil solid phase is attributed to orthophosphate anions’ strong binding abilities. Inositol phosphates (phytate) display even stronger binding abilities than orthophosphate anions, making phytate the main organic form of phosphorus in soil. Substrate availability (sorbed vs. precipitated phosphorus) and the presence of microbially-produced phytase enzymes are prime factors in the release of phytate-phosphorus for plant nutrition.

The chemical behavior of phosphorus in soil solution also depends upon pH and temperature. Soil pH influences the form inorganic phosphate anions take in soil solution (the more protonated form, $H_2PO_4^-$, in acidic soil solution, and HPO_4^{2-}in alkaline soil solution), as well as affecting the soil microbe population. As soil microbes and fungi are the major producers of the enzymes catalyzing the P-release reaction, the pH and temperature tolerances of the microbiota is also taken into account. The majority of microbes prefer a near-neutral pH of 6-7. Fungi are typically tolerant of more acidic conditions. Soil temperature affects the microbial population with optimum microbial activity occurring between $25 - 35$℃, thus influencing the rate of reaction for hydrolysis reactions and the transformation of phosphorus (Sylvia, *et al* 2009).

Phosphorus levels in soils differ by soil type. The dominating soil type in the Georgia Piedmont region is the Ultisol, a highly weathered, acidic, soil with high 1:1 clay content (predominately kaolin). Havlin et al. (2013) define typical parameters of solution phosphorus concentrations as 0.003 ppm (very low concentration) to 0.3 mg kg^{-1} (very high concentration), with average solution phosphorus at a value of around 0.05 mg kg^{-1}. Havlin et al. (2013) also show guidelines for phosphorus amounts for soybean yields as 0.025 mg kg^{-1}for 75% of the maximum crop yield and 0.200 mg kg^{-1}for 95% of the maximum crop yield. The UGA soil testing lab defines parameters for the Southern Piedmont soils as 0-22.4 kg ha^{-1} (0-20 lb A^{-1}) as low, 23.52-44.8 kg ha^{-1} (21-40 lb A^{-1}) as medium, 45.92-84 kg ha^{-1} (41-75 lb A^{-1}) as high, and 84+ kg ha^{-1} (75+ lb A^{-1}) as very high. These values were found using the Mehlich I extraction method

(Hancock & Hitchcock, 2015). Phosphorus levels in Piedmont Ultisols are generally classified as low. As little as 1% or less of total phosphorus found in soils is available to plants as orthophosphate. Highly weathered soils tend to have very little apatite parent material left and so have little native phosphorus in the soil. (Sylvia, *et al.* 2005). This extremely low amount of available native phosphorus requires supplementation by other P-sources.

Alternative Phosphorus Sources

Manures provide an extremely renewable source of nutrients and historically are the primary fertilizer source used by farmers. Sources include cattle manure, swine effluent, and poultry litter, the latter of which contains feces, feed, feathers, and bedding materials. Each manure source contains differing amounts of inorganic phosphate, releasable organic phosphorus forms, and non-labile organic phosphorus forms. The average organic phosphorus content for poultry litter, swine effluent, and cattle manure is 41.5%, 33.1%, and 28.1%, respectively (ZhengJuan, et al. 2015). Organic phosphorus is found in such high concentrations in these manures due to the high phytate content of grains and seeds found in animal feed. Phytate comprises 50-70% of nutritional phosphorus in commonly used animal feeds (Šarapatka, 2003). Lack of phytase (phytate-releasing phosphatase) production in the gut of mono-gastric swine and poultry prevents the animals from digesting and using the phytate stored in the grain as a source of dietary phosphorus to satisfy their own needs.

As in soils, the inorganic phosphate found in manure is already in a plant-available form while the organic forms require chemical transformation to become available for plant uptake. Hydrolytic cleavage of orthophosphate anions from the ring structure of phytate and other inositol phosphate isomers is required to transform organic phosphorus into available, inorganic anions. The reaction is catalyzed by extracellular enzymes (phosphatases) produced by a variety of soil microbes (Sylvia *et al*, 2009). Because the majority of phosphorus in manures is not available for immediate plant uptake, it is necessary to take into account phosphorus levels in soil, inorganic and organic phosphorus levels in the manure, and the phosphorus requirements of a particular crop. The general ratio of N: P found in manures (around 2: 1 to 4: 1) may lead to excessive phosphorus application when manures are applied to meet the nitrogen demand based upon hydrolyzable and available phosphorus. Nutrients not immediately available or not available through the same growth season nutrients are applied are subject to accumulation within the soil, runoff, and leaching. Over-application of phosphorus fertilizers, including manure, is a serious environmental and economic problem in agriculture.

CHAPTER 2

EFFECTS OF LEM TREATMENT ON SOIL PHOSPHORUS

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Abstract

Organic phosphorus is plentiful in broiler litter; however, the organic phosphorus is unavailable for crops as a nutrient source. Phosphatase enzymes catalyze a reaction that hydrolytically cleaves orthophosphate anions from a six-carbon ring, thus creating ionic forms of inorganic phosphorus crops can take up. Three microbial liquid treatments were designed and applied to composting broiler litter and agricultural test plots: Local Effective Microorganism (LEM), False LEM (FLEM), and Water-only (WO). Treated compost and the liquid treatment were applied to test plots of summer soybean (*Glycine max,* cultivar Butterbean) cover cropped with cereal rye (*Secale cereal L.*) over winter. Soil tests analyzed the quantities of total phosphorus, inorganic phosphorus (as Mehlich 1-extractable P), and acid phosphatase activity. Productivity was measured as biomass and bean yield, with significance found for biomass production for year 1 LEM plots. Soil P data showed a significant increase in post-application inorganic P availability in LEM plots, as well as an increase in total P and organic P from year 1 post-application samplings to year 2 pre-application samplings (soy biomass and cover crop tilled back in to soil) suggests a higher uptake of inorganic P in LEM plot crops.

Introduction

In the Georgia Piedmont region, soils are typically classified as ultisols, which tend to have high levels of 1:1 clays (particularly, kaolinite) and low levels of plant-available phosphorus (Havlin, et al. 2013). Phosphorus is the second most growth-limiting nutrient for crop production (Ågren et al. 2012), and so, most agricultural soils, including Piedmont ultisols, require application of P-fertilizer to properly supply plants with adequate nutrition. Phosphorus fertilizer is produced by mining rock phosphate from deposits in the ground, but reserves of rock phosphate have low lifespan projections at 50-100 years (Cordell, et al. 2009). Livestock and poultry manure can serve as a sufficient, renewable source of phosphorus for crops, but has high amounts of organic phosphorus unavailable to plants, with poultry litter ranking among the highest in levels of organic P at 41.5% organic P out of total P (ZhengJuan, et al. 2015). As it is not available for plant uptake, organic P is susceptible to being lost in runoff (Schroeder, et al. 2004), eventually causing environmental problems such as eutrophication. Another issue with the use of animal manure high in organic P is the general nutrient ratio of nitrogen to phosphorus. Manures tend to show an N:P ratio between 2:1 and 4:1, leading to excess phosphorus application when manures are applied to meet N requirements. If more organic P could be hydrolyzed, freeing the orthophosphate anions for plant uptake much less would be plant-unavailable and would in turn, make manure a more dependably available nutrient source to apply as a fertilizer. Enzymes called phosphatases catalyze the hydrolytic release of orthophosphate anions bound to a 6-carbon ring (the organic P molecule) and are produced by plant roots and primarily, soil microbes (Sylvia *et al*. 2009). Previously, the inoculation of soils with lab-grown cultures has been of interest (Higa & Wididana, year unknown; Ramesh *et al*, 2009). Higa and Wididana proposed the use of a mix of microbial cultures (Effective Microorganisms, or EM) to be applied to soil to increase sustainability in agriculture and decrease the use of plant growth regulators, chemical fertilizers, and pesticides applied to plants with the goal of including a more biological factor to farming: harnessing the power and processes of soil microbes to build more disease-suppressive soils, zymogenic, and synthetic soils. Higa defines his three soil types based upon the microbial activity within them. Disease-suppressive soils refers to soils with a high population of plant pathogen suppressing microbes, including high levels of actinomycetes, *Trichoderma, Penicillium,* and *Psendomonas*. Higa found a significant increase in total fungi numbers over three successive cropping seasons which reduced the occurrence of *Fusarium,* as well as other bacterial and fungal diseases. Higa characterized zymogenic soils as having high populations of Lactobacillus, yeasts, starch-digesting bacteria, cellulose-digesting bacteria, and other fermenting microbes. Synthetic soils were defined as having large populations of N-fixing bacteria (*Azotobacter, Beljerinckia, Derxia,* and *Spirillum*) facultative anaerobes (*Bacillus, Enterobacter, Klebsiella,* and *Clostridium*), and photosynthetic bacteria. Higa inoculated soils with a mixed culture of these microbial groups and found that both soil and foliar applications of these soils increased yield and quality of various horticulture crops (Higa, 1988). Higa preferred to use mixed cultures do the difficulties in getting pure culture to thrive in the soil (Higa & Wididana, Year Unknown) and his research focused more holistically on the whole soil ecosystem. For the scope of this research, it is of more interest to determine how best to increase P availability in fertilizers that come from renewable, sustainable sources. A study by Ramesh *et al.* (2009) used mixed *Bacillus* isolates to specifically increase P-nutrition in soybean. Ramesh *et al.* inoculated soil with *Bacillus* isolates to increase microbial phosphatase and phytase production in the soil rhizosphere. Because the pool of inorganic P is so low for many agricultural soils, plants and the surrounding biota of the rhizosphere have adapted to increase their ability to obtain sufficient P. Among these, the ability to solubilize inorganic P and hydrolyze organic P are the most important and are achieved through mycorrhizal relationships between plant roots and bacteria, fungal symbiosis, production and release of root exudates, and production of phosphatase enzymes. For agricultural researchers or farmers, the ability to either isolate or produce a biodiverse culture for application to soil and/or a fertilizer source is the most useful aspect of a microbial culture inoculation. After inoculating their test soils with the *Bacillus* cultures, Ramesh *et al.* found an increase in phosphatase activity over control soils, as well as an increase in plant-available P in comparison to non-inoculated controls. While the research of Higa and Ramesh focused on inoculating soil only, this thesis investigates the effectiveness of inoculating composting high organic P broiler litter to increase nutrient availability prior to incorporation into the soil as well as inoculation of the soil concurrently with the litter application. Rather than using lab cultured effective microorganisms or a soil-isolated mix of specific strains, the inoculant made for this research project was harvested locally, in an effort to have less competition between existing microbial communities and the newly-introduced microbial communities in the soil, as well as use microbes already acclimated to the climate. These locally harvested microbes were collected from a healthy organic layer of a local virgin forest and private land where the soil history was well known. This inoculant was mixed with nutrient sources, including carbon and sugar, and fermented. The solid ferment was weighed into cloth bags and steeped in a molasses-well water mixture. Well water was used to prevent residual chlorination from a city water source, which would have harmed the microbial population in the ferment. The molasses tea was made fresh and steeped for two weeks prior to each monthly application to the compost and the pre-planting application to the soil test plots. This inoculant of effective microorganisms was named Local Effective Microorganisms (LEM). In addition to the LEM treatment, a false LEM (FLEM) was made including the growth media used in LEM, but lacking the microbial inoculant. A third treatment, consisting only of well water, was used on the Water Only compost, which functioned as a control treatment. The broiler litter used for this research was collected immediately following the collection of the broilers from a local, organic broiler operation. The litter was transported to the research site, separated by weight into twelve compost huts, and treatments were randomly assigned (four replications of each treatment). Composting litter was turned once monthly and at each turning, another dose of treatment was added to the compost. Research objectives of this thesis begin with increasing the quantity of plant-available, inorganic P in finished broiler litter compost so sufficient levels of plant-available P could be incorporated into the ground prior to planting of a crop, much like a synthetic fertilizer would be applied. The second objective for this thesis is to inoculate the soil with the same microbial inoculant applied to the composted broiler liter to continue the hydrolytic release of organic P-bound orthophosphate anions throughout the crop growth season. This thesis follows the hypothesis that higher levels of microbial phosphatase activity will increase the hydrolytic release of orthophosphate anions, allowing the crop sufficient access to available P, resulting in a more nutritious crop and/or higher productivity in treated broiler litter/soil systems compared to false-treatment or control litter/soil systems. Hypothetically, post-application soil samples would show a higher level of inorganic, plant-available P, or a lower ratio of organic to inorganic P, as more inorganic P becomes available and organic P pools lessen. This increase in the available inorganic P would be taken up by the crops and stored within the plant biomass. An increase in total P for pre-application samplings may indicate the stored P was found in the biomass or an increase in total P for LEM plot harvested soybeans would indicate storage of P in the fruit of the crop.

