To the Graduate Council:

I am submitting herewith a thesis written by Gregory B. Hardison entitled "An Analysis of Bioavailable Phosphorus in Manured Soils". I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

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An Analysis of Bioavailable Phosphorus in Manured Soils

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Gregory Blake Hardison
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ABSTRACT

In the past and to a lesser extent today, plant nutrient requirements were met by land applying livestock manure. Animal manure is the world's oldest fertilizer yet it is no longer used completely on agricultural lands despite its being produced in increasing quantities. Applications of manure can beneficially affect soil nutrient status. Manures accomplish this by serving as an energy source for soil microorganisms that release soil nutrients. For this reason manure is considered a slow release fertilizer. The slow release nature of manure nutrients allows them to accumulate in soil following a single application. The slow release nature of manure nutrients coupled with their accumulation in soil organic nutrient pools complicates fertilizer nutrient recommendations. In the past available P (AP) was based solely on a single, chemically extracted P_i pool. This practice fails to account for contributions to the AP pool from mineralization of labile organic P (P_o) pools. Failure to account for contributions from P_o coupled with continued manure P inputs has led to a build-up of soil test P levels which, in turn, has become an environmental rather than an agronomic concern. Non-point sources of P leaving the agricultural landscape are increasingly responsible for the eutrophication of surface waters and the associated growth of undesirable aquatic plants. To this end researchers advocate the inclusion of P_o in soil P tests. Thien and Myers (1992) introduced the use of soil microorganisms as a bioextractant for use in development of a bioavailable P (BAP) index. One of the objectives of this

work was to evaluate this BAP method developed by Thien and Myers (1992) for use in soils receiving dairy cattle manure at two locations in Tennessee. Soils were collected from the Martin Agricultural Experiment Station (MAES) and the Dairy Experiment Station (DES) in Lewisburg. The MAES site had not received manure prior to the onset of this work, whereas the DES site had received periodic manure applications over the previous 35 years. Manure applications stopped in 1996 but sampling continued in 1997. The sites at these two locations provided insight into the effects of short and long-term manure management on soils susceptible to nutrient losses by surface runoff and leaching which contribute to pollution problems. Total nitrogen treatments included four rates of liquid dairy manure (126, 252, 378, and 504 kg manure N ha^{-1}), one NH_4NO_3 rate (218 kg N ha^{-1}), and a control (0 kg N ha^{-1}). The 378 kg manure N ha^{-1} treatment was not included at DES due to the lack of space. The MAES sites were cropped to no-till silage corn in the summer followed by an annual ryegrass-crimson clover winter cover and hay crop. Plots at DES were cropped to no-till silage corn followed by a winter wheat cover. Available P was determined by Olsen's 0.5 *M* $NaHCO_3$ extract (Kuo, 1996), Mehlich I extract (Kuo, 1996), and BAP (Thien and Myers, 1992). In 1991 Mehlich I and BAP extracted significantly greater concentrations of AP than Olsen did ($\alpha = 0.05$). This resulted primarily from the Olsen extract containing P_i only. The AP concentrations extracted by Mehlich I and BAP were statistically the same in all but the 378M and 504M in the 0-7.5 cm depth and the AN in the 7.5-15 cm depth at MAES in 1996 ($\alpha = 0.05$). Both Mehlich I and BAP extracted greater quantities of AP in the 0N and

126M than Olsen in the 0-7.5 cm depth. In plots getting the highest manure rates, Olsen and BAP extracted less AP than Mehlich I. At DES Mehlich I extracted significantly greater quantities of AP in the 0N, AN, and 504M in the 0-7.5 cm depth and Mehlich I and BAP extracted similar concentrations of AP in the manured plots in the 7.5-15 cm depth ($\alpha = 0.05$). In 1997, the AP concentrations in Mehlich I extracts were greater than those extracted by Olsens from all but the AN in the 7.5-15 cm depth and BAP except for the 504M in the 0-7.5 cm depth at MAES. At DES in 1997, Mehlich I extractable AP was greatest for all treatments. Each method had a strong positive correlation with silage corn yields in 1996 at MAES with Mehlich I > Olsen > BAP ($r = 0.77^{\dagger\dagger\dagger}$, $0.74^{\dagger\dagger\dagger}$, $0.68^{\dagger\dagger}$ respectively). These results indicate that Mehlich I extractable AP concentrations best reflect the amount of AP in Tennessee soils. We failed to see the kinds of results obtained by Thien and Myers with the BAP method (Thien and Myers 1992) and recommend the use of AP in Mehlich I extracts determined on the ICAP for estimating fertilizer rates in Tennessee.

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LIST OF ABBREVIATIONS

AP	Available Phosphorus
BAP	Bioavailable Phosphorus
BAP_o	Bioavailable Organic Phosphorus
BMP	Best Management Practice
CEC	Cation Exchange Capacity
FP	Iron hydroxide Impregnated Paper P Test
ICAP	Inductively Coupled Argon Plasma Emission – Spectrophotometry
DES	Dairy Experiment Station
MAES	Martin Agricultural Experiment Station
OM	Organic Matter
PI	Phosphorus Index
P_i	Inorganic Phosphorus
P_{i+m}	Inorganic + Microbial Phosphorus
P_{immob}	Immobilizable Phosphorus
P_m	Microbial Phosphorus
P_{min}	Mineralizable Phosphorus
P_o	Organic Phosphorus
P_t	Total Phosphorus
USEPA	United States Environmental Protection Agency
WSOC	Water – Soluble Organic Carbon

CHAPTER I

INTRODUCTION

Land disposal of animal manures has been practiced for many years. In the early 19th century soil fertility was maintained by land applying organic waste materials such as livestock manure. During that time period most of the fertilizer utilized originated from organic materials. Presently less than 1% of total fertilizer N is supplied by farmyard manure (McVickar, 1970). The latter percentage is astounding considering that the application of animal manure improves soil physical, chemical, and biological properties and serves as an important source of energy and nutrients for the soil ecosystem (Lampkin, 1994), in addition to seemingly completing the nutrient cycle (Brady and Weil, 1999).

Agricultural production is burdened by many environmental problems. One problem is the accumulation and resultant disposal of animal wastes from concentrated livestock operations. In 1995 (TASS), Tennessee supported approximately 2.6 million cattle and 160,000 milk cows. Given these figures and assuming 44 kg wet manure head^{-1} day^{-1} (Wells et al., 1978), wet dairy manure production alone would be approximately 2.6 *10^{6} Mg annually. Using a factor of 2.5 g of Nitrogen per kg of dairy slurry, this translate into approximately 6.5 *10^{3} Mg manure-N year^{-1}. Total P and K would also be high relative to N with approximately 1.5*10^{3} Mg of P and 7.3*10^{3} Mg of K produced yearly, using a factor of 0.58 and 2.8 g kg^{-1} manure for P and K respectively (Gilbertson et al., 1979). Many livestock operations managers dispose manure by land application and farmers often also apply commercial fertilizers to supply crops nutrient

requirement (White and Safley, 1984). This practice results in excessive amounts of nutrients which can lead to off-site water quality problems. Nutrient transport results from a combination of inappropriate manure applications and additions of mineral fertilizers. Animal wastes contribute to off-site nutrient transport via water runoff, leaching, and erosion. Off-site nutrient transport contributes to surface and groundwater pollution.

Animal manure is the world's oldest fertilizer yet it is no longer utilized completely by farmers on agricultural lands despite its being produced in increasing quantities. With the current emphasis on pollution control, the concept of manure disposal is changing to one of utilization, which simultaneously accomplishes disposal. Brady and Weil (1999) reported that utilizing animal manure would more than adequately meet plant nutrient requirements. Manure supplies nutrients at rates commensurate with plant needs by serving as an energy source for the microorganisms that release soil nutrients (Abbott, 1978). Due to the role that microorganisms play in nutrient availability many soil scientists advocate the measurement of more than a single extractable P pool for fertilizer recommendations (Thien and Myers, 1992; Tiessen, et al.,1984; Bowman and Cole, 1978).

Objectives of the Study

The working null hypothesis for this study was that there are no effects of manure applications on the P pools, mineralizable N, soluble organic C and chemical properties in the soil. The objectives of this work were:

1. To evaluate the bioavailable P method of Thein and Myers (1992) for use in soils receiving dairy cattle manure applications at two locations.
2. To evaluate impacts of dairy manure applications at two locations on soil C, N, and extractable nutrients.

CHAPTER II

REVIEW OF THE LITERATURE

Manure Applications to Soils

Applications of manure, and other biosolids, can be beneficial to the soil. Manures and biosolids provide organic C and nutrients to the soil. The addition of organic C to the soil enhances soil structure by providing the cementing substances, such as long chain polysaccharides, that bind soil separates to create stable aggregates (Miller and Donahue, 1995). Formation of stable aggregates provides erosion resistance, and promotes infiltration. Increased aggregate stability and infiltration decrease above ground transport of plant nutrients. Cation exchange capacity (CEC) of the soil is also increased by the accumulation of organic material and humic colloids (Ndayegamiye and Cote, 1989). A higher CEC results in greater storage and availability of essential plant nutrients. Increased soil organic matter provides soil microorganisms with a source of carbon thus increasing their activity. The ability of microorganisms to utilize the carbon in organic materials is dependent upon the C:N and C:P ratios of the material. Manure has low C:N and C:P ratios therefore carbon utilization would occur relatively easily and net mineralization of N and P would result from microbial activities. The mineralization and resultant availability of plant nutrients from manure applications has spurned research to evaluate the feasibility of manure to supply plant nutrient requirements.

Brady and Weil (1999) summarized those findings with the comparison that 2 Mg of an average manure will provide the amount of N, P_2O_5, and K_2O as 0.1 Mg of a 10-15-10 fertilizer.

In practice it is not uncommon for manure to be added to soils at rates as high as 90 Mg ha^{-1} (approximately 225 kg N ha^{-1}) (Young, et. al., 1985). Many research studies have been conducted with manure loading rates greater than 90 Mg ha^{-1} (Phillips, et al., 1981; Sutton, et al., 1986). Supporting research indicates an accumulation of mineralizable N and P over years of manure applications as evidenced by a continued increase in crop yields even after the practice of manure application has been discontinued and with no commercial fertilizers additions (Beri et al., 1995).

Since organic P and N accumulate in soils following long-term manure additions it can be difficult to determine nutrient recommendations. Therefore, many researchers believe that including a measurement of mineralization in manure/fertilizer application recommendations is important.

Phosphorus Availability

Agricultural soils often receive large amounts of P inputs annually through commercial fertilizer additions, above and below ground plant material, and manure and sludge applications. Over applying plant nutrients contributes to environmental issues. Specifically, nonpoint sources of phosphorus leaving agricultural landscapes can cause eutrophication of surface water and growth of undesirable aquatic plants.

The USEPA has identified agricultural runoff as the cause of impairment of 55% of surveyed river length and 58% of surveyed areas still having water quality problems (Sharpley,et al., 1994). Eutrophication is a result of runoff and erosion from agricultural non-point sources and is an increasing resource management concern. It occurs when excessive P or N in the aquatic environment results in uniformly distributed algal blooms through the water column or formation of dense scums or surface mats. Growth of undesirable aquatic weeds results from excessive additions of P through inorganic or organic P amendments (Lemunyon and Gilbert, 1993). The combined impact of eutrophication and undesirable weed growth is the depletion of oxygen (O_2) in the water body due to the heavy demand of microorganisms as they decompose this extra organic matter. The other primary impacts of eutrophication are decreased water clarity, algal scums, unsightly decaying algal clumps, discoloration, which is potentially caused by increasing phytoplankton populations and large diatoms, and filamentous algae can clog water treatment plant filters. Likewise, extensive growth of larger plant forms can interfere with recreation, navigation, and limit fisheries potential (Wingfield, 1985; Sharpley, et al., 1994).

Transport of P through the terrestrial environment can occur as either soluble or particulate P. Particulate forms of P typically represent the greatest portion of P transported from agricultural production systems. Particulate P provides a long – term source of P to aquatic biota. Sharpley et al. (1992), discovered that 10 – 90% of particulate P transported in agricultural runoff was bioavailable (available for algal growth) and a function of watershed management. Soluble forms of P generally represent P transport through forested and grassland ecosystems. Soluble P is primarily

composed of orthophosphate and is immediately available for algal uptake. Particulate P in agricultural runoff therefore poses a long–term threat to surface waters even after the implementations of P management strategies.

Regions where intense animal manure production occurs are especially susceptible to eutrophication for various reasons. The efficiency of livestock operations requires the confinement of large numbers of animals and ultimately the production of substantial volumes of manure. In most cases, manure is land applied as a means of disposal and application rates are based on nitrogen with little consideration given to the potential for excessive P applications. For example, when dairy manure was used to meet the N needs for fescue production in Arkansas, an excess of 17 kg ha^{-1} of P was applied (Sharpley, et al.,1994). Phosphorus excesses are even greater when application rates are adjusted for N losses attributable to volatilization. In light of this example one can conclude that the inherent characteristics of animal manure and nutrient uptake by crops promotes P build-up with a corresponding increase in potential P loss (Sharpley, et al., 1994).

Increases in soil test phosphorus levels following manure N applications in excess of crop needs is directly linked to the N:P ratio of the manure. The average N:P ratio for dairy manure is 3.4. The average N:P requirement of the major grains and hay crops is 8. The rapid build-up of soil test P resulting from the higher concentrations of P in manure relative to N indicates that a manure application strategy based on P levels may be appropriate. Several models and a P-index have been proposed to evaluate the impacts of nutrient loading on the environment (Sharpley, et al., 1994).

There are four types of modeling approaches used to evaluate the impact of nutrient loading on natural waterways. These are empirical correlations, budget models, nutrient-food chain models, and land-water models. Investigations indicate that a major deficiency in most of these models is their inability to discriminate between available and nonavailable forms of nutrients. Sharpley defined available forms of nutrients as those that when supplied to algae in surface waters promote rapid growth. Evidence for increasing incidences of P pollution have prompted the USDA to develop a P-index (PI) for identifying soils, landforms, and management that could have unfavorable impacts on water bodies because of non-point source P transport.

The purpose of the PI was to provide field researchers and land users with a tool to assess the various landforms and management practice for potential risk of P movement to water bodies. P-index rankings identify areas where the risk of P transport may be higher or lower than other areas within a watershed. The magnitude of the PI classifies a site's vulnerability for off-site P movement as low, medium, high, or very high. A higher score indicates a higher potential for significant transport of P from the site. The P site index is the sum of the products of a site characteristic weighting value and a P loss rating value for eight site characteristics (Pierzynski and Logan, 1993).

Many soil, hydrology, and land management site characteristics describe the landform to which the P-index is applied. Parameters used in the index have an influence on P availability, retention, management, and uptake. The parameters include erosion rate, irrigation erosion, runoff class, soil P test, P fertilizer application rate, P fertilizer application method, organic P application rate, and organic P application method. Each site characteristic has been assigned a weighting value that is based on the relative

importance it plays in the transport of P. These eight site characteristics were chosen for their ease of data acquisition and their impact on P movement (Stevens, et al.,1993).

The ability to determine the mechanisms which contribute most to P loss by utilizing the P-index make it an invaluable tool for targeting watersheds at risk for fertilizer pollution. However, it can offer no insight into soil P dynamics that could enable land managers to better estimate manure P application rates. Many soil scientists advocate the measure of phosphorus availability to better determine appropriate manure P application rates.

Availability of phosphorus is a function of: 1) the P concentration in the residue added to a soil, 2) the amounts and forms of P in the soil, 3) soil texture 4) the placement of the residue, 5) and environmental conditions such as pH, soil temperature, aeration, and soil moisture. Recycling of P through decomposition has long been recognized for its contributions to the available P pool in natural ecosystems, yet in cultivated ecosystems where P-fertilizers are utilized this aspect is frequently overlooked (Dalal, 1979). Phosphorus availability depends on mineralization, immobilization, solubilization, and precipitation reactions that occur in the soil.

Concentrations of P in the residue added to the soil play a significant role in the availability of P to soil solution and subsequently to plants by impacting rates of mineralization or immobilization. Phosphorus mineralization requires extracellular enzymes from the phosphatase group. Phosphatases catalyze hydrolytic reactions to release P from nucleotides, nucleic acids, phospholipids, and inositol phosphates. The mineralization of these compounds results in the release of orthophosphate into the soil solution. Net mineralization of P from organic inputs is dependent upon the C:P ratio of

the organic material. However sole reliance on the C:P ratio for estimates of P mineralization is unreliable due to the existence of P_i in organic amendments (Mullen, 1998).

Previous research has established the optimum C:P ratio for mineralization at < 200:1 (Mullen, 1998). Mineralization occurs when there is more P than is necessary for the residue C to be assimilated by microorganisms. Mineralization results in an increase of solution P available for plant uptake. Immobilization of phosphorus occurs when the decomposing organic compound lacks the amount of P required to assimilate all of the available carbon. The accepted C:P ratio at which P immobilization occurs is >300:1 (Mullen, 1998). In the latter scenario, microbial decomposers will scavenge P from soil solution in order to assimilate all of the C from the organic compound resulting in a decrease in plant available P.

The availability of P to plants is dependent upon the amounts and forms of P present in soils (Tiessen, et al., 1984). Primary minerals that contain P, such as apatite, are highly insoluble at pH >7.2. However the P in these minerals becomes increasingly mobilized as hydrolytic reactions proceed and pH values begin to drop. Research indicates that organic matter production is limited by P availability and the solubilization of primary inorganic P (P_i) is accompanied by organic matter and organic P (P_o) accumulation. The formation of secondary mineral P_i maintains labile plant available P in soils of an intermediate weathering status. In soils with a low pH, available P becomes limited by the formation of sparingly soluble, crystalline, or occluded Al and Fe phosphates. In such soils available P is limited and may be dependent on P_o mineralization (Tiessen, et al. 1984).

The dynamic relationship between the biology, mineralogy and chemistry of soils make it difficult to analyze the different P pools and has lead to the development of sequential extraction methodologies to characterize P_i and plant available P(Kuo, 1996). These sequential extraction procedures characterize readily available P to the least available through the utilization of bases and acids. Sequential extraction procedures that examine labile and more stable P_i and P_o in the vicinity of roots of incubated soils were developed by Hedley, et al. (1982). Resin and bicarbonate extractable P_i are thought to represent P_i adsorbed on the surfaces of crystalline P compounds, sesquioxides or carbonates. Sodium hydroxide extractable P_i is considered to be associated with amorphous and some crystalline Al and Fe phosphates. The latter extractable pool is less plant available as a result of those associations. Calcium bound P_i is, for the most part, represented by the acid extractable fraction of P_i. Soils with a high pH and base saturation tend to have lower proportions of P in extractable organic and inorganic forms, and somewhat higher acid P contents (Tiessen, et al., 1984). Plant available P is characterized by the readily mineralizable labile bicarbonate extractable P_o.

Weathering processes that accompany the development of soils like leaching of bases, removal of carbonates, and increasing activity of Al and Fe, cause a shift from primary to secondary P_i forms and influence the stabilization of organic matter and its associated P_o (Tiessen, et al., 1984). The abundance of and activity of the forms of P_i and the turnover of P_o control the replenishment of the amorphous and crystalline Al and Fe phosphates following plant uptake. Amorphous P_i replenishes the labile P pool while stable crystalline species act as a sink and long-term reservoir of P. Dalal and Hallsworth (1976), reported that hydroxide and acid extractable P_i may act as the factor that buffers

the more labile forms. Controlled mineralization of organic P fractions may perform a similar function. Analysis of P, based on an accepted concept of the soil P cycle, indicates that available P in highly developed soils is closely related to the organic P fraction, and indicates that the study of labile forms of P_o could contribute to advances in soil test procedures (Dalal and Hallsworth, 1976).

Huffman, et al. (1996) reported that P transformations could be related to soil textural properties. Ninety percent of the variability in the total P content of a Mollisol could be explained by texture. Increasing silt and clay contents have been significantly correlated with increasing P_o and $NaHCO_3$-extractable P pools. Likewise, hydrochloric acid-extractable P_i was positively correlated with increasing sand contents. Residual P was the only form not correlated with soil texture. Soil texture affects labile P_i, microbial P, and labile P_o pools. Soil textural effects were related to the percentage of water filled pores and the habitable pore space. Soils with finer textures support higher microbial biomass than do soils with more coarse texture. This indicates that transformations of labile P_i, microbial P, and labile P_o are directly linked to microbial activities. Phosphorus transformations are directly related to carbon flows. Residues and soil organic matter supply the energy for microbes to immobilize and mineralize P.

Residue placement and the subsequent increase in carbon and microbial dynamics of soils have been related to P transformations. Effects of residue placement on P transformations are dependent on the concentrations of nutrients in the soil at the time the residue is added. Research studying the effects of residue placement on P distribution, fertility, leaching, and mineralization showed that all increased when residues were allowed to decompose on the soil surface rather than when incorporated (Huffman, et. al.

1996). Residues and soil C supply most of the energy to drive nutrient cycling processes. Plants and microbes acquire nutrients from the residue, and soil inorganic and organic pools. The responsibility for residue decomposition and element cycling falls on the microbial biomass and saprophytic biomass pools. The differences between no-till and conventional till on P release from organic residues was more important during the earliest stages of decomposition (Huffman et al., 1996).

In the past available P was based solely on soil test levels for P_i (Abbott, 1978). This practice overlooked any P contributions from the P_o fraction (Thein and Myers, 1992) by measuring P already in soil solution and not the amount of P potentially available through mineralization. Thein and Myers (1992) demonstrated that soil tests reporting only the P available from the P_i fraction can greatly underestimate available P and contribute to environmental damage by overestimating commercial fertilizer recommendations. The P_o fraction can account for 30-50% of the total soil P. Estimates of readily mineralizable P_o would be beneficial for highly weathered soils that have low native P_i concentrations, making P_o a greater contributor to the amount of P available for plant utilization. The inclusion of P_o by soil test labs would provide information that could prevent excessive fertilizer application, enhance long-term fertility, improve monitoring of P dynamics in soils receiving little to no inorganic P fertilizer, and aid in the quantification of rate and extent of P_o mineralization (Thein and Myers, 1992). In general the measurement of bioavailable phosphorus would provide insight into the dynamics of the inorganic and organic P soil fractions.

The absorption of essential elements by plants grown in soils has been the focus of intensive research in soil chemistry and biochemistry. The development of a definition

for bioavailability is essential. Broadly speaking, an essential element is bioavailable if it is in a chemical available form that plants can absorb readily and if, once absorbed, it affects the life cycle of the plant (Sposito,1989). "Readily" refers to a time scale relevant to the continued growth of a plant. The phrase "affects the life cycle of the plant" means the element produces growth and development.

Bowman and Cole (1978) conducted research to examine the transformations and the bioavailability of organic P substrates. At that time little to no correlation had been made between the labile P_o pool and crop response. These researchers used the $NaHCO_3$ method of extracting P_o developed by Olsen (Kuo, 1996). They concluded that there is a strong positive correlation between total plant P uptake and the $NaHCO_3$ extractable P_o pool after three years of continuous cropping. To verify that the $NaHCO_3$ extraction method yielded a measure of the mineralizable P_o-P fraction they added various P substrates, similar to those found in soils, to soils to determine whether known amounts could be recovered. They calculated net mineralization by taking the difference between the increase in P_i of the incubated and the initial soil P_i levels. Their conclusion was that the $NaHCO_3$ extraction method is suited for evaluating P_o mineralization of various P_o containing substrates and in assessing P_o-P pool contribution to plant total P uptake.

Another relatively new approach to evaluate AP in soils is the iron hydroxide impregnated paper (FP) method developed by Menon, et al. (1988). This test uses iron hydroxide impregnated filter paper strips as a sink to sorb and retain P mobilized in a soil suspension thereby avoiding the limitations of chemical extractants. Phosphorus is extracted from soil solution by its high affinity for oxides. It has also been demonstrated that oxides have a higher affinity for P than any other soil anion with the exception of

hydroxide, and they sorb P to the filter paper surface through a process involving ligand exchange (Menon and Chien, 1995). As orthophosphate is removed from soil solution the concentration of solution orthophosphate is replenished from the labile P pools by the desorption of soil P and dissolution of slightly soluble compounds (Menon, et al., 1988). The FP test results have been correlated to yield where different fertilizers were used in soils with highly variable soil properties. The results showed that yield and P uptake were best correlated with FP in the order of FP>Olsen>Mehlich I (Menon and Chien, 1995).

Continued research on P, available from the P_o-pool, has been conducted by Thein and Myers (1992). Their research was guided by the premise that P_i and P_o pools in soils are in dynamic equilibrium. Therefore when available phosphorus (AP) from the P_i pool is depleted by plant uptake the $NaHCO_3$ extractable P_o pool can be mineralized. The conversion of P_o to P_i in soils is for the most part microbially mediated suggesting that chemically extractable-labile P_o and microbial activities should be strongly related. The conversion of P_o to AP, as is influenced by rhizosphere microorganisms, and the positive relationship between phosphatase activity and the available P in the rhizosphere are known and elude to a strong correlative index of AP, but the concept has not been further developed (McLaughin and Alston, 1986). One of the mechanisms that allow plants to adjust to low soil AP is by increasing phosphatase production. Thein and Myers (1992) deduced that in order to realistically evaluate the contribution of P_o to AP one must measure the P readily mineralized by soil microorganisms. Thus they proposed to design a P-extraction scheme that would index P availability by including P_i and P_o dynamics.

Inorganic P-organic P dynamics were evaluated by treating one set of soils with an enriched C + N source to stimulate microbial activities and to create a biological sink for P while another set of soils were left untreated. They measured the quantity of $NaHCO_3$ extractable P_i, total P (P_t), and microbial P (P_m) in both sets of soils and used the data to determine the size of the bioavailable P (BAP) pool. Treated soils always had less P_i following incubation indicating the probability of microbial immobilization. Labile P_o in the treated soils was on average 2.4 times greater than in untreated soils. Labile P_o, after incubation, averaged 2.8 times greater than the P_o level prior to incubation in the treated soils while remaining essentially the same in the untreated soils.

These results clearly showed that microbial activity enhanced P_o availability which thereby increased overall AP. The same results would not be apparent in soil tests where a test only measures P_i. Thein and Myers (1992) went a step further to combine the P_i, P_o, and P_m pools to create a BAP index.

Nitrogen and Carbon

Nitrogen Availability. Increases in crop yield have been the direct result of mass applications of mineral fertilizers. Nitrogen containing compounds are applied at the highest rates. Presently, concerns are being raised over the environmental risks involved in excessive fertilizer-N applications, in particular the leaching of nitrates to groundwater. As a consequence researchers are now faced with the challenge of rationing N fertilizer use while increasing yield profitability. The basis for this research

should be to determine the reserves of N in soil and what is the overall availability of those reserves for plant uptake. The determination of soil N reserves is relatively simple, the problem lies in estimating the amount of organic N that is going to be mineralized in the soil from the moment N is applied to the end of the growing season (Sanchez et al., 1998). Fertilizer estimates are further complicated by the forms of N that are applied.