Materials and Methods

The research site was located at the Org-2 plot at J. Phil Campbell Research Station in Watkinsville, GA. The site featured a randomized block design of four test plots per treatment for a total of sixteen plots. Two years of a summer soybean crop, *Glycine max*, cultivar Butterbean, were planted and harvested. The plots measured 0.0037 ha (20x20 feet) in size, with 0.0018 ha (10x20 feet) of the plot occupied at a time with the soybean crop. The two consecutive soybean crops occupied the same half of the plots for both years. Cereal rye (*Secale cereal L.*) was planted as a cover crop on soybean plots over winter. Broiler litter was composted until finished and treated with its respective treatment (LEM liquid, FLEM liquid, or well water) at each turning. The finished compost was then applied to the corresponding plots, along with liquid treatment, and tilled into the soil at a depth of 15 cm. The application rate used for both years was 8.45 lb acre^{-1} of compost per plot with liquid treatments applied at 10 gallons per plot. The Water-Only, F-LEM, and LEM test plot soils (all growth seasons) were analyzed to quantify total phosphorus, inorganic phosphorus, and organic phosphorus. A nitric acid-perchloric acid digestion was used to determine the total phosphorus content of the experimental soils. In accordance with the University of Georgia Soil Test Lab protocol, Mehlich I procedure was used to determine the inorganic phosphorus levels. The difference between the total P and inorganic P for each test plot was used to find the level of organic phosphorus in the plot soils. Soil acid phosphatases were quantified using Tabatabai & Bremner's (1969) phosphatase assay.

Soil Sampling

Soil sampling was performed twice during a crop season for year 1-2015 and year 2-2016. One sampling occurred before application of the composted broiler litter, but during the same week as application for a pre-application, seasonal baseline. The second sampling occurred four weeks post-application of broiler litter and liquid treatments and crop planting, around flowering began for the soybean crop. Soil samples were taken to a depth of 15 cm using a push-probe. These samples were air dried, ground to pass through a 2-mm sieve and used for analysis.

Soil Total Phosphorus- Nitric acid-perchloric acid digestion

The digestion reagent was made by mixing four parts nitric acid to one part perchloric acid (Palmer *et al.* 2015). Soil samples were weighed to 0.25 g and placed in a micro Kjeldahl digestion tube. To the tube, 5 mL of the mixed acid reagent was added, tubes were placed in a digestion block and heated to 200℃. The samples were heated until frothing subsided, and then the temperature was increased to 375℃. The solution was held at 375℃ until clear and then digested for another hour. After digestion, the samples were cooled and brought to a volume of 50 mL for soy bean and a 25 mL total volume for soil and compost samples. Total phosphorus results were visualized using the Murphy-Riley molybdenum blue procedure and analyzed spectrophotometrically at 882 nm on a TECAN infinite M200 pro (Tecan Austria GmbH, 2014).

Soil Inorganic Phosphorus- Mehlich 1

Mehlich 1 extractant was prepared by diluting 8.5 mL of HCl and 1.4 mL of concentrated H_2SO_4 to 2 L with deionized water. Test plot soils were weighed to 5.0000 g and placed in 50-mL centrifuge tube with 25 mL of Mehlich extractant. The tubes were shaken for five minutes and filtered through a Whatman #42 filter paper. Extracted samples were analyzed using the Murphy-Riley Molybdenum-blue method and read at 882 nm on a TECAN infinite M200 pro spectrophotometer (Tecan Austria GmbH, 2014).

Organic Phosphorus

Organic P was quantified by simply subtracting Mehlich 1-extracted P (the procedure used for quantifying inorganic P) from the total P found in a soil or compost sample.

Soil Acid Phosphatase Activity

Acid phosphatase activity was characterized using p-nitrophenyl phosphate degradation over 1 hour of incubated time (Tabatabai & Bremner, 1969). One gram of air-dried soil was placed into a 50-mL Erlenmeyer flask. This was repeated twice for each soil sample as follows: 1.0000 g acid phosphatase, 1.0000 g acid phosphatase control. To each set of reactions, 4 mL of the pH 6.5 MUB, 0.2 mL of toluene, and 1 mL of p-nitrophenyl phosphate solution were added. To the controls, only the MUB and toluene were added. The flasks were stoppered and incubated at 37℃℃ for one hour. After incubation, the stoppers were removed and 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added to the flasks to halt the reaction. The solutions were swirled to mix, filtered through a Whatman #40 filter paper, and analyzed spectrophotometrically at a 410 nm wavelength using a TECAN infinite M200 pro (Tecan Austria GmbH, 2014).

Statistical Analysis

Results were statistically analyzed using JMP Pro 13.0.0 (JMP Pro 13.0.0. 2016). A one-way ANOVA followed by a means comparison using the Tukey HSD All Pairs test was run to determine significance at α-level 0.05. If results were not normally distributed, they were also analyzed non-parametrically using the Wilcoxon test for each pair.

Results and Discussion

Composting helps to reduce the mass of material, but also influences the hydrolytic release of inorganic P anions from organic P molecules. LEM-inoculated compost was expected to show higher levels of acid phosphatase activity, influencing the levels of both Total P and inorganic P (as Mehlich 1-extracted P) found in finished compost. Soils treated with LEM were expected to exhibit lower inorganic P levels for pre- and post-application sampling due to increased initial availability and plant uptake. LEM soils were expected to have higher total P and organic P levels for pre-application samplings caused by decomposition of biomass from cover crop (and year 1 soy biomass for year 2 pre-application soil samples) and for post-application soils, a lowered ratio of organic: inorganic P was expected due to microbial hydrolytic release of the inorganic P from organic P in the soil. Higher acid phosphatase activity was predicted for LEM plots, corresponding with the lowered ratio of organic to inorganic P. Higher productivity in harvested soybean and biomass, or higher total phosphorus in analyzed soybean from LEM plots was also hypothesized.

Compost Analysis

Statistical analysis of compost yielded a significant difference in organic P (Figure 2.1) among the treatment compost, as well as significantly higher acid phosphatase activity (Figure 2.2). FLEM compost was significantly lower (p=0.0472) in organic P than Water-Only treated compost, with LEM compost not significantly different from either. It is noted that both FLEM and LEM were lower in organic P and higher in inorganic P than Water-Only treated compost. LEM-treated compost showed significantly higher acid phosphatase activity (p=0.0251) and of the treated, finished compost, had the highest level of inorganic P, although not significantly higher than Water-Only or FLEM treated compost.

Soybean Year 1 Baseline, Post-Application, and Harvest

Data was statistically analyzed by samplings to determine significance for two years of soybean crops (Figure 2.3). Baseline samples were taken prior to application. Post-applications samples were taken four weeks after application of compost, liquid treatments, and crop planting. Baseline soil samples (Figure 2.4) were used to determine application rate of compost, which was applied at 8.45 lb acre^{-1}. Post-application sampling showed significantly lower ($p=0.0047$) acid phosphatase activity in FLEM and LEM plots compared to plots amended with Water-Only-treated compost (Figure 2.5). Organic and inorganic P was also found to be significantly different (Figure 2.4). Organic P was significantly higher ($p=0.0304$) in FLEM plots compared to Water-Only and LEM, and consequently, had the lowest level of inorganic P between treated soils. LEM soils showed a significantly lower level of organic P with a significantly higher ($p=0.0085$) level of inorganic P, indicating some interaction between LEM application and conversion of organic to inorganic P. Water-Only treated soils showed similar results to LEM plots with regards to significance in soil P. This Water-Only similarity to LEM data, plus the higher levels of acid phosphatase activity in Water-Only, suggests a possible occurrence of biostimulation of the original microfauna found in the non-inoculated plots following the incorporation of the water-only treated broiler litter.

Soybeans were harvested and analyzed for productivity by measuring biomass, yield, and total P found in the soybeans. Total P was analyzed in beans to determine if the crop was storing excess P in the soybeans. No significant differences were noted between yield or total P in the beans, but LEM soils showed significantly higher biomass production than FLEM plots (p=0.0265, Figure 2.6), and while not significantly higher, LEM plots did produce more biomass than Water-Only plots. Following the harvest and biomass measurement, some biomass was left on the plots and tilled in prior to planting of the cereal rye cover crop. When soils were being prepared for the year 2 soybean crop, the cover crop was also tilled into the soil.

Soybean Year 2 Pre-Application, Post-Application, and Harvest

Soybean soils sampled for year 2 pre-application (Figure 2.7) sampling showed no significance regarding acid phosphatase activity between treatments, but did show significant differences in inorganic soil P with Water-Only significantly higher (p=0.0709) than FLEM and LEM soils not significantly different from either. Total P, organic P, and acid phosphatase were not significant for this sampling. While total P and organic P were not statistically significant between treatments for the pre-application sampling, the levels of total P and organic P were approximately 400 mg kg^{-1} higher than post-application sampling from year 1. The difference in total P between year 1 post-application sampling and year 2 pre-application sampling was significantly different in LEM plots (p=0.0096) with post-application total P averaging 447.6 mg kg^{-1} and pre-application levels averaging 844mg kg^{-1}. It was surmised the significantly greater jump in total and organic P came from the tilling-in of soy biomass and the winter cover crop, increasing both total and organic P, but not inorganic. Of the three treatments, the total and organic P was highest for LEM (although not significantly), indicating that more P was stored in the biomass of the LEM-grown crop than in Water-Only or FLEM plot crops. This implies more inorganic P was available in LEM plots than the other two treatments, and rather than being lost in the soil to adsorption or runoff, was stored in the biomass and cycled back into the soil.

For year 2 post-application soil sampling, no significance in total P, organic P, inorganic P or acid phosphatase activity was found.

The year 2 soybean harvest (Figure 2.8) showed significant differences in productivity: biomass (p=0.0224) and yield (p=0.0295) were significantly lower for LEM plots compared to Water-Only plots, while FLEM plots were not statistically significantly different from either Water-Only or LEM soils. Total P in the soybeans was not significantly higher for any treatment, but was higher in soybeans harvested from LEM-plots. The LEM plots showed obvious pest activity (in this case, deer) which adversely affected both biomass and productivity measures. No other indications of any insect pest or pathogen causing the loss in biomass and yield were detected and only LEM-treated plots showed evidence of deer grazing.

In conclusion, compost analysis showed a significantly higher acid phosphatase activity in LEM-treated compost (p=0.0251). The LEM compost also showed higher levels of inorganic P and lower levels of organic P, although these differences were not significant. Based upon phosphatase activity, the LEM treatment did enhance the conversion of organic to inorganic P in the compost.

Soil and harvest analysis provided similar insight into how the LEM treatment worked: significantly lower organic P was found in LEM and Water-Only plots, with correspondingly higher inorganic P levels. Water-Only plots showed significantly higher acid phosphatase activity than FLEM and LEM soils, despite LEM soils having similar levels of organic and inorganic P as Water-Only plots. The year 1 harvest biomass was significantly higher for LEM plots. Some biomass from year 1 soy was tilled in prior to cover cropping, and once again, the cover crop was tilled in prior to planting the year 2 soybean crop. This resulted in year 2 pre-application soils showing much higher levels of total and organic P compared to year 1 post-application soils. While not significantly higher by treatment for the year 2 pre-application sampling, LEM plots had higher levels of both organic and total P. The major spike in total and organic P was associated with the tilling in of biomass and cover crop, leading to the conclusion that more inorganic P was available to crops grown in LEM-treated soil, was stored in the biomass, and was not lost, but cycled back into the soil in the form of organic (and total) P.