Livestock manure is an inexpensive fertilizer resource because animals utilize only 20-25% of ingested N. Therefore most imported N, in the form of feed, remains in the area in the form of manure. Most of the N in manures is in the organic form (Pratt and Castellanos, 1981; Felipo and Garau, 1987). Before manure N can be available for plant uptake it must be mineralized, converted to ammonium or nitrate forms. Thus mineralization , or the amount of N that can be mineralized during a specified period, is the factor controlling the availability of manure N. Furthermore, the physical form of manure affects quantities of N that are potentially mineralizable during a growing season. Pratt and Castellanos (1981) stated that 75% of the N in a manure slurry was available in the first year after application, whereas only 45% of the N from solid manure was available in the first year. Solid manure, aged by wetting and drying as they accumulate on corral floors or under pens or coops lose N by volatilization of ammonia, the remaining N at application time is more stable and resistant to mineralization (Pratt and Castellanos, 1981).

Research indicates that animal wastes are a valuable nutrient amendment when applied to soil at rates commensurate with crop uptake (Chang and Janzen, 1996). This guideline, however, does not consider the heterogeneous nature of N in manure. Approximately half of the manure N is immediately available for plant uptake, NH_3-

volitalization, or leaching. The remaining N in manure is in organic forms that are slowly mineralizable (Chang and Janzen, 1996). Yet when rates of application exceed crop nitrogen requirements, the excess N can leach into groundwater or be lost to the atmosphere. High costs of transporting manure and the resulting tendency to apply high rates of manure close to production sources results in the areas with intensive livestock production being extremely susceptible to environmental damage. Therefore, best management practices (BMP) are required to exploit the agronomic benefits of manure and to minimize release of excess nutrients into the environment

Keeney (1982) defines available nitrogen (N) as N in the root zone in a chemical form readily absorbed by plant roots. Available nitrogen is derived from many sources, some are internal eg. N_2 fixation and OM , others are external eg. animal manure and crop residues. Nitrogen availability depends on the inputs, losses, and transformations of the N-cycle and the dynamic equilibrium between organic and inorganic N species within the soil system (Keeney, 1982). Significant amounts of organic N are mineralized during a growing season. One estimate reports that surface soils contain 0.08-0.4 % total N, with most of it being organic N. If only 1-3 % of this is mineralized during a growing season then approximately 8-120 kg of N ha^{-1} could be available for crop utilization (Keeny, 1982). Other research reports the proportion of N from cattle slurry that is available to plants during a single growing season lie within a range of 45-75% (Pratt and Castellanos, 1981; Thompson, et.al., 1987). An experiment comparing the availability of mineral N with cattle slurry N + nitrapyrin, an NH_3-volatilization inhibitor, found that the availability figures of 72% and 66% respectively compare favorably (Chang and Janzen, 1996). Sequential additions of manure to soils

and the accumulation of slow-release organic-N over long time periods makes it difficult to estimate available N for any particular growing season.

In the past, and to a lesser extent today, agricultural producers depended on N mineralization from legume residues, animal manure and soil organic matter to supply crop N requirements. Mineralization is the biological transformation of organic N to ammonium (NH_4^+), this is also referred to as gross nitrogen mineralization or ammonification. The transformation of organic N compounds to ammonium is enzymatically mediated in the soil by microorganisms. Extracellular enzymes are responsible for the degradation of organic N polymers in the first step in the mineralization process. These enzymes depolymerize proteins, microbial cell walls, nucleic acids, and urea (Myrold, 1998). The monomers that result from the depolymerization reactions pass across microbial cell membranes and are further metabolized by multi-step reactions involving intracellular enzymes. Ammonium produced by these reactions is released to the soil solution. Microbial degradation of N containing organic compounds result from the energy and carbon needs of heterotrophic microorganisms (Myrold, 1998).

Heterotrophic microorganisms need to acquire carbon and other nutrients from organic matter in the soil for growth and cell synthesis, therefore the production and release of ammonium can be viewed as an indirect benefit. Agricultural producers need to be conscientious concerning soil management in order to take advantage of ammonification. For gross mineralization to occur the carbonaceous compounds providing C and energy to microbial communities must contain a sufficient amount of N. Soil amendments with C:N ratios typically < 20:1 will promote mineralization. When

carbon and energy sources with C:N ratios > 30:1 are added to the soil, microbial synthesis results in soil N deficiency (Tate, 1995). An example of a carbon and energy source with a high C:N ratio is plant biomass. Plant biomass has C:N ratios ranging from 60:1 to 80:1. Research has shown that long-term management of soils with N containing organic amendments increases soil organic matter and microbial biomass, as compared to fertilized or unammended soils (Hadas, et al., 1996).

Long-term fate of nitrogen from annual feedlot manure applications was determined by Chang and Janzen (1996). The examination of yield and plant tissues revealed that higher rates of manure did not increase N uptake beyond the 30 Mg manure ha^{-1}. Total NO_3^--N in the soil profile increased with time in all manure treatments. Organic N concentrations in the soil increased gradually over time in all manure amended plots. Organic N accumulations were twice as great in soils receiving higher manure rates compared to the control plots. There were no significant N-leaching losses which suggests that the applied N was balanced by crop removal and increases in soil NO_3^--N and organic N. Chang and Janzen (1996) discovered that 56% of the applied manure N was available over time. This study showed that manure applied N was accounted for by crop removal, total soil N and soil NO_3^--N, indicating that losses via leaching, denitrification, and ammonia volatilization were negligible.

Indices of soil N availability can be determined by employing biological and chemical tests. Biological methods are considered to be more reliable because living organisms are used. Biological methods involving the estimation of N released during incubation with living organisms provide valid measures of the ability of a soil to release N for plant growth. Longer incubation periods are influenced less by sample

pretreatment and give more useful results. Incubation is also considered to be a disadvantage when using biological indices because it increases the amount of time required to determine available N over chemical methods.

The incubation period is performed under either aerobic or anaerobic conditions (Keeney, 1982). Most researchers choose to use the anaerobic method for N mineralization studies because of their simplicity and reproducibility. Anaerobic N mineralization methods have many advantages over aerobic incubation methods. The advantages to utilizing anaerobic incubation methods includes: 1) researchers only have to measure NH_4^+-N 2) problems normally associated with the establishment of an optimal water content during incubation do not arise 3) more N is mineralized during a given incubation period than is when aerobic methods are employed 4) higher incubation temperatures can be used since optimum temperatures for nitrification is not a concern. The latter advantage also contributes to more rapid mineralization rates.

Anaerobic methods are easily reproducible, requires no elaborate laboratory supplies, and have been shown to give good predictions of soil N availability. Anaerobic methods involve the incubation of a soil sample under anaerobic conditions in an enclosed test tube with as little head space as possible. During the incubation period biological activity maintains the anaerobic environment. The maintenance of anaerobic activity eliminates the possibility of nitrification and denitrification reactions at the soil water interface. Should nitrification and denitrification proceed then overall mineralization results would be significantly reduced. Mineralizable N determinations are made by taking the difference between the amount of NH_4^+-N in the soil prior to and following incubation.

Early evaluations of this mineralizable-N procedure found the results satisfactory. Later publications report that the effects of sample pretreatment may be the cause of poor correlations with field evaluations of available N (Bundy and Meisinger, 1996). However, insignificant correlations did not appear to represent a contradiction between anaerobic incubation tests and field results but instead differences in N transformations quantified by the two methods. Field available N measurements reflect the net effects of mineralization and immobilization while anaerobic incubation measures N mineralized from aerobic microbes lysed by the anaerobic test conditions (Bundy and Meisinger, 1996).

Water-Soluble Organic Carbon. Applications of organic wastes to agricultural lands has received growing criticism in recent years because of environmental problems associated with disposal problems. Animal manures have well been established for their contributions to improving soil fertility for crop production. One of the immediate impacts of the application of manures onto agricultural lands is an increase in water-soluble organic carbon (WSOC). Natural soil processes helping to generate WSOC include: substrate fragmentation, depolymerization, and solubilization, microbial lysis in inhospitable environments, and faunal grazing on microorganisms (Wang and Bettany, 1993). Water-soluble organic C has been well established as the immediate energy source for soil microorganisms (Huang and Schenau, 1996; Liang, et al., 1996; Boyer and Groffman, 1996).

Researchers have conducted considerable research to determine the effects of WSOC on heterotrophic microbial processes. Research has concluded that microbial processes in soils are frequently limited by the availability of C. Water-soluble organic

carbon has been identified as an important energy source for heterotrophic decomposers and denitrifiers in agricultural surface soils (Bufford and Bremner, 1975). Also suggested in their work is that WSOC assessment could be used as an indicator of the soil's ability to denitrify. Water-soluble organic carbon in decomposing plant residues is recognized as a readily degradable fraction with a strong influence on N immobilization and residue persistence in soils. The factors that affect the production, transport, biovailability and degradation rate of WSOC are important in understanding nutrient cycling and pollution dynamics in soils and groundwater (Boyer and Groffman, 1996).

Water-soluble organic carbon is derived from many internal and external sources within the soil environment. The production of WSOC depends upon plant type, soil type, amount of primary production, nutrient dynamics, and temperature. One important external source of WSOC is from manure applications onto soils. Water-soluble organic C in manure contains a fraction of labile C that can stimulate microbial growth (Liang, et al., 1996). Research was conducted to evaluate the effects of WSOC extracted from dairy manure on C mineralization in soils of varying texture. Mineralization rates of WSOC is a function of microbial biomass, temperature, water, and nutrient availability, as well as the concentration and bioavailability of the substrate. The decomposition of organic matter can generally be described as occurring in two phases; an initial rapid mineralization of added substrates and derived microbial cells, accounting for 60 - 80% of the added C, is followed by a slower mineralization of stable microbial products and undecomposed materials (Liang, et. al., 1996). Much of the WSOC produced at the soil surface is mineralized within the top 10 cm by soil animals, fungi, and bacteria. In an experiment, conducted by Liang, et al. (1996), three soils with

textures ranging from 3 - 54 % clay were amended with WSOC extracted from dairy manure at rates of 0 - 80 mg WSOC kg soil^{-1} and incubated for 16 weeks at 23° C. The differences in amount of mineralized C, with and without added WSOC, were approximately 13 - 16 times, 4.8 - 8 times, and 7.5 - 8 times greater than the amount of C added to clay, loam, and sand soils respectively. The total amount of C mineralized was greater than the amount of C added in the extracts in the three soils. This research resulted in the conclusion that immediately following manure applications, C mineralization rates increase, and that most of the C mineralized is derived from the indigenous soil organic C pool. The amount of WSOC found at any given time represents the equilibrium between synthesis and destruction. These results suggest that the value of manuring for the maintenance of soil organic matter levels may depend on the percentage of WSOC in the manure. Besides changes in C mineralization resulting from manure WSOC extracts Liang et. al. (1996) examined the effects of these extracts on N mineralization.

In addition to containing labile C, soluble organic extracts from manures contain labile N that may contribute to microbial stimulation. Understanding the effects of WSOC on N-mineralization could improve agronomist's knowledge of the short - term N turnover in soil. Using the same soil samples and treatments as described above, Liang, et al. (1996) incubated the soils for 11 weeks at 23°C. Water soluble organic carbon additions were found to enhance net N-mineralization only in the soil containing the highest clay content, and the increased net N-mineralization was similar to the amounts of organic N added in the water extract. The WSOC extracted from the manure had little effect on net N-mineralization, especially in coarse-textured soils. They concluded that

the short-term effect of WSOC inputs on the net N-mineralization in soils may depend on the mineral N concentration of the water extract.

Agricultural soils reportedly have greater concentrations of WSOC than do forest soils despite the latter having higher concentrations of soil organic carbon (SOC) (Boyer and Groffman, 1996). The differences between corn and wooded soils were related to the litter quality of corn versus forest vegetation. Litter quality is influenced by C:N ratio and the presence of recalcitrant compounds. Highly fertilized, annual plants have higher litter quality than forest vegetation. High litter quality materials have been found to decompose and mineralize faster than low litter quality materials. Higher litter quality materials have been found to provide greater amounts of WSOC to the soil environment. Mineralization decreases the amount of WSOC available to leach to deeper zones in well structured soils.

Water-soluble organic C can be responsible for the transport and loss of organic nutrients from a soil profile. Rolston and Liss (1989) reported significant movement of WSOC to a depth of 0.8 m associated with high manuring rates and irrigation. Recent studies in Canada have demonstrated significant leaching of WSOC (Wang and Bettany, 1993). The WSOC concentrations found in subsoil contained high concentrations of N and P, and greater than 50 % of water soluble P was associated with WSOC in the A horizons of certain Luvisols. This water soluble organic P was not adsorbed by the soil horizons and it was suggested that organic nutrients may move within the profiles and be lost in some soils through the movement of WSOC. Also reported has been the significance of the role that WSOC plays in the translocation of some pesticides (Boyer and Groffman, 1996).

Freeze-thaw and flooding have been identified as increasing leaching losses of WSOC from soils. Freeze-thaw cycles contribute to additional sources of WSOC through lysis of microbes during freezing. This destruction of the microbial population and resultant increase in the release of cellular components significantly increases the WSOC pool. Shrinking and expansion of soil organic matter during freeze-thaw cycles enhance fragmentation and surface exposition of the OM. This in turn may facilitate the biochemical and biological degradation of the fragmented materials. The importance of the above process in the translocation of WSOC was recognized during periods of flooding during spring months. These results suggest that great care is needed in the interpretation of field data taken at a single point in time because chaotic climatic events can alter the quantity of WSOC (Wang and Bettany, 1993)

Water-soluble organic carbon studies are typically conducted using a water extraction procedure. A few studies have utilized a K_2SO_4 extraction method to quantify soluble organic carbon in soil. Research by Huang and Schoenau (1996) found that both the water and K_2SO_4 extraction procedures are reproducible with low coefficients of variation. They conducted regression analysis of the two extraction methods and the results indicate a strong relationship ($r = 0.93$). The concentrations of WSOC were higher for the K_2SO_4 extraction method than for the water extraction. This suggested to researchers that the K_2SO_4 extraction remove additional organic C from the solid phase and that the K_2SO_4 may partially lyse microbial cells thereby releasing additional organic C into the soil solution. A second reason for finding higher concentrations of organic C using the K_2SO_4 extraction procedure is that K_2SO_4 may induce changes in pH that will affect the charge of soil mineral surfaces and soil organic

matter. The water extraction procedure more closely represents the composition of soil and is therefore considered to be a better method to employ when evaluating WSOC. In addition, the water extraction method is simple, requiring only water and no chemicals. Therefore the water extraction method will be utilized in this study.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

Field plots were established at the Martin Agricultural Experiment Station (MAES) in May, 1991 and at the Dairy Experiment Station (DES) in Lewisburg in April, 1992. Martin is located in a region with highly erodable upland loessal soils where conservation tillage is required to maintain the productivity on these soils. Surface runoff is a major concern in this region. The experimental site is located on a Loring silt loam soil (fine, silty, mixed, thermic, Typic Fragiudalf) with 5% slopes. The Dairy Experiment Station is located in an area of relatively shallow, undulating clayey and silty soils over limestone. Problems may occur with both runoff and leaching to shallow groundwater through karst parent materials. The Lewisburg experimental site is located on a Huntington silt loam (fine, silty, mixed, mesic, Fluventic Hapludalf) with slopes of less than or equal to 2%. The Martin sites had not been manured prior to the onset of their establishment, whereas the Lewisburg sites have received periodic manure applications over the previous 35 years. Manure was applied at both sites up to 1996. No manure was applied in 1997. These sites provide insight into the effects of short and long-term manure applications on soils susceptible to nutrient losses by surface runoff and leaching contributing to pollution problems.

Manure treatments are given in Table 1. Total nitrogen treatments included four rates of liquid dairy manure (126, 252, 378, and 504 kg manure N ha^{-1}), one NH_4NO_3 rate

Table 1. Manure and fertilizer treatments used at MAES and DES

Treatment	Treatment ID	N Rate
		------kg ha^{-1}------
Control	0N	0
Inorganic Fertilizer	AN	218
126 kg Manure N	126M	126
252 kg Manure N	252M	252
378 kg Manure N	378M	378
504 kg Manure N	504M	504

(218 kg N ha^{-1}), and a control (0 kg N ha^{-1}). These rates were split into spring and fall applications. Spring N treatments were 84, 168, 252, or 336 kg N ha^{-1} as manure, 168 kg N ha^{-1} as NH_4NO_3, and the control. Fall nitrogen treatments were 42, 84, 128, and 168 kg N ha^{-1} as manure, 50 kg N ha^{-1} as NH_4NO_3, and the control. At Lewisburg the 378 kg N ha^{-1} treatment was omitted due to the lack of space. These applications cover the deficient to the excessive N application range. However, the high application rate is not uncommon for dairy operators in these areas. Inorganic P and K were applied to the NH_4NO_3 fertilizer plots at soil test recommended levels. Treatments were replicated three times at each site. The plots were arranged in a completely randomized design at both sites. Plot sizes were 0.007 and 0.013 ha at Martin and Lewisburg respectively. Martin was cropped to no-till corn silage in the summer followed by an annual ryegrass-crimson clover winter cover and hay crop. The plots at Lewisburg were cropped to a no-till corn silage crop followed by a wheat winter cover. These rotations are common for the respective areas of the state.

Manure was obtained as slurry from holding tanks at each location. Total Kjeldahl N was determined prior to application in order to determine the volume of manure to apply to each plot. The slurry was homogeneously mixed by a tractor driven pump prior to removal. The slurry was transported to the field in a 1500 L tank and pumped onto the plots through a hose using a submersible sewage pump. Slurry homogeneity was maintained by an agitator in the tank that continuously mixed the slurry during application. Treatment volumes were monitored using a calibrated dipstick in the tank. Subsamples of the fresh slurry were taken during the application for the determination of total Kjeldahl N, total P, and K in $HClO_4$ digests, pH, and % dry matter.

Analytical Procedures

Soil samples were collected from each plot in the fall of 1991, 1996, and 1997 at the Martin Experiment Station and fall 1992, 1996, and 1997 from the Lewisburg sites. Samples used for the purposes of this research were obtained from the 0-7.5 and 7.5-15 cm depths. Two soil samples were taken in each plot using a 7.6 cm bucket auger. These samples were mixed and the composite was used for subsequent analysis. Soil samples from Lewisburg 1992 could not be located and were excluded from the following analytical procedures. Composited soil samples were air dried, sieved to < 2 mm, and stored at room temperature. Samples were analyzed for pH in a 2:1 (water: soil) suspension by the glass electrode method (Thomas, 1996). The elements Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn were extracted using Mehlich-I double-acid extract and the extracts were analyzed using inductively coupled argon plasma emission-spectrophotometry (ICAP) (Soltanpour et. al., 1996). Total C and N were determined with the LECO CNS-2000 (Matejovic, 1997).

Bioavailable phosphorus was determined using a procedure developed by Thien and Myers (1992). Their phosphorus index requires the quantitative analysis of inorganic, organic and microbial soil P pools. Extractable inorganic and organic P were determined using Olsen extraction (Kuo, 1996). Microbial P was determined later using a modification of the Olsen procedure (Thien and Myers, 1992). Three grams of each soil sample were extracted with 30 ml 0.5 *M* $NaHCO_3$ to allow measurement of these soil P pools. The 0.5 *M* $NaHCO_3$ solution extracts labile inorganic P (P_i) and organic P (P_o). Bicarbonate releases the readily available P_i into solution by forming precipitates with

Ca^{2+}. Total P (P_t) was determined by digesting an appropriate aliquot of the extract with potassium persulfate and 2 M H_2SO_4 in an autoclave for 30 min. at 121°C. Phosphorus concentrations were determined colorimetrically at 880 nm using the ascorbic acid method (Kuo, 1996). Organic P was calculated by taking the difference between the total and inorganic P fractions.

Bioavailable P was measured using the method of Thien and Myers (1992). Prior to an incubation step, the P_i and P_o concentrations were determined with 0.5 *M* $NaHCO_3$ extraction procedure developed by Olsen (Kuo, 1996). These samples provide a baseline and are referred to as preincubation P_i and P_o. Three grams of soil were placed in each of four centrifuge tubes. Two tubes were treated with 1.5 mL of 10:1 C+N solution and the remaining two with water. The C+N solution was composed of 0.44 *M* dextrose and 0.24 *M* NH_4Cl (Thein and Myers, 1992) and stored under refrigerated conditions. Addition of C+N to the incubated samples stimulates microbial activities which, in turn, creates a biological sink for P. The centrifuge tubes receiving the C+N solution were labeled bioactive (+CN) because this solution was intended to stimulate microbial activities. The centrifuge tubes treated with water only were labeled untreated (-CN). The four tubes were separated into two sets (set A and B) with one of each treatment per set. The tubes were loosely capped and placed in an incubator at 36°C for 7 days. At the end of the incubation period the samples in set A were analyzed for P_i, P_t, and P_o by the technique described in the preceding paragraph. The set B samples underwent fumigation with 2 mL of 1-hexanol to lyse microbial cells (Thein and Myers, 1992). The centrifuge tubes were capped for 24 h, then uncapped and evaporated for 36 h. This step is intended to solubilize any P immobilized by the microbial biomass during the incubation step. All

extractable P was quantified by 0.5 *M* $NaHCO_3$ extraction following fumigation. The P value obtained through $NaHCO_3$ extraction represents P_i plus P_m (P_{i+m}) released by the lysing step. A value for P_m, to be used later to help describe BAP, was obtained by subtracting the post-incubated P_i concentration from the P_{i+m} concentration then dividing by a recovery factor of 0.4 (Thein and Myers, 1992).

Inorganic N was measured using a method for microscale determination of inorganic N (NH_4^+ and NO_3^-) in soil extracts (Sims, et al., 1995). In this procedure NH_4^+ and NO_3^- extracted from soil using 0.5 M KCl were determined by measuring the intensity of the green color that forms upon treatment of an aliquot of the extracts with citrate, salicylate-nitroprusside, and hypochlorite at 650 nm on a microplate reader (Sims,et al., 1995). Mineralizable nitrogen was measured by incubating a 5 g soil sample in 10 mL deionized H_2O at 36° C for 7 days. Ammonium-N was determined as described above for incubated and non-incubated samples. The difference between the two measures was interpreted as the N mineralized during the time period (Mulvaney, 1996).

Water soluble organic carbon was determined utilizing the procedure developed by Bufford and Bremner (1975). Extracts used in the analysis were obtained by shaking 10 g of soil with 30 ml of reagent grade water for 30 minutes in a 50 ml polyethylene centrifuge tube. The soil-water suspensions were centrifuged. Centrifugation was carried out in a two step process. The first centrifugation was performed for 5 minutes at 1,490.7 *g*. The supernatant was decanted into a second centrifuge tube which was centrifuged a second time for 60 seconds at 1,490.7 *g*. The clear supernatant resulting from the final centrifugation step was filtered through a 0.45 μm filter. This step removed microorganisms from the filtrate that could lead to the overestimation of

WSOC. The filtrate was analyzed by the automated UV persulfate oxidation method for total C on a Dohrman automatic carbon analyzer (Santa Clara, CA).

Data Analysis and Interpretation

The data obtained from this research was analyzed for significance using the statistical procedures available in the SAS statistical software package (SAS, 1990). Data were evaluated with respect to the manure and fertilizer N application rates, depth. Data were evaluated for differences in total, inorganic, and organic P, available P (BAP, Olsen, and Mehlich I), mineralizable N, and water-soluble organic carbon using analysis of variance procedures. When analysis of variance procedures indicated the existence of significant treatment effects means were separated using Fisher's protected LSD.

CHAPTER IV

RESULTS AND DISCUSSION

Soil Chemical Properties

pH. The soils tested had variable pH, organic C, N, Ca, K, Mg, and P from year, location and depth of sample. In 1991, at the Martin Agricultural Experiment Station (MAES), the 126M pH value was greater than that found in all the other treatments in depth two (Table 2). The AN pH values were significantly lower than all other treatments in 1996 and 1997 at 0-7.5 cm depth and in the 7.5-15 cm depth for 1997. There were no significant differences in pH values at DES during 1996 (Table 3). In 1997, the AN treatment was statistically lower than the other treatments for depth one. In depth two, the AN was significantly lower than the 504M (Tables 2 and 3). The recurrence of statistically lower pH values in the AN plots is attributed to nitrification of NH_4^+. Manure, on the other hand, has the ability to moderate soil acidification by increasing soil OM and CEC (Tisdale et al, 1993). Increasing OM and CEC increase a soils pH buffering capacity thereby manured soils can sustain higher pH despite H^+ producing reactions occurring in the soil.

Carbon and Nitrogen. There were no differences among treatments for carbon and nitrogen concentrations in 1991. By 1996, differences were apparent in all soils at both locations (Tables 2 and 3). At MAES, concentrations of C and N were significantly greater in the 378M than the 0N, AN, and 126M. At DES the 0N had significantly lower

Table 2. pH and total carbon and nitrogen for MAES

	pH			C			N		
Treatment	1991	1996	1997	1991	1996	1997	1991	1996	1997
				g kg soil^{-1}			g kg soil^{-1}		
0-7.5 cm									
0N	5.15	5.88 a †	5.81 a	13.5	11.9 b	11.3 b	6.8	1.3 b	1.5 ab
AN	5.22	5.25 b	5.16 b	12.5	14.0 b	9.5 b	6.4	1.3 b	1.1 b
126M	5.73	6.04 a	5.79 a	14.1	13.0 b	15.6 ab	7.2	1.4 b	1.9 a
252M	5.16	6.25 a	5.92 a	18.1	17.7 ab	14.0 ab	6.1	1.8 ab	1.7 ab
378M	5.22	6.23 a	6.15 a	14.5	21.9 a	15.0 ab	7.6	2.2 a	1.7 ab
504M	5.18	6.10 a	5.88 a	15.8	17.3 ab	19.8 a	8.1	1.7 ab	2.1 a
7.5-15 cm									
0N	5.59 b	6.27 ab	6.19 a	6.9	6.7	7.1	3.5	0.7	0.9
AN	5.67 b	5.92 b	5.50 b	8.4	6.7	7.4	4.2	0.7	1.1
126M	6.49 a	6.56 a	6.37 a	8.1	7.0	7.3	4.1	0.8	1.0
252M	5.91 b	6.66 a	6.21 a	8.0	7.0	7.1	4.1	0.8	1.0
378M	5.84 b	6.63 a	6.37 a	7.0	6.7	8.0	3.6	0.7	1.1
504M	5.82 b	6.43 a	6.21 a	7.5	8.0	7.9	3.9	0.8	1.2

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

Table 3. pH and total carbon and nitrogen for DES

Treatment	pH		C		N	
	1996	1997	1996	1997	1996	1997
			----------10^5 g kg soil^{-1}----------		----------10^3 g kg soil^{-1}----------	
0-7.5 cm						
0N	6.47	6.52 a †	15.4 b	17.2	1.4 b	1.5 b
AN	6.18	5.94 b	17.9 ab	19.1	1.7 ab	1.6 ab
126M	6.54	6.64 a	18.1 ab	21.2	1.6 ab	1.9 a
252M	6.62	6.49 a	20.0 ab	18.0	1.8 ab	1.6 b
504M	6.62	6.79 a	23.9 a	21.3	2.2 a	1.9 a
7.5-15 cm						
0N	6.40	6.57 ab	13.1	12.8	1.2	1.2 c
AN	6.47	6.42 b	13.7	13.5	1.3	1.2 c
126M	6.47	6.67 ab	12.3	14.1	1.1	1.5 ab
252M	6.66	6.61 ab	13.9	13.6	1.3	1.3 bc
504M	6.78	6.86 a	13.1	15.8	1.2	1.6 a

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

C and N concentrations than those in soils receiving the 504M (Table 2). Carbon accumulations in soils under manure treatments were expected. Fewer differences at the DES probably resulted from long-term manure applications to all plots prior to this study.