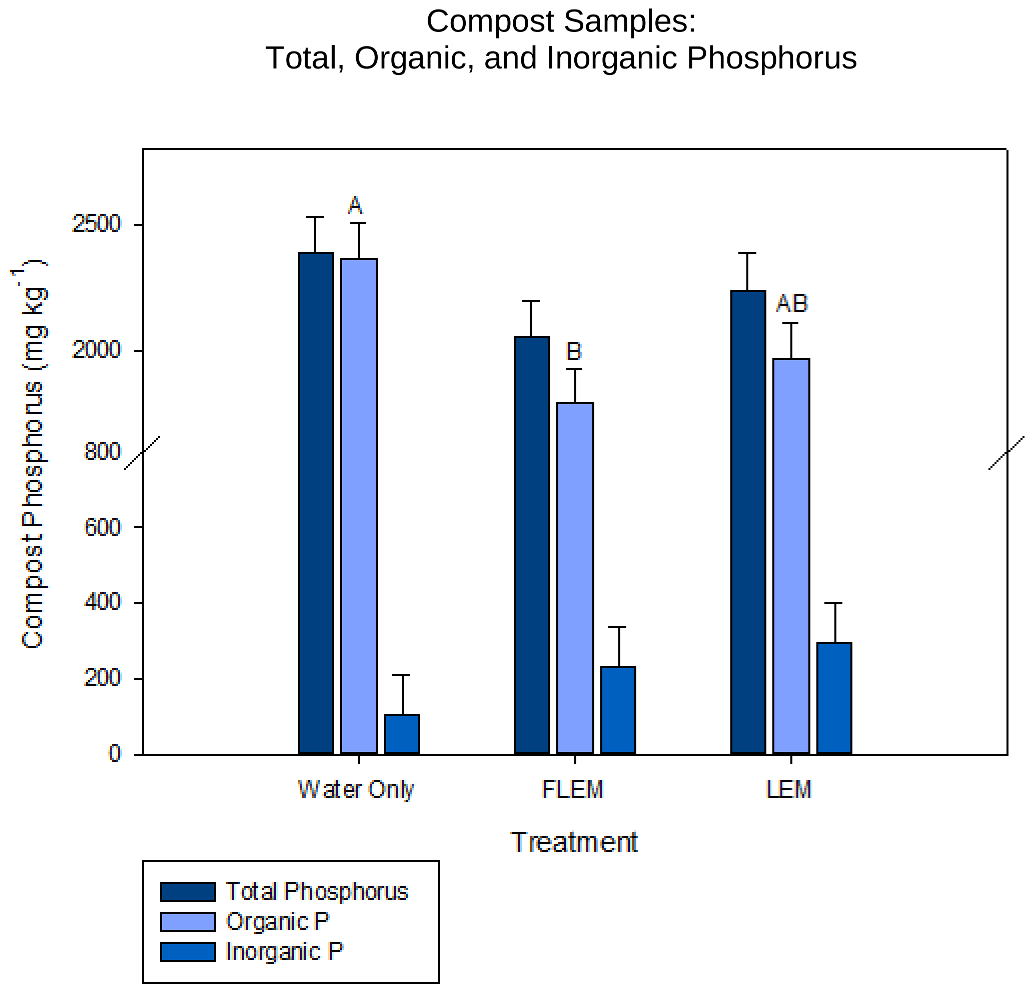

Figure 2.1: Samples were analyzed for total, organic, and inorganic P content by treatment using one-way ANOVA and Tukey HSD All Pairs means comparison at a 95% confidence level. Letters not connected by the same letter are significantly different. Significance was noted for organic P: Water-Only treated compost significantly higher in organic P than both FLEM compost. LEM compost is not significantly different from Water-Only or FLEM for organic P, although it is noted both FLEM and LEM have lower organic P and higher levels of inorganic P than Water-Only treated compost.

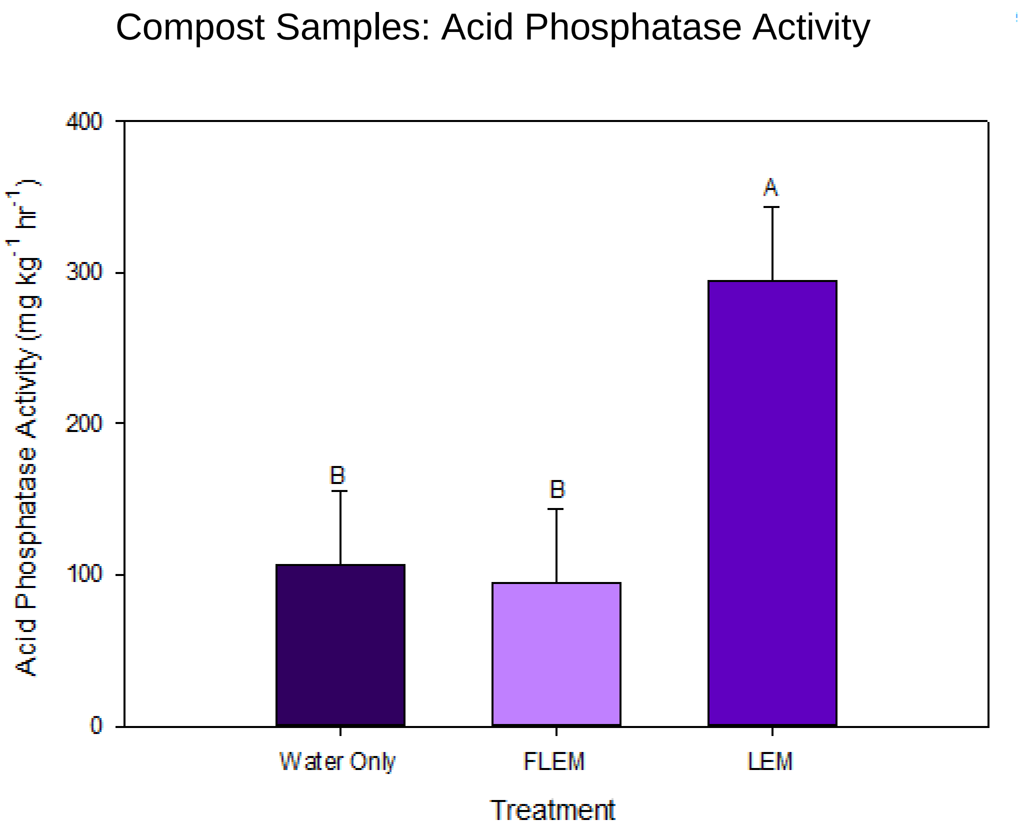

Figure 2.2: Acid phosphatase activity was statistically analyzed by treatment using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. LEM treated composts showed significantly higher acid phosphatase activity compared to Water-Only and FLEM treated compost. Higher acid phosphatase activity in LEM compost was due to the microbial inoculant, suggesting the microbes in the inoculant did flourish and acclimate to the compost environment.

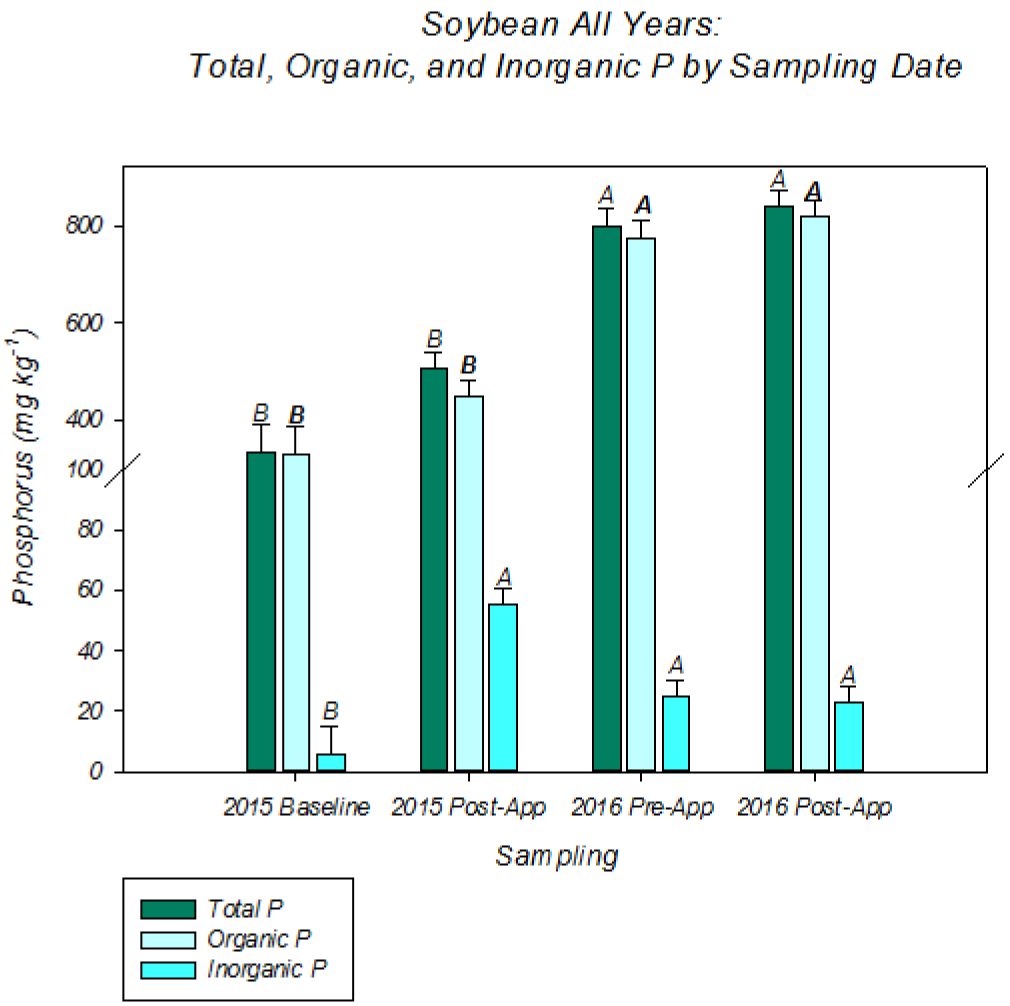

Figure 2.3: Soils were analyzed statistically to determine any significant differences across sampling dates for the three soil P analyses and is denoted by P fraction. using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. Total P significance is uppercase, organic P is uppercase and bold, and inorganic P is uppercase and italicized. Acid phosphatase activity was also analyzed by sampling, but was not significant across treatments. Significance was found across all sampling dates for total, organic, and inorganic phosphorus, with 2016 pre-app soils showing a significant increase over 2015 post-application soils. This increase in total and organic P from post-

app to pre-app suggests that much of the increased available inorganic P in LEM plots was taken up by the crop, stored in biomass, and when tilled in to the soil prior to application, began to be broken down by microbes and was extracted in the total P digestion.

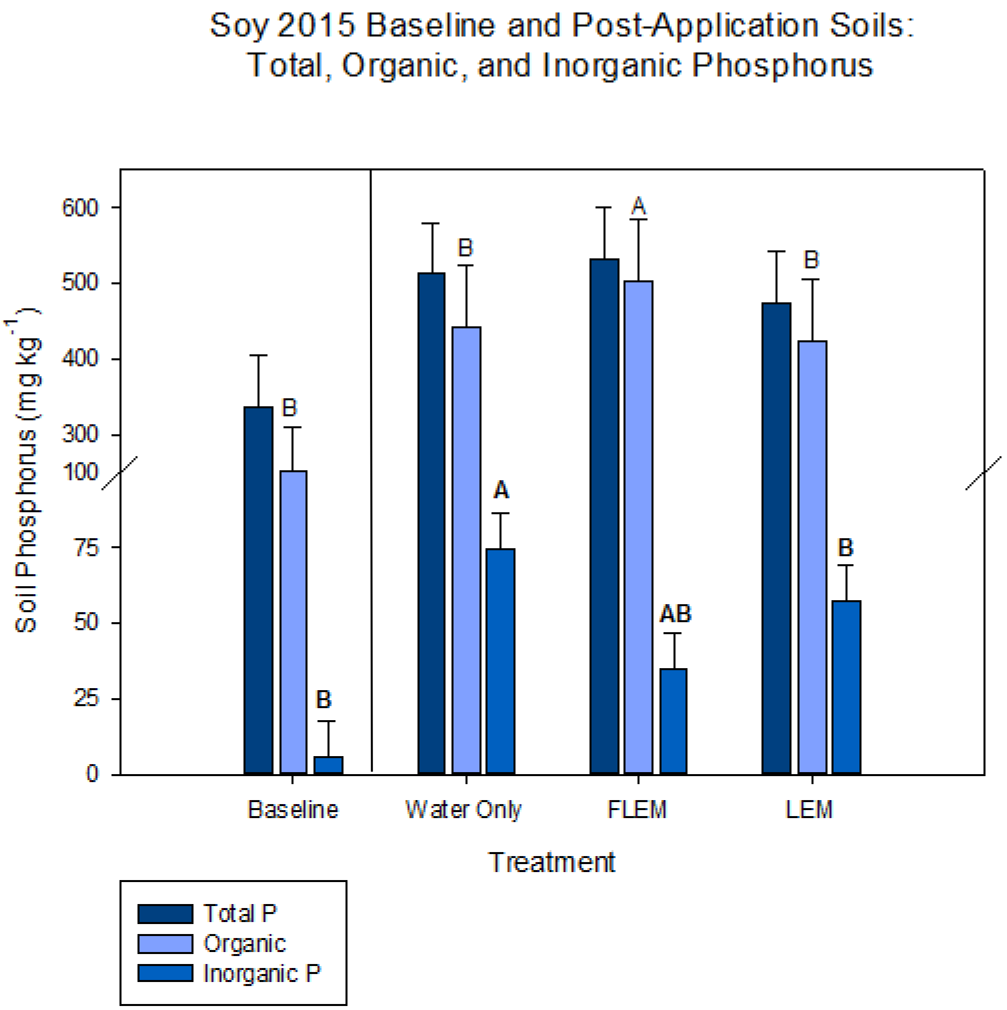

Figure 2.4: Significant differences were found across sampling dates; thus, sampling dates were analyzed by treatment using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. Significance is shown by P fraction: organic P is uppercase and inorganic P is uppercase and bold. No significant differences were found in total P levels for post-application sampling. Organic P is significantly lower in Water-Only and LEM treated plots, with LEM and FLEM treated plots also lower in inorganic P at the post-application sampling.