In 1997, at MAES, carbon concentrations in the surface soil were significantly greater in the plots receiving the high manure rate (504M) than the 0N or AN plots. There were no significant differences in C concentration among treatments at DES. Nitrogen concentrations in the surface soils of the 126M and 504M were significantly greater than those in the AN at MAES and the 0N at DES. The 126M and 504M N concentrations were greater than those in the 0N and AN in depth two at DES. The differences in C and N concentrations during 1997, reflects the persistence of organic C and N with repeating manure applications. Carbon data for MAES revealed that increasing rates of manure from 126M to 378M significantly affected C concentrations. Lower manure rates did not affect soil C concentrations by comparison to 0N and AN treatments. Chang and Janzen (1996) found that organic N concentrations gradually increased over 18 years of manure applications. Increases in N concentrations resulted from incomplete utilization of manure-N during the growing season that immediately followed manure application. The MAES N data agree with these findings. The establishment of N reserves was a benefit from manure application and could be detected after six years of manure utilization. Furthermore, there were no treatment applications in 1997 so differences in N concentrations in soils receiving the 504M and AN provide evidence that organic N fertilizers have a sustained nutrient supplying ability. When compared, the lowest manure-N application rate resulted in greater quantities of total N despite having the application of 92 kg N ha^{-1} much less N over five years than the AN-N rate. Fewer

differences among treatment soil N concentrations at DES could have resulted from 35 years of uniform manure applications across the research area prior to treatment applications. This could have resulted in a uniform distribution and accumulation of soil C and N across all plots thereby minimizing manure treatment effects. Differing N concentrations in depth two at DES indicate that N is mobile in the soil profile under long-term manure management. Others have reported that approximately one half of the manure-N is immediately available for plant uptake, NH_3 - volatilization, and leaching (Chang and Janzen, 1996). They show that under long-term manure management NO_3^- leaching increased with prolonged manure application. When applied in the fall, NO_3^- nitrogen accounted for 29-36% of the applied manure-N (Chang and Janzen, 1996). Differences in N concentrations in depth 2 at DES were expected given the long-term accumulations of N in the soil surface.

Mineralizable Nitrogen. Mineralizable-N was quantified to examine the amount of N potentially available to growing plants from soil organic N pools. Mineralizable-N was interpreted as the amount of N equivalent to applied fertilizer-N which should reduce annual fertilizer N applications (Bundy and Meissinger, 1996). For the purposes of this research mineralizable-N was determined by the method developed for evaluating ammonium production under waterlogged conditions by Waring and Bremner (1964). This method was recommended by Keeney (1982) because it uses the same microbial agents active under field conditions. In 1991, at the MAES site, mineralizable-N concentrations were not significant in depth one (Table 4). At depth two, 378M mineralizable–N concentrations were significantly greater than those measures for other treatments (Table 4). Insignificance among the treatments in depth one and the slight

Table 4. Mineralizable nitrogen at MAES and DES

	Mineralizable N				
	MAES			DES	
Treatment	1991	1996	1997	1996	1997
	mg kg^{-1}				
0-7.5 cm					
0N	77.5	44.9 b	40.7bc	58.8 b	40.9
AN	55.9	37.7 b	27.0 c	65.6 ab	39.4
126M	81.6	55.3 ab	75.3 a	60.0 b	64.9
252M	68. 7	69.7 a	53.7 abc	65.7 ab	52.6
378M	68.6	66.7 a	62.3 ab	††	††
504M	93.9	51.5 ab	52.2 abc	83.3 a	49.11
7.5-15 cm					
0N	25.9 b †	14.9 b	13.2	51.4	32.3
AN	30.4 b	18.8 b	16.6	50.1	26.3
126M	25.3 b	17.2 b	20.4	56.5	44.7
252M	30.4 b	20.9 ab	9.7	49.2	30.4
378M	64.1 a	19.5 ab	18.3	††	††
504M	25.8 b	27.3 a	21.3	55.5	46.5

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

†† DES did not get the 378M

differences in depth two were probably the result of a single treatment application at prior to sampling or soil heterogeneity.

Significant differences were observed in 1996 among samples evaluated for mineralizable-N at MAES and DES (Table 4). Both medium manure rates at MAES had mineralizable-N concentrations that exceeded those in the AN in depth one (Table 4). In depth two 504M plots had the highest rates at 27.3 mg N kg^{-1} soil ($\alpha = 0.05$). At DES, 504M concentrations were statistically greater than the 126M and 0N (Table 4). Significance of mineralizable-N concentration among treatments in depth two could have resulted from N transport through the profile in the plots receiving high manure applications.

Mineralizable-N concentrations were significant in 1997 at MAES only (Table 4). There, mineralizable-N concentrations were greater in the 126M and 378M than those in the AN ($\alpha = 0.05$). We expected to see higher mineralization rates in the 126M than in the AN because of the concomitant addition of C in the plot receiving the manure treatment. Greater concentrations of available N in the 126M at MAES reflects the residual benefits of long-term manure application over mineral fertilizers on organic matter accumulation and soil microbial communities (Hadas, et. al., 1996). Research by Chang and Janzen (1996) on the long-term fate of manure nitrogen discovered increasing amounts of N mineralization with increasing manure-N rates. This finding was concurrent with the 1996 MAES and DES depth one results.

Water-Soluble Organic Carbon. The consequences of nutrient losses from agricultural production systems are numerous and a major source of non-point source pollution. Many attempts to identify causes for these losses have focused on soil

microbial activities. Carbon availability is the factor that commonly limits soil microbial activities (Boyer and Groffman, 1996). Identifying factors that affect the production, transport, bioavailability, and degradation of soil organic C is therefore important in understanding nutrient cycling in soils. To this end research has shown that approximately 50% of water soluble P is associated with WSOC in surface soils (Wang and Bettany, 1993). Water-soluble organic carbon (WSOC) obtained from subsoil contained large concentrations of P and N. Further research demonstrated that water-soluble organic P was not adsorbed by soil horizons, which suggests that organic nutrients may translocate within profiles and be lost from some soils through movement of WSOC (Wang and Bettany, 1993). Quantifying WSOC concentrations could aid in our understanding of nutrient losses from agricultural systems, in particular those that incorporate organic fertilizers as part of their nutrient supplying strategy. The water extraction method used in this study was described by Bufford and Bremner (1975). The results were highly varied but roughly followed the expected pattern of the control and fertilizer treatments being less than the 126M, 252M, 378M, and 504M treatments in ascending order for the Martin Lysimeter plots.

In 1991, MAES plots were more varied than expected given that the plots had received only one treatment application. The values for WSOC ranged from 170.98 to 231.52 mg C kg^{-1} soil in the AN and 504M respectively (Table 5). The AN had significantly lower WSOC concentrations than the 126M, 378M and 504M ($\alpha = 0.05$). Values for WSOC were not significant in depth two.

In 1996 the MAES depth one 378M concentrations for WSOC were significantly greater than those found in the 0N (Table 5). At DES, the 504M treatment supplied

Table 5. Water-soluble organic carbon at MAES and DES

	Water-soluble Organic C				
	MAES			DES	
Treatment	1991	1996	1997	1996	1997
	----------	----------	mg kg^{-1}	----------	----------
0-7.5 cm					
0N	197.64 ab †	164.99 b	264.99 ab	164.0 b	151.9
AN	170.98 b	271.96 ab	208.61 ab	225.3 ab	148.2
126M	217.56 a	195.32 ab	350.28 a	142.1 b	187.0
252M	217 ab	261.09 ab	227.79 ab	254.8 ab	158.5
378M	231.52 a	280.59 a	173.92 b	††	††
504M	228.63 a	210.64 ab	184.49 b	338.3 a	166.4
7.5-15 cm					
0N	103.83	98.74	158.46	161.2	122.8
AN	108.20	141.15	81.29	158.6	102.9
126M	105.90	120.43	162.5	177.0	145.1
252M	118.15	123.73	117.93	164.5	116.9
378M	117.28	145.21	121.75	††	††
504M	110.16	129.53	81.00	196.1	136.5

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

†† DES did not get the 378M

significantly greater WSOC concentrations than the 0N and 126M. There were no differences among treatment concentrations in depth two at either location (Table 5). Differences were expected between all plots getting manure, the control, and fertilizer plots. The absence of differences may have resulted from the qualitatively observed response of the crimson clover winter cover crop. Plant residues on the 0N and AN could have compensated for enough of the difference between C additions on the manure plots because crimson clover appeared to flourish in the 0N and 126M plots and not in the heavily manured plots and the AN plots, where ample N was available for grass growth.

In 1997,WSOC concentrations in the 378M and 504M were significantly lower than those in the 126M at MAES ($\alpha = 0.05$). There were no significant differences in WSOC concentrations among treatments in depth two at MAES or at DES in 1997 (Table 5).

Discrepancies in the WSOC data might be explained by the research of Rolston and Liss (1989). They reported findings that rewetting and incubation of air dried soil samples has been known to create a burst of CO_2 production when compared to the same soil kept continuously moist. Three possible causes of such high CO_2 evolution are an increase in the solubility of organic substrates, an observed higher concentration of amino acids in air dried soils, and an increase in rapidly decomposable material released from the protective association with clay minerals by the action of drying (Rolston and Liss, 1989). Rolston and Liss (1989) also reported that the fall addition of 45 Mg ha^{-1} of manure significantly affected the level of WSOC only until the following fall. Given these results, it could be assumed that the period from 1996 to1997 was long enough for WSOC concentrations to become insignificant among treatments.

There were no significant correlations between nitrogen mineralization and WSOC in either depth at MAES in 1996 or 1997 (r = 0.26 and r = 0.41). These results were similar to the findings of Liang et al. (1996). However in 1996 at DES a strong correlation existed between N-mineralization and WSOC (r = 0.71††). The relationship was still apparent yet not as strong (r = 0.66††) in 1997.

Extractable Elements. There were no differences among treatments for extractable elements at MAES except for Ca concentrations in 1991(Table 6). In depth one, 252M the Ca concentration was significantly lower than that in 126M. Depth two Ca concentrations in 126M were statistically greater than the AN and 504M treatments. Because there were no differences for the other elements in 1991 we can assume that treatment differences for Ca were the result of field heterogeneity since there had been only one treatment application.

In1996, differences in all element concentrations had developed in depth one at both locations (Table 6 and 7). The highest manure rates (378M and 504M) resulted in significantly greater K, Mg, and P concentrations than in plots receiving 0N and AN in depth one at MAES. Calcium concentrations in the 378M and 504M at MAES were greater than those in the AN. At DES, Mg and P concentrations in the 504M were significantly greater than those found in the AN, and K concentrations were greater in the 504M treatment than in either the 0N and AN of depth one. Calcium in the surface soil was significantly greater in the 504M than in the AN and DES. Phosphorus was the only extracted element with differences in depth two in 1996 at MAES (Table 6). Concentrations of P were greater in the 378M and 504M than in the 0N and AN treatments. At DES concentrations of K in the 126M and 504M were significantly

Table 6. Mehlich I extractable Ca, K, Mg, and P for MAES

	Ca			K			Mg			P		
Treatment	1991	1996	1997	1991	1996	1997	1991	1996	1997	1991	1996	1997
	g kg soil^{-1}			mg kg soil^{-1}								
0-7.5 cm												
0N	15.0 ab†	14.4 bc	13.5 ab	123	63 c	58	162	119 c	109 c	32.4	25.6 b	18.9 c
AN	14.7 ab	10.9 c	8.9 b	85	82 c	74	158	105 c	189 abc	27.8	49.2 b	23.6 c
126M	16.3 a	16.8 bc	16.0 a	90	84 c	72	151	162 bc	162 bc	26.7	41.4 b	45.4 bc
252M	13.4 b	17.6 ab	15.5 a	95	95 bc	58	154	237 ab	177 abc	21.2	74.1 b	48.3 bc
378M	14.8 ab	22.8 a	17.1 a	112	141a	78	170	315 a	237 ab	25.9	139.3 a	72.9 b
504M	14.6 ab	18.2 ab	18.3 a	122	129 ab	74	175	299 a	261 a	26.6	137.2 a	114.9 a
7.5-15 cm												
0N	14.5 ab	13.7	12.8 ab	51	53	54	196	132	116	8.0	9.5 d	7.8 b
AN	13.2 b	11.9	10.3 b	39	69	55	186	140	120	5.8	11.6 cd	16.3 ab
126M	17.4 a	14.5	14.0 a	44	57	54	170	125	122	6.8	11.9 cd	13.5 b
252M	15.1 ab	13.5	12.9 ab	44	65	54	153	161	146	6.3	19.8 bc	14.1 b
378M	14.6 ab	13.8	13.7 a	44	70	61	145	181	172	6.3	22.5 b	17.4 ab
504M	12.2 b	12.7	12.1 ab	48	72	63	137	196	176	4.9	39.4 a	27.2 a

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

Table 7. Mehlich I extractable Ca, K, Mg, and P for DES

Treatment	Ca		K		Mg		P	
	1996	1997	1996	1997	1996	1997	1996	1997
	g kg^{-1}		mg kg-1					
0-7.5 cm								
0N	19.0 ab †	24.5 ab	182 c	136 d	108 c	116 c	36.6 b	83.2 b
AN	16.2 ab	18.0 b	223 bc	209 cd	99 c	101 c	47.6 b	63.0 b
126M	19.6 ab	19.9 ab	332 b	293 bc	158 b	168 b	35.8 b	93.4 b
252M	20.3 ab	21.0 ab	282 bc	305 b	171 b	179 b	46.2 b	68.4 b
504M	22.9 a	25.5 a	463 a	442 a	250 a	254 a	100.2 a	138.1 a
7.5-15 cm								
0N	19.1	18.9	119 c	87 c	90 b	77 c	27.7	36.2 b
AN	19.7	22.1	119 c	105 c	99 b	93 bc	25.1	29.3 b
126M	19.1	17.3	178 b	216 b	106 b	115 b	22.8	45.2 b
252M	19.9	19.9	167 bc	179 bc	116 ab	112 b	23.4	37.8 b
504M	19.2	23.2	339 a	327 a	141 a	151 a	41.7	71.9 a

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

greater than those in 0N or AN plots and the 504M Mg concentration was significantly greater than plots getting the 0N, AN, and 126M (Table 7). Significant concentrations of these nutrients in subsurface layers reflect their apparent movement from the soil surface when applied at the high manure rates. Early reports found P movement into the subsurface soil to depths of 0.2- 0.4 m following single manure applications at rates in excess of 90 Mg ha^{-1} (Abbott and Tucker, 1973; Thangudu, et al., 1981). Movement of P through soil to a depth of 0.8m was more recently reported (Rolston and Liss, 1989). Much of the P at this depth is in the water-soluble form and is associated with water-soluble carbon. Ryan et al. (1994) found that, when applied to soils in a pot study, farmyard manure reduced P sorption. The mobility of phosphorus under different farming systems depends on the kinds of bonds between P ions and the solid phase of soil. Organic anions from the applied manure form complexes with mineral components of clay surfaces, which would normally attract and retain P anions (Ryan, et al., 1994). Additionally, researchers demonstrated that manure applications modify the kinds of bonds that can occur thereby rendering more soil P to labile pools (Oberson, et al., 1993). A final explanation is that P contained in microbial cells and cellular debris can move physically and account for marginal amounts P redistribution (Abbott and Tucker, 1973).

In 1997, concentrations of Ca continued to be significantly greater in all plots receiving 378M and 504M treatments than in plots receiving AN at MAES (Table 6). Concentrations of extractable Mg, in the plots receiving highest manure rate at MAES, exceeded those in the 0N and 126M in depth one and 378M and 504M P concentrations were significantly greater than those in the 0N or AN. At DES, differences in Ca were observed between the 504M and AN plots (Tables 7). The highest manure rate at DES

also resulted in greater concentrations of extractable K, Mg, and P than those found in the 0N and AN. Given that no further treatment applications were made in 1997 at either location, the data demonstrates the residual benefits gained by utilizing manure fertilizer.

Phosphorus Changes and Availability

Nutrient management is becoming important in agricultural production. Knowledge of P status in soil is a necessary aspect of nutrient management due to potential environmental impacts. To better assess soil P status emphasis has been placed on including estimates of available P (AP) in soil P tests for fertilizer recommendations.

Traditionally, AP indices have been based exclusively on inorganic P extracted in Olsen or Mehlich I extracts. Olsen's, or 0.5 *M* $NaHCO_3$, extract typically extracts less P than Mehlich I (Tisdale, et al., 1993; Kuo, 1996). The occurrence of great differences between extractable levels of AP can be attributed to the effects of soil P buffering on AP extractability (Kuo, 1996). The 0.5 *M* $NaHCO_3$ method of Olsen was primarily developed to extract P_i and to correlate this pool with plant response. The Olsen extraction accomplished this by simulating root extraction in calcareous soils. The theoretical basis for the extraction is that solubility of calcium-phosphate increases as a result of decreasing Ca^{2+} activity in extracting solution. This method has the advantage of efficient surface extraction, and decreases secondary precipitation and adsorption reactions (Bowman and Cole, 1978). Olsen test levels of 10 mg kg $soil^{-1}$ are considered in the high category (Kuo, 1996).

The Mehlich I P test was developed for use in acid soils, here $AlPO_4$ is the primary P mineral controlling solution P concentrations. Chlorine anions in the extraction solution form complexes with aluminum cations decreasing its solution concentration. As solution Al^{3+} decreases, aluminum phosphate dissolves to replenish solution Al^{3+} and release P into solution. Phosphorus that is released into solution is measured and represents the amount of P in soil that is potentially plant available. The HCl in the extract also releases P from Ca-P minerals in neutral to acid soils. Mehlich I extracts large amounts of nonlabile P in soils that have a pH greater than 6.0 (Kuo, 1996). The Mehlich I procedure is inaccurate in alkaline or calcareous soils, soils that have just received rock phosphate applications, and in soils with a high CEC or base saturation. Mehlich I phosphorus levels of 20 to 30 mg kg $soil^{-1}$ are considered to be high (Kuo, 1996). Some researchers have suggested that adjustments for different soil types need to be made. Mehlich I is the primary extractant used by soil testing laboratories to determine AP in Tennessee soils. In Tennessee, soil P test levels greater than 30 mg kg $soil^{-1}$ are considered high. Mehlich I-AP soil test levels were presented in tables 6 and 7 and discussed in the previous section.

Research indicates that using chemical extracts to evaluate AP may be inadequate because they do not include the most labile forms of P_o (Bowman and Cole, 1976; Abbott, 1977). An initial approach to determine available P was to include the fraction of organic P most likely to be mineralized during a growing season. (Bowman and Cole, 1976; Abbott, 1978). The rate at which P_o becomes available for plant uptake is largely dependent upon the rate of organic matter decomposition and is a function of microbial activities. Because of this role, Thien and Myers (1992) developed a method for

measuring AP that includes microbial biomass P. Evaluation of changes in soil P_i and P_o concentrations were estimated prior to and following a seven day incubation at 36°C. In addition to using incubation, microbial activities were stimulated by the addition of a C+N solution in an attempt to better assess P changes in the root zone. Inorganic and organic P were estimated using the 0.5 *M* $NaHCO_3$ extraction step described previously (Kuo, 1996). This method was utilized because $NaHCO_3$ was found to extract the most labile forms of P_i and P_o (Bowman and Cole, 1978). Microbial biomass P (P_m) was determined using a modification of the Olsen method (Thien and Myers, 1992).

No differences in preincubation 0.5 *M* $NaHCO_3$ extractable P_i or P_o (hereafter referred to as pre-P_i and pre-P_o) concentrations were evident in 1991 at MAES except for pre-P_o depth two (Table 8). Here, pre-P_o concentrations were significantly lower in the 0N plots than in the 504M plots. This difference could be the result of heterogeneity of P in the soil given that only one treatment application had been made at the time of this sampling.

In 1996, differences in preincubation $NaHCO_3$ P_i and P_o concentrations had developed in the surface soils at both locations. (Tables 8 and 9). Concentrations of pre-P_i at MAES were significantly greater in the 378M and 504M plots than in the 0N and AN plots. Differences in pre-P_o concentrations occurred between the higher manure rates (Table 8). Depth two, pre-P_i and pre-P_o concentrations in 504M were significantly greater than in plots getting AN treatment. At DES, pre-P_i concentrations in the 504M treatment were greater than for all other treatments (Table 9). Inorganic and organic P concentrations were statistically the same for the AN and 126M, and 252M treatments at both locations in the surface soils. This indicated that manure and mineral fertilizers have

Table 8. Preincubation $NaHCO_3$ extractable inorganic and organic phosphorus for MAES

	Pre-Inorganic P			Pre-Organic P		
Treatment	**1991**	**1996**	**1997**	**1991**	**1996**	**1997**
	mg kg^{-1}					
0-7.5 cm						
0N	16.5	4.3 c †	3.4 d	8.5	12.4 ab	10.9
AN	13.2	10.6 bc	7.1 cd	8.7	12.9 ab	12.4
126M	13.9	10.5 bc	12.5 c	9.2	11.0 ab	13.8
252M	11.9	25.6 b	12.0 c	9.8	13.1 ab	12.5
378M	15.7	45.1 a	22.9 b	10.4	14.9 a	11.9
504M	17.3	45.6 a	31.9 a	7.2	9.1 b	13.0
7.5-15 cm						
0N	2.5	0.2 bc	1.1 b	6.9 a	9.2	6.7 ab
AN	1.7	0.5 c	1.9 ab	4.9 ab	8.7	5.2 b
126M	2.5	0.8 c	2.5 ab	5.0 ab	6.9	5.3 b
252M	1.9	3.4 b	3.9 ab	6.5 ab	9.0	6.1 ab
378M	2.8	3.7 b	4.7 ab	6.2 ab	9.2	7.7 ab
504M	2.5	12.9 a	5.7 a	4.4 b	8.5	10.1 a

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

Table 9. Preincubation $NaHCO_3$ extractable inorganic and organic phosphorus for DES

	Pre-Inorganic P		Pre-organic P	
Treatment	**1996**	**1997**	**1996**	**1997**
	mg kg^{-1}			
0-7.5 cm				
0N	15.7 b †	16.7 b	10.2	8.5
AN	15.9 b	18.3 b	13.1	12.3
126M	16.8 b	22.6 b	13.1	19.3
252M	17.7 b	19.6 b	13.1	10.0
378M	43.6 a	38.7 a	7.2	8.3
7.5-15 cm				
0N	9.1	8.0 bc	9.5	9.9
AN	7.7	6.6 c	9.3	9.2
126M	8.2	11.2 b	10.2	13.2
252M	9.6	8.3 bc	12.9	8.4
378M	15.1	18.2 a	8.7	8.6

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

comparable P supplying abilities when applied at the same P rate and no additional P benefits were achieved from increasing manure applications rates. Other researchers have found that the form of soil P, as determined by similar $NaHCO_3$ extraction procedures, was affected by agricultural management (Oberson, et al., 1993). They reported increases in P_i pools by fertilizer and manure P additions, however, P_o pools were affected by manure P applications only.

In 1997, concentrations were significant in both at MAES and DES (Tables 8 and 9). MAES pre-P_i concentrations were significantly greater in the 378M and 504M plots than in the 0N and AN plots. Depth two 504M pre-P_i concentrations were greater than those in 0N extracts only. DES pre-P_i Concentrations were statistically greater at both depths in the 504M than in the AN. There were very few differences in pre-P_o among treatments at either location.

Soil phosphorus dynamics were further evaluated using the analytical scheme developed by Thein and Myers (1992). Soils getting the C+N solution were termed bioactive (+CN), and were intended to simulate the energy-rich conditions of rhizosphere soils where elevated biological activity would increase the demands for P from both inorganic and organic P pools. The C+N enrichment allows the evaluation of the soil's potential to supply P to actively growing roots. The non-bioactive (-CN) soils are indicative of bulk soil bioavailability. Adding C+N to half of the soil samples during incubation stimulated and subsequently increased microbial activity which, in turn, created a sink that placed demands on the most biologically available forms of P.

In 1991, significant differences were observed in the +CN P_i concentrations only. The 252M +CN P_i concentration was significantly less than those in the AN plot

(Table 10). The differences in the incubated soils were likely caused by stimulated microbial activities because there was no significance among treatments in the samples that were not incubated. The latter indicates that the significance in the incubated samples is more a reflection of soil heterogeneity than of improvements in the soil microbial community receiving manure instead of mineral fertilizer.

In 1996, significant differences in P_i and P_o concentrations were observed in both sets of soils at both locations. The MAES P_i concentrations in the 378M and 504M significantly exceeded concentrations in 0N and AN for both sets of soils and at both depths (Table 10) . Organic P concentrations were significantly greater in the 378M plots than in the 0N in depth one of the +CN soils. In depth two, the +CN P_o concentrations were greater in the 378M and 504M plots than those in the 0N, AN, and 126M plots. No significant differences occurred in P_o concentrations among treatments in the -CN soils (Table 10). At DES the 252M +CN P_i concentrations were greater than those found in the 0N and AN plots for depth one (Table 11). In depth two, the 252M +CN P_i concentrations were greater than those in 0N. In the -CN soils the 252M P_i concentrations exceeded those for all other treatments in depth one. There were no significant differences in the -CN P_i concentrations in depth two. Bioactive and non-bioactive soil organic P concentrations lacked significance in the surface soil at DES. In depth two the 252M +CN P_o concentrations were significantly greater than P_o concentrations found in 0N (Table 11).