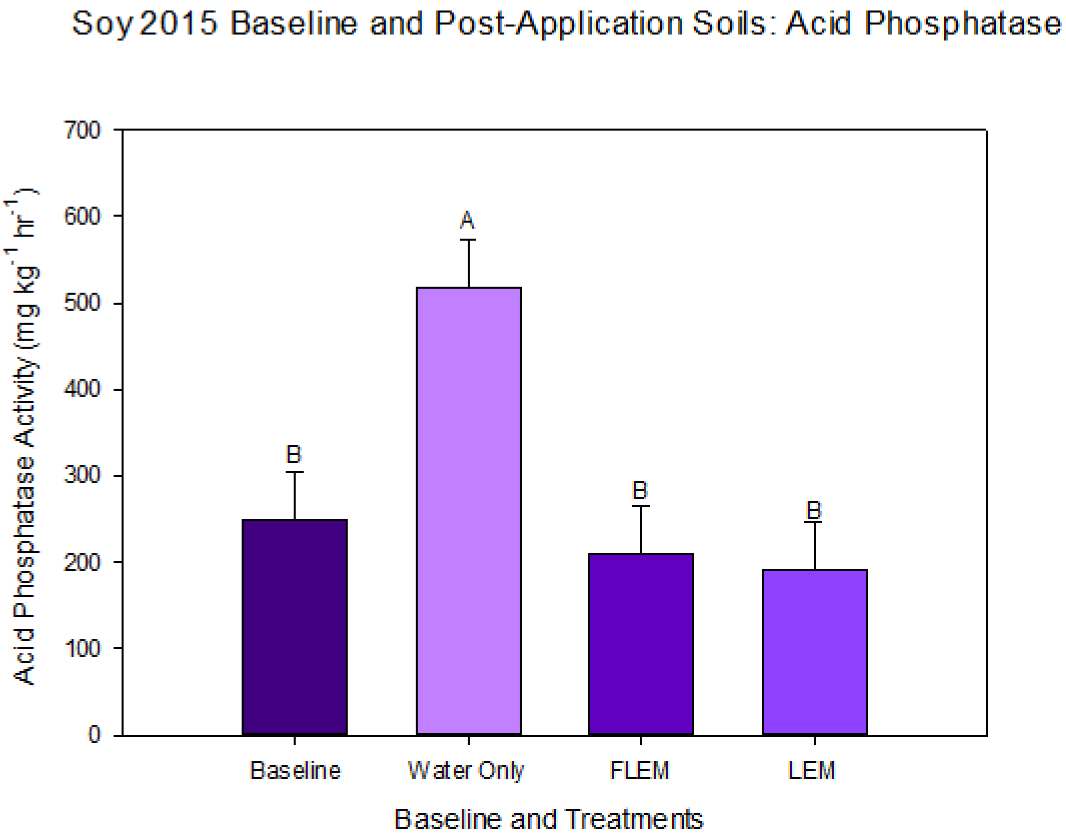

Figure 2.5: Phosphatase activity was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. Acid phosphatase activity was significantly high in Water-Only plots at post-application sampling. Water-Only also showed significantly higher levels of inorganic P at this sampling (Figure 2.4).

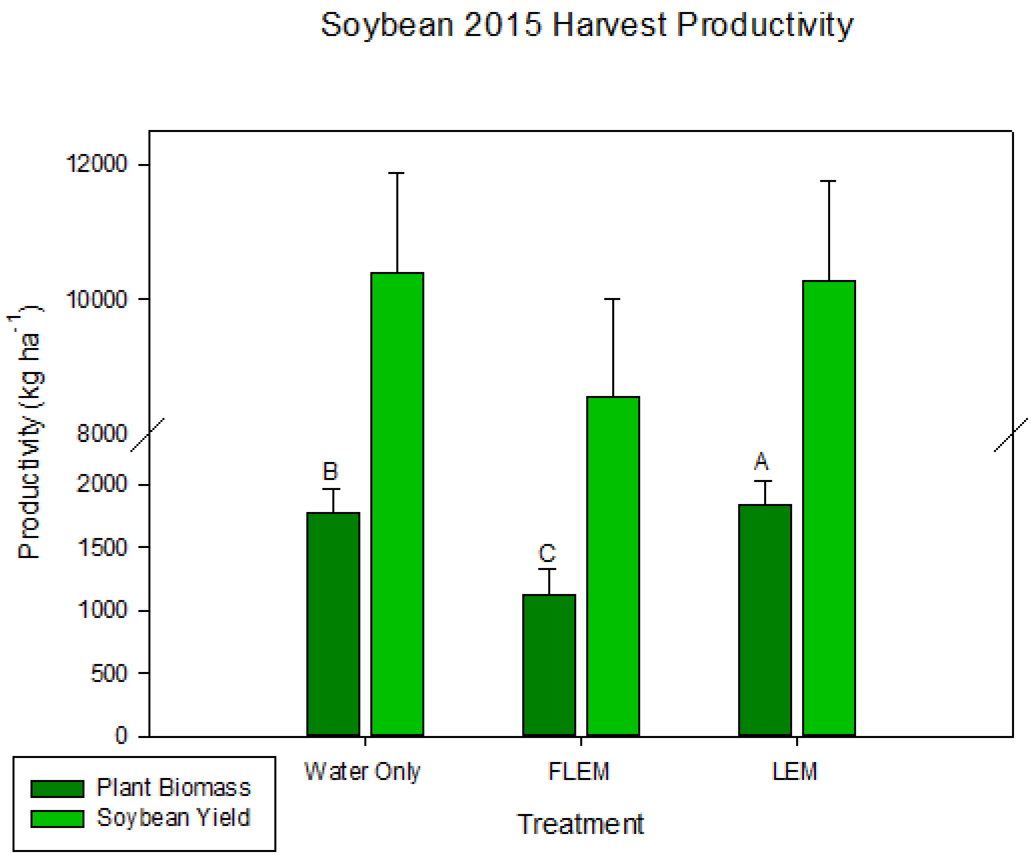

Figure 2.6: Treatments were analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. Treatments showed no significant differences in yield, but LEM soils produced a significantly higher biomass than Water-Only and FLEM treatments. Some of the biomass from the soybean crop was tilled into the soil for planting of the cover crop, which in turn, was also tilled into the soil prior to the next application and planting of the year 2 soy crop. Pre-application soil samples of LEM plots (Figure 2.3) showed significantly higher total P and organic P in year 2 post-application soils, indicating a nutrient cycle: P was not lost nor was it found in the bean, but found in the biomass.

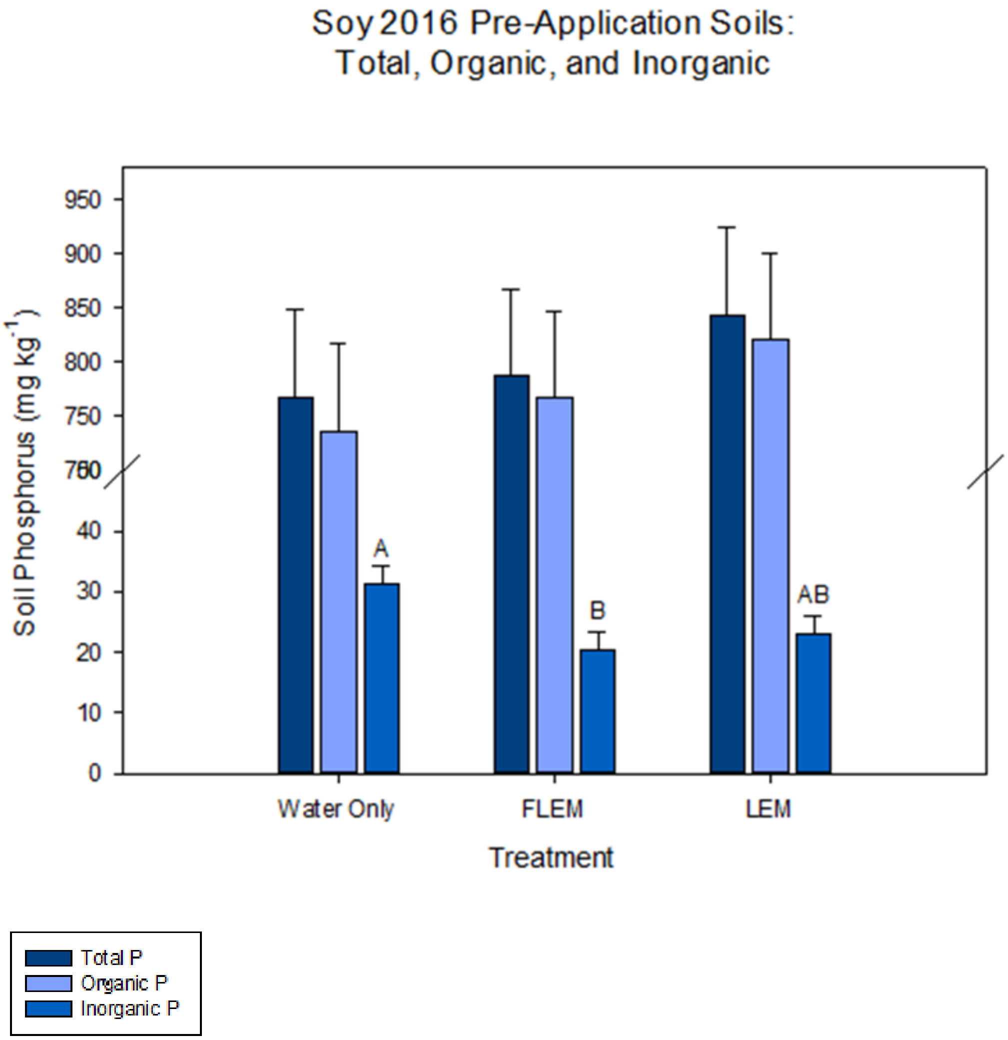

Figure 2.7: Data was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. Significance is noted by P fraction for each treatment. After one summer crop of soybean and one winter cover crop of cereal rye, pre-application sampling showed significantly higher inorganic P in Water-Only plots (which showed significantly higher acid phosphatase activity at the last 2015 sampling) with FLEM soils showing the lowest inorganic P values, and LEM soils not significantly differing from either other treatment. While not significant across treatments, the increase in total P and organic P in LEM treated soils prior to any application of nutrients is surmised to have

come from the decomposition of soy biomass and winter cover crop tilled in to soils, indicating more available P was taken up by crops in LEM plots and stored in the biomass, rather than the bean.