At MAES in 1997, 378M and 504M +CN P_i and –CN P_i concentrations were greater than those in the 0N and the 504M concentration exceeded that of the AN in the 0-7.5 cm depth (Table 10). In depth two, the AN and 504M P_i concentrations were in

Table 10. $NaHCO_3$ extractable inorganic and organic phosphorus for post-incubated bioactive (+CN) and non-bioactive (-CN) treated soils at MAES

	1991		1996		1997	
Treatment	+CN	-CN	+CN	-CN	+CN	-CN
	mg P kg soil^{-1}					
			Inorganic P			
0-7.5 cm						
0N	14.1 b	19.1	6.1 c	8.5 d	3.2 c	4.2 c
AN	12.2 a	14.7	9.3 bc	10.4 d	6.1 bc	5.8 c
126M	10.9 ab	17.4	8.2 bc	15.7 cd	6.0 bc	10.4 b
252M	7.5 b	14.7	20.2 b	25.6 bc	5.1 bc	9.6 b
378M	11.5 ab	18.3	35.1 a	37.3 ab	9.5 b	13.4 b
504M	10.3 ab	16.9	37.9 a	41.5 a	15.5 a	18.8 a
7.5-15 cm						
0N	4.1 a	5.9	2.3 c	3.5 d	1.1 b	2.2 b
AN	2.9 ab	4.4	2.5 c	3.4 d	2.7 a	6.4 a
126M	3.3 ab	5.2	2.7 c	4.5 cd	2.1 ab	3.7 ab
252M	2.6 b	4.9	4.1 bc	7.8 bc	2.2 ab	3.6 ab
378M	2.2 b	4.9	4.9 b	8.7 b	2.7 a	4.3 ab
504M	2.3 b	4.5	8.9 a	14.8 a	3.6 a	6.1 a
			Organic P			
0-7.5 cm						
0N	15.5	16.8	12.5 b	11.1	6.4 b	6.7 c
AN	15.9	13.7	20.5 ab	11.7	12.9 ab	9.2 c
126M	22.4	14.9	16.3 ab	9.7	12.3 ab	10.8 b
252M	21.1	14.3	17.6 ab	12.8	10.7 ab	10.2 bc
378M	20.9	15.9	23.8 a	15.0	12.5 ab	11.6 ab
504M	20.7	13.1	18.9 ab	14.7	16.2 a	15.4 a
7.5-15 cm						
0N	6.8	4.7	9.8 b	9.7	3.6	3.8
AN	7.0	5.2	10.3 b	10.1	4.2	4.1
126M	6.3	3.6	10.2 b	8.9	4.1	4.4
252M	10.5	5.1	11.1 ab	8.4	4.2	4.3
378M	9.2	5.8	12.9 a	9.2	4.5	4.7
504M	7.1	3.8	12.9 a	8.8	5.2	5.8

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

Table 11. $NaHCO_3$ extractable inorganic and organic phosphorus for post-incubated bioactive (+CN) and non-bioactive (-CN) treated soils at DES

Treatment	1996		1997	
	+CN	-CN	+CN	-CN
	mg kg^{-1}			
	Inorganic P			
0-7.5 cm				
0N	3.9 c †	11.8 b	16.3 b	23.8 b
AN	8.3 b	10.3 b	24.7 ab	26.9 b
126M	6.9 b	13.7b	17.2 b	24.9 b
252M	22.7 a	31.4 a	33.0 a	39.5 a
504M	5.6 bc	10.7 b	16.1 b	22.6 b
7.5-15 cm				
0N	2.0 b	7.4	9.6 b	12.4 bc
AN	3.9 ab	6.8	12.5 ab	16.4 b
126M	1.6 b	6.1	8.1 b	11.4 c
252M	7.8 a	12.1	16.8 a	22.5 a
504M	1.3 b	5.7	8.2 b	10.6 c
	Organic P			
0-7.5 cm				
0N	14.2	11.4	12.4 b	8.4 ab
AN	11.0	10.2	16.9 ab	9.7 ab
126M	16.0	12.6	11.8 b	8.9 ab
252M	17.2	12.3	13.6 b	6.7 b
504M	17.7	12.5	20.2 a	10.9 a
7.5-15 cm				
0N	11.8 c	10.4	9.6	7.8
AN	15.9 ab	11.2	13.7	9.2
126M	13.5 abc	10.7	10.3	8.1
252M	16.4 a	10.4	11.9	7.3
504M	12.2 bc	10.3	12.6	7.6

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

excess of those extracted in the 0N for both sets of incubated soils. Significance in incubated P_o concentrations occurred in depth one soils samples at MAES (Table 10). In the +CN soils, the AN and 504M concentrations were greater than the 0N. For the –CN soils the 126M and 504M P_o concentrations were greater than the 0N and AN. At DES, 252M + and –CN P_i concentrations were greater, at both depths, than the 0N, and also the AN in the –CN treated soils (Table 11). The +CN P_o concentrations in the 504 were significantly greater than 0N for depth one only. Thein and Myers (1992) found that upon comparison P_o concentrations increased in +CN soils with incubation by an average factor of 2.6 times. They also reported that concentrations of P_o extracted from soils treated with C+N were consistently greater than those treated with water. Abbott (1978), also reported increasing P_o concentrations in extracts following incubations when compared to nonincubated soils. These increases in P_o can be accounted for by the mineralization of P, followed by its immobilization into microbial biomass. Immobilized P from the biomass pool eventually enters the soluble P pool through cellular turnover. A comparison of the data presented above for pre- and post-incubation concentrations revealed that incubation very seldom increased P_o concentrations in either set of incubated samples ($\alpha = 0.05$). The P_o concentration differences in +CN and -CN treated soils were seldom significant when compared across treatments ($\alpha = 0.05$). In cases where there was significance, neither the +CN nor -CN soils had predominantly higher P_o concentrations. The lack of similarities among +CN nor -CN soil P_o concentrations may be attributable to C inputs by manure applications supporting enhanced levels of organic P mineralization. Evidence exists to support this in that there were few significant differences among pre-P_o and +CN nor -CN P_o concentrations in the AN plots ($\alpha = 0.05$).

In cases where there was significance, the pre-P_o concentrations were greater than those in the incubated soil (α =0.05). The results indicate that the incubation period could have been too short for native soil microbial response. Reddy et al. (1996) reported a decrease in 0.5 *M* $NaHCO_3$ extractable P in soils receiving manure during the first 6 weeks of incubation. They observed that soluble P was temporarily immobilized during that time period.

Contributions to extractable AP from the P_o pool have been demonstrated by increases in P_o during incubation (Abbott, 1978; Thein and Myers, 1992). The increase in P_o represents labile forms of P and should also be considered plant available. Turnover of the more labile components of the organic P pool emphasize the importance of its inclusion in AP estimations. Evidence exists that mineralization of organic P from manured soils continues over a period of eight weeks after the initial immobilization period (Abbott, 1978; Reddy, et al., 1995). The persistence of manure phosphorus availability over phosphorus from commercial fertilizers in soil is explained by the increases in microbial activity and greater activity in the P cycle between the inorganic and organic P forms and depends on conditions favorable to OM decomposition. Therefore, an index for describing the bioavailability of P should include P_i, P_o, and the highly labile microbial biomass P (P_m) pools in existence at the end of incubation.

Microbial biomass P was determined using the analytical scheme developed by Thein and Myers (1992). Tables 12 and 13 show a high degree of variation among treatment values for P_m concentrations in +CN and -CN soils. In 1991, P_m concentrations at MAES were significantly greater in the 252M and 504M treatments than in treatments getting either 0N or AN (Table 12). In depth two, P_m concentrations were significantly

Table 12. Microbial biomass phosphorus following incubation for bioactive (+CN) and non-bioactive (-CN) treated soils at MAES

Treatment	1991		1996		1997	
	+CN	-CN	+CN	-CN	+CN	-CN
	mg P kg soil^{-1}					
0-7.5 cm						
0N	-3.5 b †	2.4	2.4 ab	4.8 ab	5.6 b	8.2 b
AN	-2.5 b	1.2	-2.2 b	6.9 a	3.9 b	7.5 b
126M	1.8 ab	-2.3	6.0 ab	1.1 ab	17.1 a	12.3 ab
252M	11.3 a	1.3	11.6 a	1.2 ab	9.4 b	9.2 b
378M	7.5 ab	1.3	5.1 ab	0.9 ab	13.6 ab	12.7 ab
504M	10.5 a	3.0	-1.0 ab	-9.6 b	25.5 a	17.2 a
7.5-15 cm						
0N	-0.3 c	1.3	3.5 b	3.9	5.1	6.0 ab
AN	1.9 abc	0.7	4.0 b	3.9	4.7	-2.9 b
126M	0.9 b	0.8	5.2 ab	1.3	4.5	4.5 ab
252M	1.4 bc	2.2	2.9 b	2.1	4.5	5.7 ab
378M	4.4 a	1.4	2.7 b	10.4	4.9	6.3 ab
504M	2.8 ab	1.9	10.0 a	-0.6	4.8	7.1 a

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

Table 13. Microbial biomass phosphorus following incubation for bioactive (+CN) and non-bioactive (-CN) treated soils at DES

Treatment	1996		1997	
	+CN	-CN	+CN	-CN
	mg kg^{-1}			
0-7.5 cm				
0N	3.4	1.8	-16.5	-17.5
AN	4.3	3.9	-24.7	-16.0
126M	12.8	4.1	-18.9	-22.1
252M	17.7	4.2	-15.7	-23.0
504M	7.4	5.7	-6.9	-22.9
7.5-15 cm				
0N	1.9 ab †	2.2	-13.5	-10.3 ab
AN	0.7 b	2.1	-17.5	-13.3 bc
126M	5.7 ab	4.1	-10.5	-8.7 a
252M	6.3 a	1.5	-14.1	-15.0 c
504M	3.1 a	4.4	-12.0	-8.8 a

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

lower in the 0N than in the 126m, 378M, and 504M plots. There was no significance among treatments for the -CN soils. Higher microbial biomass P concentrations were anticipated for the plots receiving manure treatments because the organic inputs could serve as a substrate for microbial stimulation. Increases in P immobilization resulting from microbial assimilation was expected immediately following the initial treatment application, and to last up to six weeks.

In 1996, at MAES the P_m concentration were significant in both the +CN and –CN treated soils in depth one and in depth two of the +CN soil only ($\alpha = 0.05$) (Table 12). In depth one, +CN soil P_m concentrations were significantly lower in AN than in the 252M plot, yet in -CN soils the AN treatment P_m concentrations are in excess of those in the plot getting the highest manure rate (Table 12). In depth two +CN, the 504M P_m concentration was significantly greater than those getting any other treatment except for the 126M. At this point in the study we expected to see well established microbial communities in the plots receiving manure compared to the 0N and AN treatments. The lack of treatment differences were inconsistent with the findings from other research (Abbott and Tucker, 1973; Thein and Myers, 1992; Oberson, et al., 1993). Dairy experiment station P_m concentrations were not significantly different among treatments in depth one during 1996 (Table 13). The absence of differences can be attributed to the persistence of manure benefits after discontinuing manure applications in the 0N and AN coupled with similar soil microbial communities across treatments resulting from long-term manure management.

The 1997 MAES P_m concentrations in the 504M are significantly greater than those found in the AN plots in depth one for both +CN and -CN soils. The 126M

treatment, in the +CN soil set, also had significantly greater P_m than the AN plots. The DES soils in 1997 were variable and P_m concentration values were uniformly negative. Depth two -CN soils had significant differences, but again, all values were negative so no conclusions were drawn here. Negative values for P_m may be caused by the 0.5 *M* $NaHCO_3$ procedure not working well for manured soils. The length of fumigation and subsequent evaporation period may have allowed P, released from cellular constituents, to be complexed by Al^{3+} in the soil solution. The formation of Al-complexes would greatly reduce the concentrations of P_{i+m} in 0.5 M $NaHCO_3$ extracts thereby negative values could arise for P_m.

Available phosphorus refers to the fraction of P in a chemically available form that plants can absorb readily and that, once absorbed, can affect the life cycle of the plant (Sposito, 1989). The ability to estimate this portion of P can greatly improve soil P test recommendations and potentially prevent excessive fertilizer P applications. Two soil P tests commonly used to evaluate AP are the Olsen and Mehlich I extractable P tests. These indices for estimating AP are based on a single, chemically extractable P_i pool. Researchers predict that soil tests measuring only P_i can greatly underestimate potential AP (Abbott, 1978; Thein and Myers, 1992).

Bioavailable P was determined using the method developed by Thien and Myers (1992). Bioavailable phosphorus concentrations were not significantly different among treatments in 1991 in the +CN or -CN treated soils (Table 14). Thein and Myers (1992) found that BAP was higher in all +CN soils than in their -CN counterparts. In 1991, no differences existed between BAP concentrations in the +CN and -CN treated soils receiving the same treatment at MAES ($\alpha = 0.05$).

Table 14. Bioavailable phosphorus for bioactive (+CN) and non-bioactive (-CN) treated soils at MAES

Treatment	1991		1996		1997	
	+CN	-CN	+CN	-CN	+CN	-CN
	mg P kg soil^{-1}					
0 - 7.5 cm						
0N	26.1	38.3	20.9 d †	24.4 b	15.3 c	19.1 d
AN	25.7	29.6	27.6 cd	28.9 ab	22.8 bc	22.5 cd
126M	35.1	29.9	30.5 bcd	26.4 b	35.4 b	23.4 b
252M	39.9	30.3	49.3 abc	39.6 ab	25.2 bc	29.4 bc
378M	39.8	35.6	66.8 a	53.2 a	35.6 b	37.8 b
504M	41.5	33.0	53.0 ab	46.5 ab	57.2 a	51.4 a
7.5 - 15 cm						
0N	10.6	12.0	15.6 b	17.0 ab	9.7	11.9 ab
AN	11.8	10.2	16.9 b	17.5 ab	11.6	7.6 b
126M	10.6	9.6	18.7 b	14.8 b	10.7	12.6 ab
252M	14.6	12.2	18.2 b	18.3 ab	10.9	13.6 ab
378M	15.8	12.1	20.5 b	28.2 a	12.2	15.4 ab
504M	12.2	10.2	31.9 a	22.9 ab	13.6	19.1 a

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

By 1996, there were significant differences among treatments for BAP concentrations in both depths and in both sets of soils except in depth two at DES -CN treated soil (Tables 14 and 15). At MAES, concentrations of BAP were significantly greater in the 378M and 504M soils than those in the 0N and AN for +CN treated soils, depth one. The 378M –CN treatment had higher BAP concentrations than the 0N and 126M at MAES in depth one. In depth two +CN, the 504M BAP concentrations were significantly greater than those in all other treatments. Depth two -CN soils exhibited differences between BAP concentrations in the 378M and the 126M (Table 14). The DES +CN and -CN treated soils exhibited a similar pattern of differences in depth one. There the 504M had the greatest concentrations of BAP. This pattern recurred in depth two for the +CN soils (Table 15). There were no statistical differences between the +CN and -CN soils indicating that no biological stimulation occurred during incubation as a result of the C+N enrichment step ($\alpha = 0.05$). The incidence of higher BAP concentrations in plots receiving the 378M and 504M than those in the 0N and AN plots in the +CN soils at MAES may reflect higher microbial activities in these manure plots. When applied at equal rates, manure and fertilizer BAP concentrations are similar (AN and 126M Table 14). The lack of significance between the +CN and –CN treated soils may indicate that the treatment differences were solely influenced by higher P application rates rather than stimulated microbial activity. Few differences between treatments receiving a low manure rate and the AN plots were inconsistent with the conclusions of other researchers. Oberson, et al. (1993), found that in plots receiving P inputs as manure only, extractable AP was higher than in plots receiving mineral P inputs.