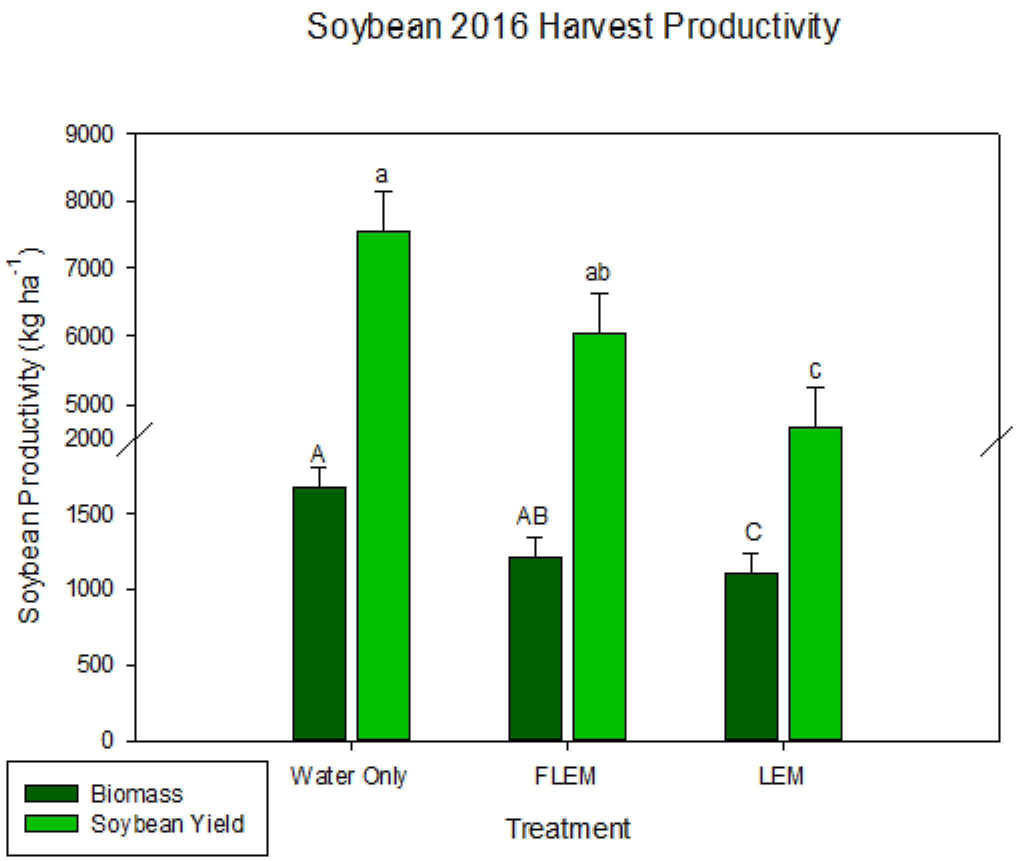

Figure 2.8: Data was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. Significance is noted by analysis for each treatment: biomass significance is uppercase; yield significance is lowercase. Productivity measurements for the 2016 soybean harvest showed significantly higher biomass and soybean yield from Water-Only plots and LEM plots showing significantly lower biomass and yield compared to the other treatments. It is worth noting the LEM plots had several instances of grazing by deer prior to the harvest. None of the plots showed any signs of insect pests nor significant weed growth. Only LEM plots were grazed by deer, which is believed to be the cause of the lower biomass and yield.

CHAPTER 3

EFFECTS OF LEM TREATMENT ON SOIL PHOSPHORUS IN TWO WINTER CROPPING SYSTEMS

Overview

Three microbial treatments were designed for application to composting broiler litter and agricultural test plots with the objective of increasing phosphatase activity, thus increasing inorganic P availability: Local Effective Microorganism (LEM), False LEM (FLEM), and Water-only (WO). Treated compost and the liquid treatment were applied to test plots of two sets of differentiating crop systems. One system consisted of a rye grass forage (*Lolium multiflorum)* over two winters and a fallow system over summer. The second system consisted of two summers of soybean (*Glycine max*, cultivar Butterbean) cover cropped with cereal rye (*Secale cereal L.* over winter. Year 3 for both systems was the winter Turkey Hard Red wheat (*Triticum aestivum)* crop. Compost and soils were analyzed for total phosphorus, inorganic phosphorus (as Mehlich 1-extractable P), and acid phosphatase activity. Productivity (grain yield, biomass, and total P extracted from grain) was also analyzed to determine effects of LEM on crop production. Wheat: Rye system showed significantly higher inorganic P in LEM pre-application soils, lower organic P in post-application LEM soils, and lower inorganic P in FLEM and LEM plots in post-harvest sampling. Acid phosphatase activity was significantly higher for LEM soils sampled post-harvest. Wheat: soy-cereal system soils showed significantly lower

total P in Pre-application soils and lower inorganic P levels in FLEM and LEM treatments. Soy-cereal soils showed significant phosphatase activity in FLEM treatment over all samplings.

Materials and Methods

The research site was located at the Org-2 plot at J. Phil Campbell Research Station in Watkinsville, GA. The site featured a randomized block design of four test plots per treatment for a total of sixteen plots. Two years of a summer soybean crop, *Glycine max*, cultivar Butterbean, were planted and harvested. The plots measured 0.0037 ha (20x20 feet) in size, with 0.0018 ha (10x20 feet) of the plot occupied at a time with the soybean crop. The two consecutive soybean crops occupied the same half of the plots for both years. Cereal rye (*Secale cereal L.*) was planted as a cover crop on soybean plots over winter. Broiler litter was composted until finished and treated with its respective treatment (LEM liquid, FLEM liquid, or well water) at each turning. The finished compost was then applied to the corresponding plots, along with liquid treatment, and tilled into the soil at a depth of 15 cm. The application rate used for both years was 8.45 lb acre^{-1} of compost per plot with liquid treatments applied at 10 gallons per plot. The Water-Only, F-LEM, and LEM test plot soils (all growth seasons) were analyzed to quantify total phosphorus, inorganic phosphorus, and organic phosphorus. A nitric acid-perchloric acid digestion was used to determine the total phosphorus content of the experimental soils. In accordance with the University of Georgia Soil Test Lab protocol, Mehlich I procedure was used to determine the inorganic phosphorus levels. The difference between the total P and inorganic P for each test plot was used to find the level of organic phosphorus in the plot soils. Soil acid phosphatases were quantified using Tabatabai & Bremner's (1969) phosphatase assay.

Soil Sampling

Soil sampling was performed twice during a crop season. One sampling occurred before application of the composted broiler litter, but during the same week as application for a pre-application, seasonal baseline. The second sampling occurred four weeks post-application of broiler litter and liquid treatments and crop planting, around flowering began for the soybean crop. Soil samples were taken to a depth of 15 cm using a push-probe. These samples were air dried, ground to pass through a 2-mm sieve and used for analysis.

Soil Total Phosphorus- Nitric acid-perchloric acid digestion

The digestion reagent was made by mixing four parts nitric acid to one part perchloric acid (Palmer *et al.* 2015). Soil samples were weighed to 0.25 g and placed in a micro Kjeldahl digestion tube. To the tube, 5 mL of the mixed acid reagent was added, tubes were placed in a digestion block and heated to 200℃. The samples were heated until frothing subsided, and then the temperature was increased to 375℃. The solution was held at 375℃ until clear and then digested for another hour. After digestion, the samples were cooled and brought to a volume of 50 mL for soy bean and 25 mL total volume for soil and compost samples. Total phosphorus results were visualized using the Murphy-Riley molybdenum blue procedure and analyzed spectrophotometrically at 882 nm using a TECAN infinite M200 pro (Tecan Austria GmbH, 2014).

Soil Inorganic Phosphorus- Mehlich 1

Mehlich 1 extractant was prepared by diluting 8.5 mL of HCl and 1.4 mL of concentrated H_2SO_4 to 2 L with deionized water. Test plot soils were weighed to 5.0000 g and placed in 50-mL centrifuge tube with 25 mL of Mehlich extractant. The tubes were shaken for five minutes and filtered through a Whatman #42 filter paper. Extracted samples were analyzed using the Murphy-Riley Molybdenum-blue method at 882 nm on a TECAN infinite M200 pro (Tecan Austria GmbH, 2014).

Organic Phosphorus

Organic P was quantified by simply subtracting Mehlich 1-extracted P (the procedure used for quantifying inorganic P) from the total P found in a soil or compost sample.

Soil Acid Phosphatase Activity

Acid phosphatase activity was characterized using p-nitrophenyl phosphate degradation over 1 hour of incubated time (Tabatabai & Bremner, 1969). One gram of air-dried soil was placed into a 50-mL Erlenmeyer flask. This was repeated twice for each soil sample as follows: 1.0000 g acid phosphatase, 1.0000 g acid phosphatase control. To each set of reactions, 4 mL of the pH 6.5 Modified Universal Buffer (MUB), 0.2 mL of toluene, and 1 mL of p-nitrophenyl phosphate solution were added. To the controls, only the MUB and toluene were added. The flasks were stoppered and incubated at 37°C℃ for one hour. After incubation, the stoppers were removed and 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added to the flasks to halt the reaction. The solutions were swirled to mix, filtered through a Whatman #40 filter paper, and analyzed spectrophotometrically at a 410 nm wavelength on a TECAN infinite M200 pro (Tecan Austria GmbH, 2014).

Statistical Analysis

Results were statistically analyzed using JMP 13.0.0. Pro (JMP Pro 13.0.0. 2016) A one-way ANOVA followed by Tukey HSD All Pairs means comparison was run to determine significance at α-level 0.05. If results were not normally distributed, they were also analyzed non-parametrically using the Wilcoxon test for each pair.

Results and Discussion

Like the soybean data, wheat data was analyzed by sampling date to determine any significance across time, then was analyzed by treatment for each sampling. Data that was insignificant does not have an accompanying figure. Significance was found in both wheat systems for inorganic P only. Soy-cereal system post-application samples were significantly higher in inorganic P than all rye system samples and soy-cereal system pre-application and post-harvest samples (Figure 3.1). FLEM acid phosphatase activity was significantly different across sampling dates for both systems, but no differences were found for Control, Water-Only, or LEM plots. Acid phosphatase activity was highest for soy-cereal system post-harvest soil samples and significantly lower than that sampling for soy-cereal pre-application and rye system pre- and post-harvest application. Because significance was found over time, the two systems were analyzed further by treatment for each sampling.

Wheat: Rye Grass System Pre-application, Post-application, & Post-harvest soils, and Wheat Harvest

The rye grass system plots had previously cropped rye grass (*Lolium multiflorum*) as a winter forage. The plots lay fallow in the summer. Though not significantly different from Water-Only or FLEM plots due to the inclusion of Control data, pre-application sampling (Figure 3.2) of the treatment plots showed LEM plots were lowest in inorganic P, indicating less was left in the soil from the previous crop. This is most likely due to microbial activity making more P available during the previous winter's crop growth. Phosphatase activity in pre-application soils was not significant for any treated soil.

Post-application samplings indicated a significantly lower (p=0.0021) level of total P in LEM-treated and Control soils (Figure 3.4). LEM soils were also lower in organic P than Water-Only and FLEM soils. Due to the lower levels of total P and organic P in LEM post-application soils, the ratio of organic to inorganic P is lower than in the Water-Only and FLEM plots, with which the inorganic P was not significantly different. This lower organic: inorganic ratio may be interpreted as increased inorganic P availability. Acid phosphatase activity measured for post-application soils was once again not significant for any treatment.

Wheat was harvested and wheat post-harvest soil samples were taken. For the wheat crop, no significance was found for productivity, which included grain yield, biomass, and total P in the wheat grain. Post-harvest soil sampling showed a significant difference (p=0.0025) in inorganic-P. Water-Only plots were significantly higher than LEM plots, while FLEM plots were not significantly different from either other treatment (Figure 3.5). No significance for organic or total P was found for post-harvest soil samples. Acid phosphatase activity was significantly higher (p=0.0427) for LEM soils. Lower inorganic P levels were expected in LEM soils, and corresponded with the significantly higher level of phosphatase activity in LEM plots (Figure 3.6). Total P and organic P showed no significant differences in post-harvest soils.

Wheat: Soy-Cereal Rye System Pre-application, Post-application, & Post-harvest soils, and Wheat Harvest

The soy-cereal system previously had two crops of soybean grown during the previous two summer seasons and was cover cropped with cereal rye over winter for both years. Pre-application sampling (Figure 3.3) showed no significant differences in organic P, inorganic P, or phosphatase activity for any treatments. Total P was significantly lower (p=0.0304) for LEM soils. The last soil sampling (soy 2016 post-application, Figure 2.11) of LEM soils had shown LEM soils with significantly lower inorganic P, a smaller organic to inorganic P ratio, and significantly higher phosphatase activity than the two other treatments, indicating that microbial activity was liberating more inorganic P from organic P molecules than in other treatment plots. For post-application sampling (Figure 3.4), no significant phosphatase activity was shown, inorganic P was consistent across all treatment plots, with only Total P significantly lower (p=<0.0001) in LEM soils. Once again, LEM soil analysis showed a smaller ratio between organic and inorganic P, indicating more organic P moved from the organic pool to the inorganic P pool and potentially taken up by the wheat. Like rye system wheat harvest statistics, harvest data for this crop system also showed no significant differences in productivity (yield, biomass, and total P in grain) across treatments. Post-harvest soil samples indicated inorganic P levels significantly lower (p=0.0433) than Water-Only plots for LEM and Control plots (Figure 3.5). Total P and organic P levels in LEM post-application samplings increased for this sampling with no further applications of compost before the post-harvest soil sampling. Potentially, warming temperatures increased microbial activity in the soil and began breaking down plant residue and broiler litter compost that had not yet dispersed through the soil. FLEM plots showed significantly higher (p=0.0004) acid phosphatase activity than phosphatase activity in Water-Only and LEM plots (Figure 3.7).

In conclusion, both wheat system's LEM plots showed a similar trend: increased total and organic P at the pre-application sampling due to tilled-in biomass from previous crop seasons. The increase in inorganic P, as evidenced by the lower organic: inorganic P ratios during post-application and post-harvest, was found in the biomass and thus created a nutrient cycle. Increased availability of inorganic P allowed crops to take up sufficient P. Analysis of the grain confirmed no significance of total P in the wheat grain between treatments, meaning any excess P was not stored in the grain. This extra P taken up was instead stored in the biomass, but did not increase biomass productivity. This is indicated by higher pre-application soil levels of total and organic P in LEM plots and returned to the LEM soils by decomp of the tilled biomass.

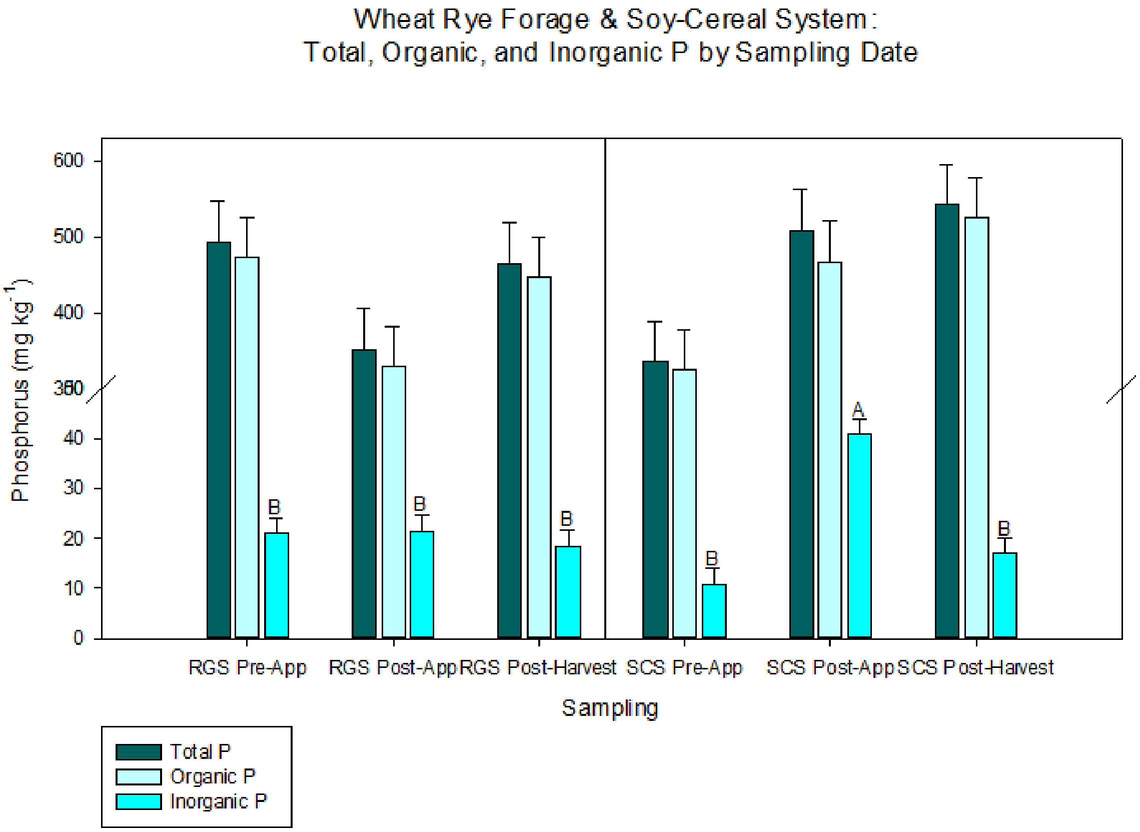

Figure 3.1: Data was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. RGS is rye grass system with the accompanying three soil samplings. SCS is soy-cereal system and samplings. Samplings of both systems occurred at the same time for each sampling point noted on the graph. Significance is noted across treatments by P analysis.

Significance for all sampling dates showed only one significant difference, with significantly higher inorganic P in soy-cereal system post-application sampling across both systems and all sampling. Statistical analysis was continued for each system and analyzed by treatment for each sampling.

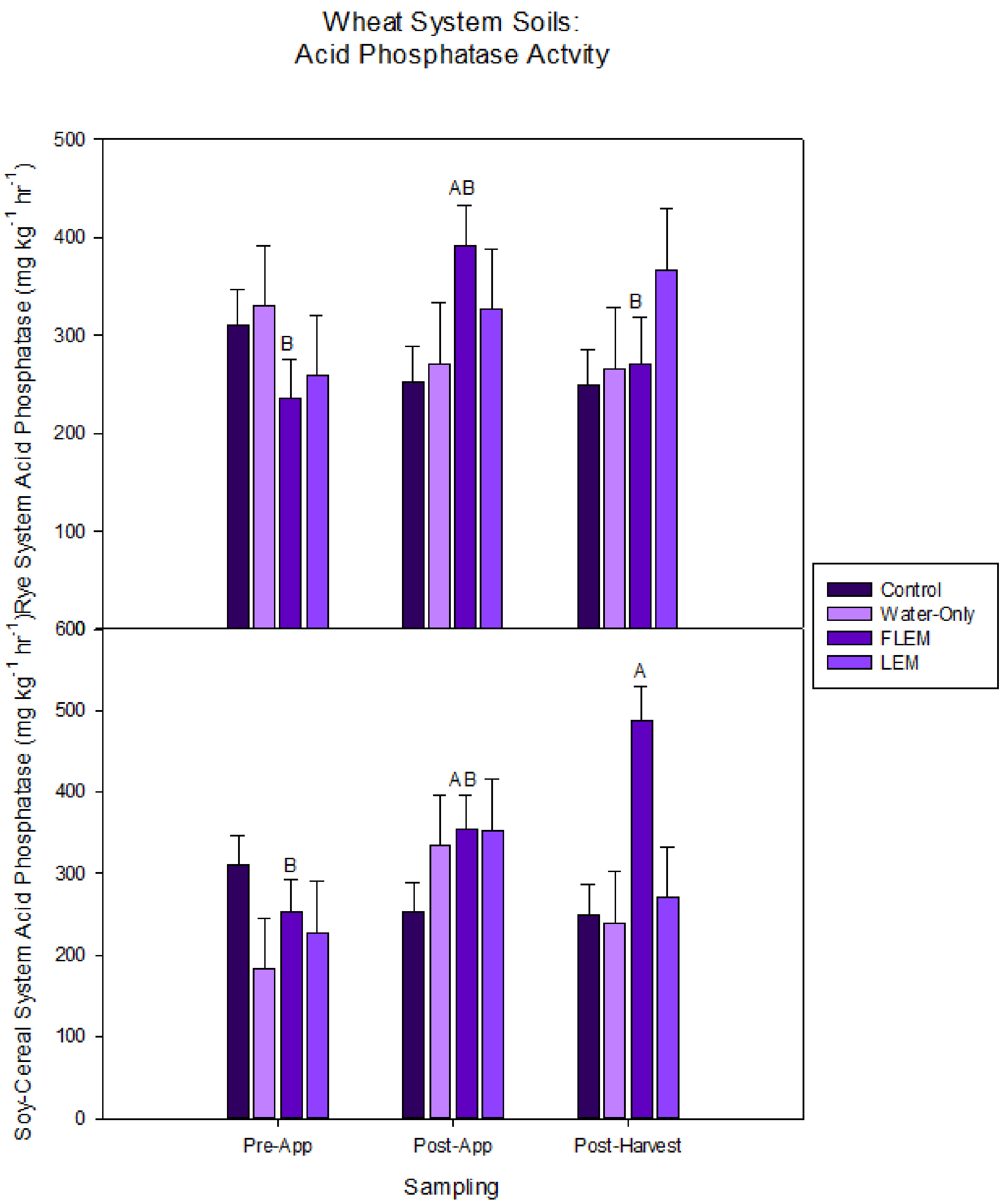

Figure 3.2: Data was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. Only FLEM treated soils showed significant fluctuations in acid phosphatase activity across sampling for both crop systems.

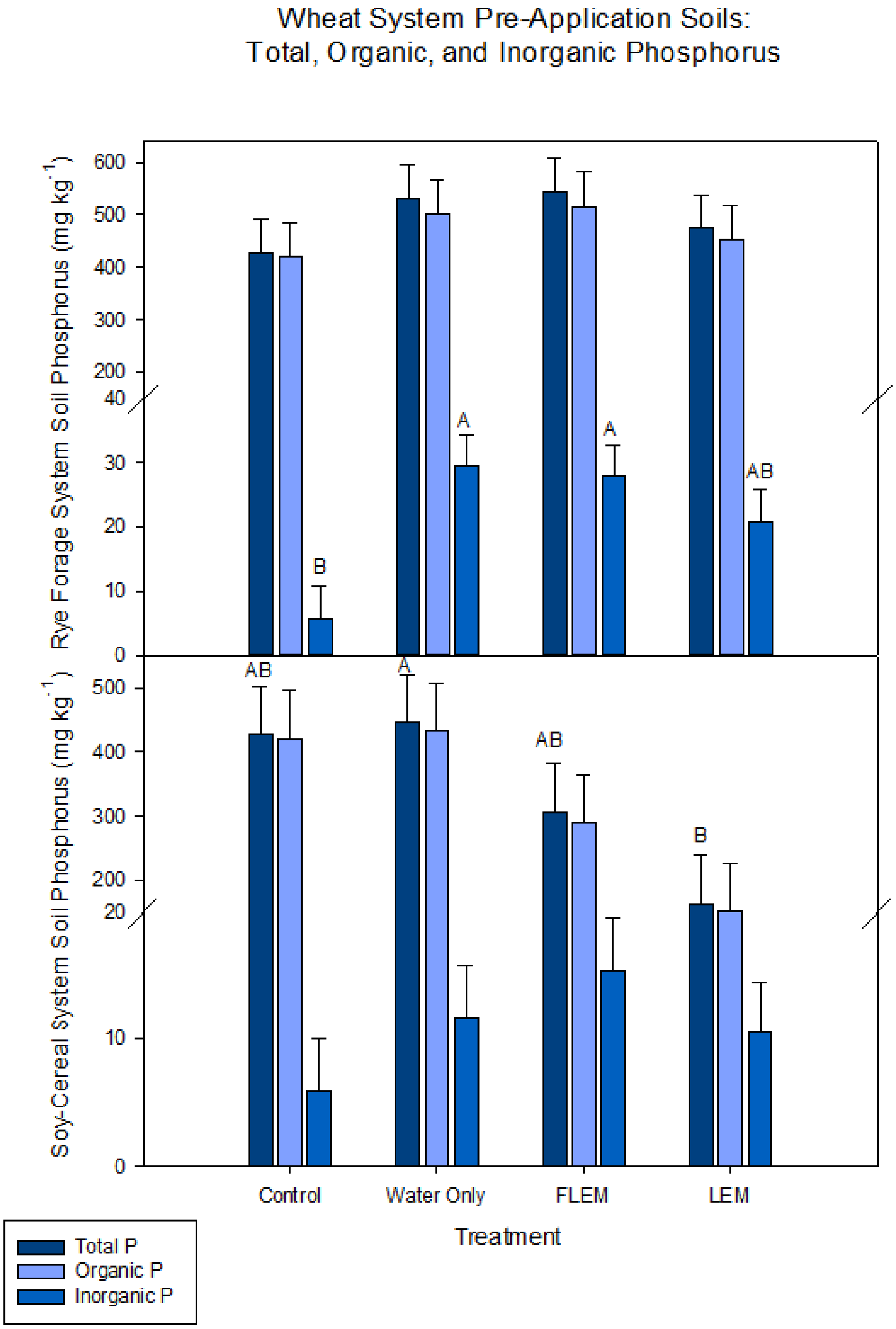

Figure: 3.3: Data was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. Significance is shown in uppercase letters between treatments. Rye system soils showed significant pre-application differences for inorganic P levels, with LEM soils holding the least amount of inorganic P of the treated soils. This is indicative of the increased availability of inorganic P during the prior growth year. Soy-cereal system soils showed significant differences in total P, with LEM soils containing the lowest level of total P. Not much biomass was tilled into the ground from the summer soy crop because of the active analysis associated with the wheat study. The significantly lower levels of total P and lower, but not significantly so, levels of both organic and inorganic P in the LEM pre-application highlight the increased uptake of the year 2 soy crop in LEM plots.

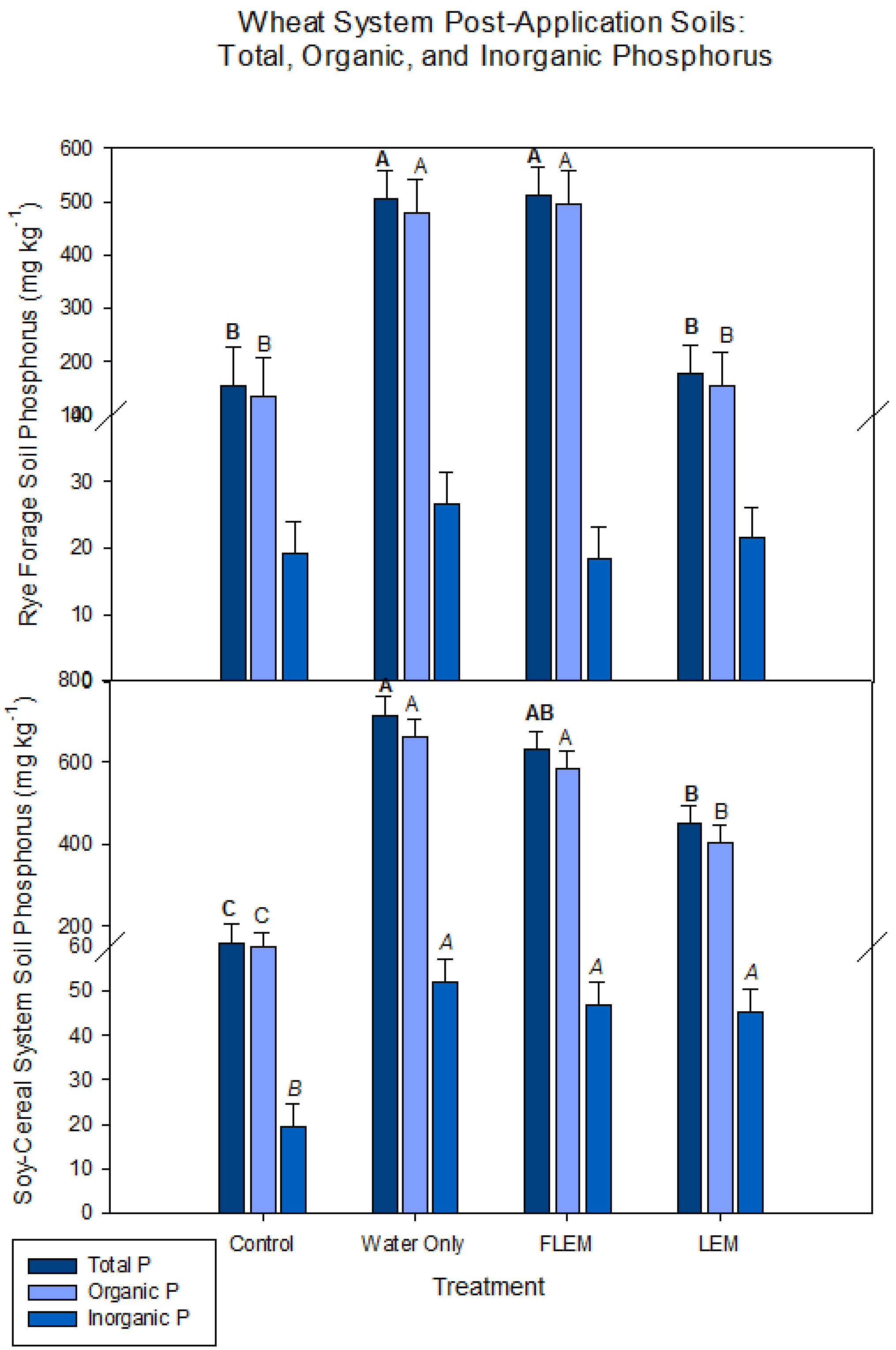

Figure 3.4: Data was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are

significantly different. Data was analyzed by treatment for each P analysis and is noted by treatment. Total P is bolded and uppercase, organic P is uppercase, and inorganic is noted by uppercase, italicized letters. Rye system soils showed significant differences between LEM soils and Water-Only and FLEM soils. LEM plots were significantly lower in total and organic P for post-application sampling. Despite being lower in total and organic P, LEM plots showed the lowest ratio between organic and inorganic P, meaning much more of the total P within LEM soils was available for plant uptake.

Soy-cereal soils also showed significant differences between treatments for total P and organic P, while inorganic P was only significantly difference between treated soils and control soils (containing no amendments). Similar to the rye system soils for this sampling, LEM soils were significantly lower in total P compared to Water-Only, but were not significantly different from FLEM soils. Organic P in LEM soils was significantly lower than that found in FLEM and Water-Only soils. Inorganic P levels were similar and not significant for any treatment. Like the rye system, this means the ratio of organic to inorganic P was lower, and more of the P in the LEM plots was available for uptake.

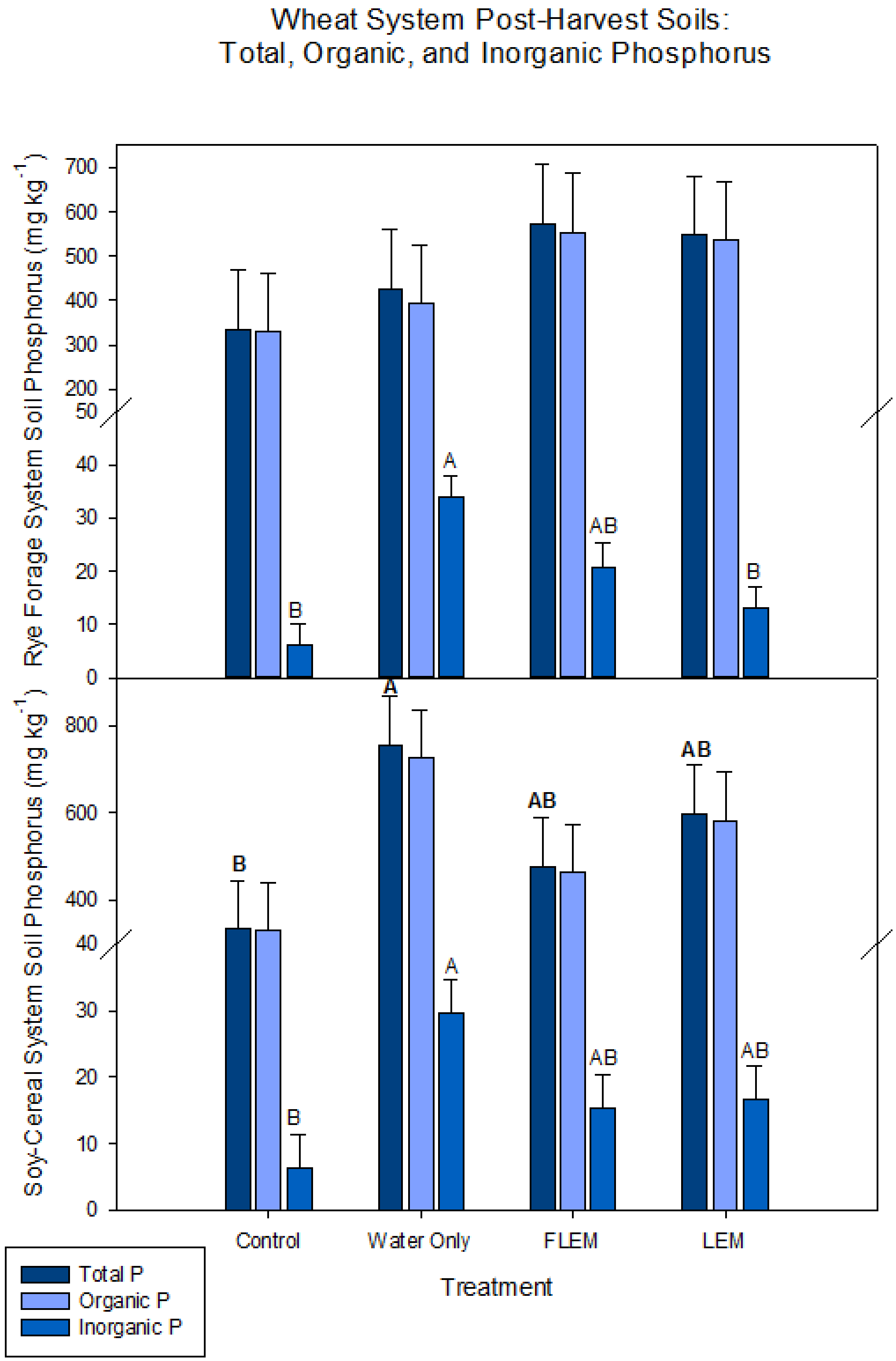

Figure 3.5: Data was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are

significantly different. Significance is shown between treatments for each soil P fraction. Total P significance is denoted by uppercase, bolded letters; inorganic P significance is denoted by uppercase letters. Organic P was not significant for either system.

Rye system soils showed significance for inorganic P at the post-harvest sampling with LEM soils showing the lowest inorganic P of treated soils. Because this sampling was taken after harvest, it can be surmised the LEM plots had a significantly lower level of inorganic P due to the lower ratio of organic to inorganic P in post-application soils, resulting in increased P uptake in LEM plots. Figure 3.7 shows significantly higher acid phosphatase activity in LEM plots, supporting the hypothesis that the lower ratio of organic to inorganic meant increased P availability and thus, increased uptake.

Soy-cereal system soils showed significance between Water-Only soils and Control soils for total P and inorganic P. Total P was highest in Water-Only plots, which also had the highest level of inorganic P. FLEM and LEM plots were not significantly different from each other, although LEM did show higher levels of total and organic P.

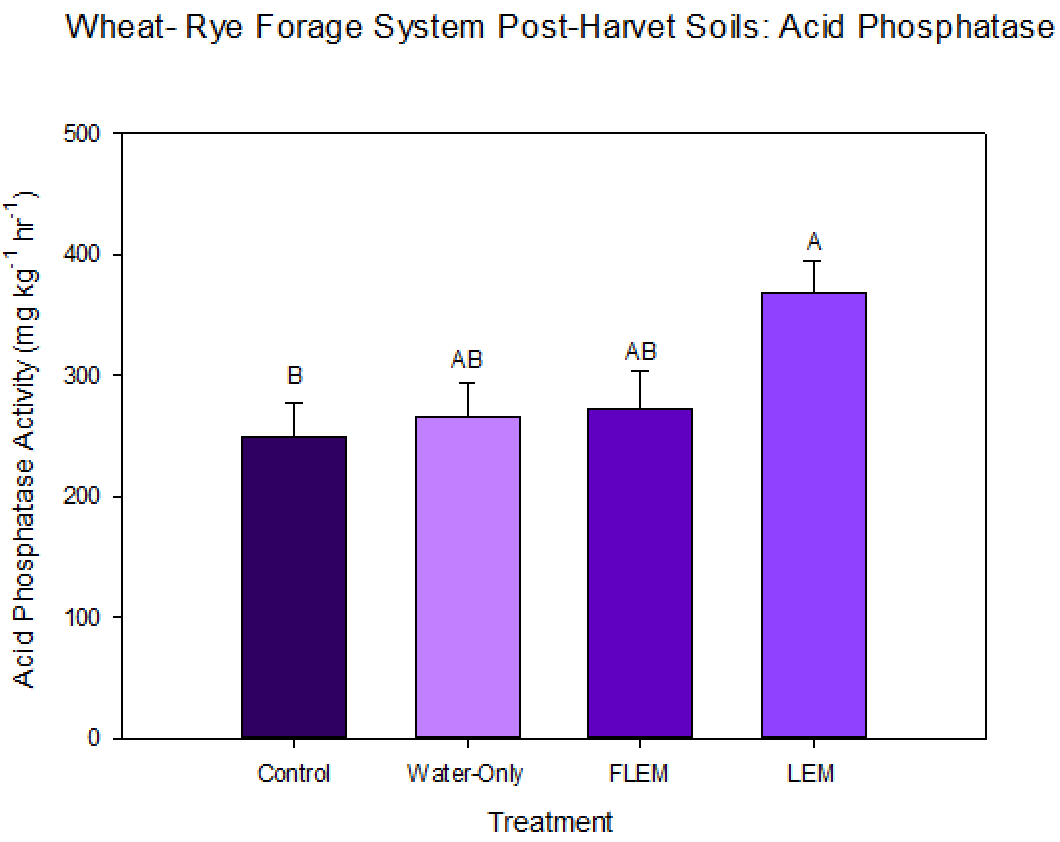

Figure 3.6: Data was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. LEM plots in the rye system showed significantly higher phosphatase activity compared to all other treatments. The higher phosphatase activity corresponds with the low level of inorganic P in post-harvest samplings. The higher activity caused more P to become plant-available, and was taken up by the wheat biomass.

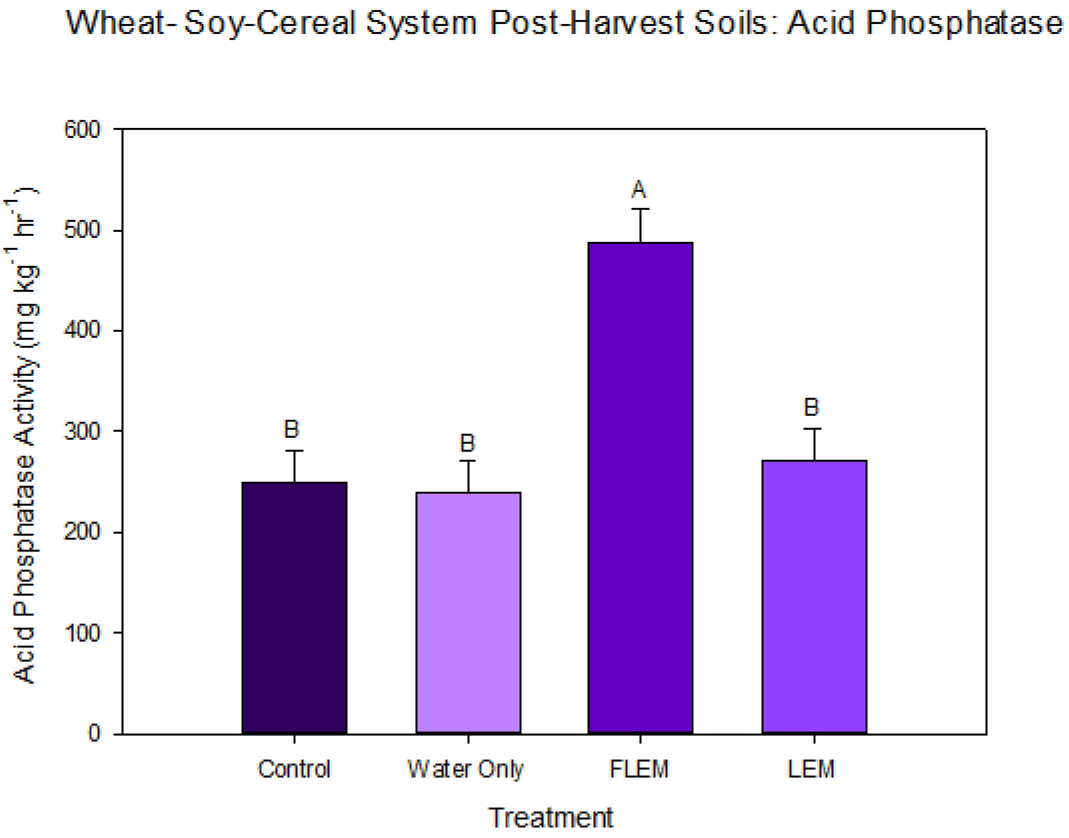

Figure 3.7: Data was analyzed using one-way ANOVA and Tukey HSD All Pairs means comparison at the 95% confidence level. Letters not connected by the same letter are significantly different. Soy-cereal system soils showed significant acid phosphatase activity in FLEM soils compared to both other treatments and controls, with relatively no difference in the phosphatase levels of the other treatments. FLEM phosphatase levels correspond with the lower ratio of organic to inorganic P found in the post-harvest soil samples. The higher activity caused hydrolytic cleavage of the inorganic phosphate anions from the organic P structure, much like the LEM plots in soy-cereal system post-application soils.

CHAPTER 4

CONCLUSIONS

Compost Study

Compost analysis showed no statistically significant differences in inorganic P levels, although LEM-treated compost did have the highest level of inorganic P at the end of composting. FLEM compost contained significantly less organic P than Water-Only treated compost, with LEM treated compost not significantly different than either FLEM or Water-Only. LEM-treated compost did show significantly higher acid phosphatase activity compared to Water-Only and FLEM treated compost. Due to the significantly higher phosphatase level and higher (although not significantly so) level of inorganic P, it can be determined the LEM treatment did positively affect the ratio of organic to inorganic P that was sought after in this portion of the project.

Soybean Study

Soybean soils were sampled pre-application of composted broiler litter and liquid treatment and again four weeks post-application, coinciding with the flowering of the soybean plants. Post-application sampling from year 1 showed a significant difference in organic P levels between FLEM soils (highest) and Water-Only and LEM soils. The post-application FLEM soils also showed a significant difference in inorganic P, having the lowest level in comparison to Water-Only and LEM soils. Acid phosphatase activity was significantly higher in Water-Only plots than in LEM or FLEM soils Hypothetically, this lack of expected results in the LEM soils may point to a problem often seen in bioremediation sites: microbes often have an adjustment period when introduced to a new site and it may take multiple inoculations for the new community to flourish. Inversely, the addition of LEM community microbes to the LEM plots may have caused competition between pre-existing microbial communities and microbes added through the LEM treatment. The Water-Only plots may have had such high acid phosphatase activity due to another theory of bioremediation. Biostimulation is another bioremediation mechanism where microbial activity is increased by addition of nutrients. Water-Only treated soils received only well water as the liquid treatment as well as broiler litter compost treated with the same well water. This may have helped boost pre-existing microbial performance in Water-Only plots. This higher phosphatase activity in Water-Only plots was reflected in the levels of organic P and inorganic P, both of which were significantly lower than in FLEM soils. LEM soils displayed the same significant differentiation from FLEM soils as did Water-Only plots, though without the higher phosphatase activity. The soybean study yielded promising results for the year 1 harvest with crop productivity (measured as bean yield, biomass, and total P in the beans), with LEM plots showing significantly higher biomass; however, bean yield and bean total P were not significantly different from either other treatment, and so it cannot be conclusively said the treatment had an effect on crop productivity. Soybean year 2 soils showed significant differences in samples from the pre-application sampling. FLEM soils were significantly lower in inorganic P than Water-Only, but not significantly different from LEM soil inorganic P. Total P, organic P, and acid phosphatase activity showed no significant difference for any treatment for pre-application or post-application samples. The soybean crop harvested during year 2 of the soybean study was significant: Water-Only treated plots produced a significantly higher biomass and yield than FLEM plots, which were also significantly higher in both biomass and yield than LEM plots. Unfortunately, the LEM plots were heavily grazed by deer during the year 2 growth season and it cannot be certain the LEM plots would have still shown less productivity or if the LEM plots would have exhibited a harvest similar to that of the year 1 harvest.

Wheat Study

The wheat study was conducted on two crop systems. The rye forage system showed no significant change in P levels (total, organic, and inorganic) across the crop season, but did show significant fluctuations in acid phosphatase activity in the FLEM soils. Pre-application for the rye system showed lower levels of inorganic P in LEM plots than in Water-Only and FLEM. Water-Only and FLEM inorganic P levels were significantly different from Control plots, and LEM soils weren't significantly different from any other plots. Acid phosphatase activity was not significant for pre-application sampling. Rye system post-application showed significantly lower total and organic P in LEM and Control soils. LEM having a lower total and organic P indicates a lower ratio of organic to inorganic P, meaning more P is moving from the organic to inorganic pool. Due to the loss of total P in the four weeks since pre-application, it was assumed phosphorus was being taken up by the plant. More phosphorus was available in LEM plots because of the decomposing biomass from the previous winter's forage crop or enhanced microbial activity in LEM-amended plots. Once again, acid phosphatase was not significant in any plots. Post-harvest soil samples for the rye grass system showed significantly lower inorganic P in LEM and Control plots, with Water-Only significantly higher, and FLEM plots not significantly different from any other plots. Total and organic P were not significant for any plots for this sampling. Post-harvest acid phosphatase activity was significantly higher in LEM plots, corresponding with the significantly lower inorganic P level. Increased microbial activity caused phosphatase activity to rise, meaning more P was taken up by and increasingly active microbial biomass, but more phosphatase activity led to more organic P to become plant available. This may explain the increase in total and organic P in post-harvest LEM soils from post-application soils. No significant productivity measures were found for rye system wheat.

Pre-application samples of soy-cereal system soils gave a significantly low level of inorganic P in LEM plots, with Water-Only significantly higher, and FLEM and Control plots significantly different from neither LEM nor Water-Only. A spike in total P was not seen in these pre-application soils, unlike the year 2 soybean LEM plots, which was the most recent crop. This is most likely due to the fact that much less soy biomass was able to be tilled into the plot, unlike the cereal rye of the previous winter. Lower overall P in LEM soils indicates increased availability and uptake by the wheat crop. Total P, organic P, and acid phosphatase activity were not significant for pre-application soil samplings, Post-application soils showed significance between treatments for organic and total P. LEM soils were significantly lower than Water-Only plots for total P, and significantly different from Water-Only and FLEM plots for organic P. This shows a lower ratio of organic to inorganic P in post-application LEM soils, meaning more P became available and was taken up by the crop. Inorganic P was only significantly lower for Control soils, Acid phosphatase was not significant for post-application soils. Post-harvest soils were significantly higher in total P in Water-Only plots compared to Control. Inorganic P showed the same significance. LEM and FLEM plots were not significantly different from

Water-Only or Control plots for inorganic and total P. Organic P was not significant for this sampling. Phosphatase activity was significantly higher in FLEM plots, corresponding with the lower level of inorganic P found in FLEM soils. LEM soils displayed a similar level of inorganic P, but lacked the significant phosphatase activity. Harvest analysis of the soy-cereal system wheat crop produced no significant differences in yield, biomass, or total P of harvested grain.

In conclusion, LEM treatment was a viable way of increasing the phosphatase activity of the compost. The LEM soils for both soybean crops showed a nutrient cycle in play. Increased phosphorus became available, was taken up by the soy biomass and the cereal rye cover crop, then returned to the LEM-treated soils in the form of tilled in biomass and became plant-available throughout the year 2 soybean season. The LEM treatments showed significantly greater production of biomass in year 1 soybean plots. Year 2 LEM soybean plots were damaged by deer. No LEM-treated wheat system plots showed an effect on productivity. Wheat soils seemed to have more inorganic P become available throughout the crop season, but this may have been due to the nutrient cycling effect seen between year 1 soy post-application and year 2 soy pre-application soils. While the LEM treatments may take several repeated years of inoculation to become completely reliable, data shows the LEM treatment does increase availability of P in soils, leading to higher crop uptake. When biomass in LEM plots is tilled back into the soil, the nutrients are regained as organic P and once again, hydrolytically released into inorganic P.

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