In 1997, BAP concentrations remained significantly different among treatments at

Table 15. Bioavailable phosphorus for bioactive (+CN) and non-bioactive (-CN) treated soils at DES

Treatment	1996		1997	
	+CN	-CN	+CN	-CN
	mg kg^{-1}			
0-7.5 cm				
0N	21.5 b †	25.0 b	12.2	14.5 abc
AN	30.7 b	28.9 b	29.5	10.5 c
126M	26.5 b	25.3 b	16.9	20.6 ab
252M	35.8 b	30.3 b	10.1	11.8 bc
504M	57.6 a	47.9 a	31.0	23.1 a
7.5-15 cm				
0N	15.7 b	20.0	5.7	9.8
AN	16.5 b	20.4	8.7	9.5
126M	20.6 b	20.1	8.6	12.3
252M	20.8 b	20.9	7.9	10.9
504M	30.5 a	24.0	14.6	14.9

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

MAES in both sets of incubated surface soils (Table 14). The 504M BAP concentrations were significantly greater than concentrations in the 0N and AN for the +CN soils. In -CN surface soils, the 126M, 378M, and 504M BAP concentrations were greater than those in 0N and AN. In depth two -CN treated soils, 504M BAP concentrations were greater than those of AN. At DES, the only significant differences among treatments occurred in the surface layer of -CN soils (Table15). There, AN and 252M BAP concentrations were greater than those in 504M. Loss of significance from 1996 to 1997 in the +CN soils at DES may have been influenced by the negative values obtained for P_m that were reported earlier. Higher concentrations of BAP in the manure plots than the 0N and AN stress the value of an AP-index for fertilizer P recommendations because of the persistence of P in soils receiving manure applications. The persistence of manure AP has been associated with the quantity of manure applied and shown to last for several years following the termination of manure applications (Abbott and Tucker, 1973). As was discussed earlier, Oberson, et al. (1993) found that manure additions affect the residual P_o pool. Contributions to residual P_o by manure applications could account for some of the AP persistence in manured soils because it is subject to relatively short-term transformations by soil flora and fauna when solution and other labile forms of P become depleted. Residual P_o could therefore be considered a reserve, to be hydrolyzed only when the content of available P_i has decreased to a low level (Oberson, et al., 1993). The later is not likely the case for these soils. Bioavailable phosphorus persistence appears to be a result of high manure P rates in conjunction with large quantities of the applied P being in a slowly mineralizable form. Few significant differences among +CN and -CN BAP concentrations within corresponding treatments in these soils may have resulted

from the high variability among values obtained for P_m.

Many soil test laboratories rely upon Olsen and Mehlich I AP indexes because they are quick, simple, and correlate well with field plant response. The BAP AP-index provides insight into the amount of P that is potentially available through mineralization of P_o and microbial turnover in addition to the traditional P_i measure for AP. To gain further insight into the significance of including the biological extraction in the estimation of AP, Mehlich I, and Olsen AP concentrations were included for comparison with concentrations of AP extracted by BAP, (Tables 16 and 17).

Comparison of mean AP concentrations extracted by each method in 1991 indicate that initial BAP-AP concentrations were significantly greater in the surface layers of the 252M and 504M plots than AP concentrations obtained using Mehlich I or Olsen. In depth two, BAP extracted higher AP concentrations in all but the 0N and 126M plots in which case BAP-AP concentrations were equal to those obtained by Mehlich I (Table 16). BAP and Mehlich I procedures extracted significantly greater concentrations of AP than Olsen.

In 1996, BAP and Mehlich I extractable AP levels were statistically the same in depth one for soils getting 0N, AN, 126M, and 252M and all but the AN in subsurface soils. In the 0N and 126M, BAP and Mehlich I-AP concentrations were significantly greater than levels extracted with Olsen in depth one (Table 16). Mehlich I extracted significantly greater quantities of AP than BAP or Olsen in depth one of soils receiving 378M and 504M. The BAP procedure extracted statistically greater amounts of AP in depth two of the AN plots. At DES, Mehlich I extracted significantly greater AP concentrations than Olsen and BAP in plots getting 0N, AN, and 504M in surface soils

Table 16. Comparison of BAP, Olsen, and Mehlich I extractable P concentrations within treatments for 1991, 1996, and 1997 at MAES

Method	Fertility Treatment					
	0N	AN	126M	252M	378M	504M
	mg P kg $^{-1}$					
1991						
0-7.5 cm						
BAP	26.1	25.7	35.1	39.9 a	39.8 a	41.5 a
Olsen	16.5	13.2 b	13.9	11.9 b	15.7 b	17.3 b
Mehlich I	32.4	27.8 a	26.7	21.2 b	25.9 ab	26.6 b
7.5-15 cm						
BAP	10.6 a †	11.8 a	10.6 a	14.6 a	15.8 a	12.2 a
Olsen	2.5 b	1.7 c	2.5 b	1.9 b	2.8 b	2.5 c
Mehlich I	8.0 a	5.8 b	6.8 ab	6.3 b	6.3 b	4.9 b
1996						
0-7.5 cm						
BAP	20.9 a	27.6 ab	30.5 a	49.3 ab	66.8 b	53.0 b
Olsen	4.3 b	10.6 b	10.5 b	25.6 b	45.1 b	45.6 b
Mehlich I	25.6 a	49.2 a	41.4 a	74.1 a	139.3 a	137.2 a
7.5-15 cm						
BAP	15.6 a	16.9 a	18.7 a	18.2 a	20.5 a	31.9 a
Olsen	0.2 c	0.5 c	0.8 b	3.4 b	3.7 b	12.9 b
Mehlich I	9.5 a	11.6 b	11.9 a	19.8 a	22.5 a	39.4 a
1997						
0-7.5 cm						
BAP	15.3 ab	22.8 a	35.4 a	25.2 ab	35.6 ab	57.2 b
Olsen	3.4 b	7.1 b	12.5 b	12.0 b	22.9 b	31.9 c
Mehlich I	18.9 a	23.6 a	45.4 a	48.3 a	72.9 a	114.9 a
7.5-15 cm						
BAP	9.7 a	11.6	10.7 a	10.9 a	12.2 a	13.6 ab
Olsen	1.1 b	1.9	2.5 b	3.9 b	4.7 b	5.7 b
Mehlich I	7.8 a	16.3	13.5 a	14.1 a	17.4 a	27.2 a

† Means within a column for a given year followed by the same letter are not different as determined by LSD at $\alpha = 0.05$.

Table 17. Comparison of BAP, Olsen, and Mehlich I extractable P concentrations within treatments for 1996 and 1997 at DES

	Fertilizer Treatment				
Method	**0N**	**AN**	**126M**	**252M**	**504M**
	mg P kg^{-1}				
1996					
0-7.5 cm					
BAP	21.5 b †	30.7 b	26.5	35.8 b	57.6 b
Olsen	15.7 b	15.9 ab	16.9	17.7 c	43.6 b
Mehlich I	36.7 a	100.2 a	35.8	46.2 a	47.6 a
7.5-15 cm					
BAP	15.7 b	20.3 b	20.6	20.8 a	30.5
Olsen	9.1 c	7.7 c	8.2	9.6 b	15.1
Mehlich I	27.7 a	25.2 a	22.9	23.5 a	41.7
1997					
0-7.5 cm					
BAP	12.9 b	29.5 b	16.9 b	10.1 b	31.0 b
Olsen	16.7 b	18.3 ab	22.6 b	19.6 b	38.7 b
Mehlich I	83.3 a	63.0 a	93.4 a	68.5 a	138.1 a
7.5-15 cm					
BAP	5.7 b	8.7 b	8.6 b	7.9 b	14.6 b
Olsen	8.0 b	6.6 b	11.2 b	8.3 b	18.2 b
Mehlich I	36.3 a	29.3 a	45.3 a	37.9 a	71.9 a

† Means within a column for a given year followed by the same letter are not different as determined by LSD at $\alpha = 0.05$.

and in the 0N and AN of depth two (Table 17). BAP and Mehlich I extracted similar quantities of AP in depth two of plots receiving manure treatments (Table 17).

In 1997, Mehlich I extractable AP concentrations were significantly greater than Olsen extractable AP in all treatments except in AN depth two (Table 16). BAP and Mehlich I-AP concentrations were not significantly different at MAES except in the surface soil of plots receiving 504M. BAP-AP concentrations were significantly greater than Olsen-AP for surface soils getting AN, 126M, and 504M and in depth two of the 0N, 126M, 252M, and 378M soils (Table 17). At DES, Mehlich I extractable AP concentrations were significantly greater than those extracted by either BAP or Olsen at both depths (Table 17) with the exception of Olsen for the AN plot. There were no statistical differences among methods within treatments for BAP and Olsen extracted AP concentrations.

Despite significant differences in the concentrations of AP extracted by these methods, treatment differences within method were significantly correlated and told similar stories at both locations in 1996 and 1997(Appendix A1-A5). At MAES each method extracted significantly higher concentrations of AP from the 504M plots than those in the 0N and AN plots in the surface soils during 1996 and 1997.(Table 16). In 1996, BAP extracts contained greater AP concentrations in the 504M than those in the AN at DES. Mehlich I and Olsen extracts contained significantly higher concentrations of AP in DES plots getting 252M than those in the AN for both years (Table 17). The results for extractable AP show that soils under all treatments contained high P levels therefore additional P applications would probably not result in increases in yield. Given

these results additional fertilizer P would be prone to off-site movement and be a pollutant of natural water resources (Sharpley, 1993).

Under short and long-term soil management regimes and for manured versus commercially fertilized soils, Mehlich I consistently extracted greater quantities of AP. There were two possible reasons for this. First, acid in the Mehlich I extracts could release P from recalcitrant organophosphorus complexes which were not available through microbial activities during the incubation period used in this study. Second, soil test labs generally determine P concentrations in Mehlich-I extracts colorimetrically. Mehlich-I extracts used in this study were quantified on the ICAP. Colorimetric evaluation of Mehlich-I extracts typically yield lower P values than those determined on the ICAP because the latter values includes both organic and inorganic P (Kuo, 1996).

There is evidence to support a claim that the Mehlich-1 or acid extractable methods overestimate the AP pool. Tiessen et al. (1984) reported that the acid extractable P pool mostly contains primary P minerals that have not yet entered the AP pool, and are therefore not readily available for the production of organic matter. It has been shown that manure additions affect soil residual P_o. It was also likely that the accumulation of residual P_o in these soils could have contributed to higher AP levels in Mehlich I extracts than in BAP extracts given that the Mehlich I extracts were determined on the ICAP. The latter is likely true because the soil P levels in each plot were excessive. Residual P_o contributes to AP through microbial activities when solution P have diminished. Following treatment applications in 1996, increases of AP concentrations in Mehlich I extracts could have resulted from the hydrolysis of greater quantities of organically bound P in addition to nonlabile portions of P. Significance in

the amounts of AP extracted by these methods in 1996 and 1997 could have resulted from not having used Cowell's modifications (Kuo, 1996) for the Olsen and BAP methods. Cowell modified the Olsen procedure in 1963 by increasing the soil/solution ratio from 1:20 to 1:50 and the reaction time from 30 minutes to 16 hours. These modifications decreased the influence of soil P buffering capacity on P extractability. Decreasing the soil's P buffering capacity was particularly significant in highly weathered soils where Al and Fe PO_4^- control solution P concentrations. Using Cowell's modifications increases the concentration of AP extracted from soil (Kuo, 1996).

Comparison of crop response with fertilizer application is the most effective way to analyze soil test procedures. Means from the yield data, for MAES 1996, are presented in table 18. Using this data we were able to evaluate whether these available phosphorus indices correlated with yield. Yields were significantly greater in all treatments than for the control. Plots getting the highest manure rates produced higher yields than those produced in plots with low manure rates. The lack of differences in yield in the AN and 126M further uphold manure as a viable fertilizer source. Pearson correlation coefficients reveal that each of the methods used were strongly correlated with yield. Mehlich I-AP results most strongly correlate with yield followed by Olsen and BAP ($r = 0.77^{\dagger\dagger\dagger}$, $0.74^{\dagger\dagger\dagger}$, and $0.68^{\dagger\dagger}$ respectively) (Appendix A2). At the time of this writing no BAP-yield correlation data existed in the literature. Thien and Myers (1992) acknowledged the need for such data.

Table 18. Yield data for MAES 1996

Treatment	Yield
	1996
	-----Mg DM ha^{-1}-----
0N	2.4 d †
AN	6.8 ab
126M	4.7 c
252M	5.6 bc
378M	7.9 a
504M	7.8 a

† Means in a column followed by the same letter are not significantly different as determined by LSD at $\alpha = 0.05$

CHAPTER V

SUMMARY AND CONCLUSIONS

Soil testing is an essential aspect of soil management. In addition to being a useful diagnostic tool for evaluating soil fertility for fertilizer recommendations, soil testing also plays a role in the prevention of environmental problems by providing guidelines to minimize loss of nutrients to surface and groundwater. Manure rates applied to soil are often based on the N-requirements of crops with little attention paid to the accumulation and fate of P, which in most cases is applied at rates that exceed crop requirements (Thangudu, et al., 1981). Chemical analysis of the MAES and DES soils revealed significant differences among P accumulations in the 7.5-15 cm depth at MAES in 1996 and at both locations in 1997. This mobility indicates that at the highest manure rate larger concentrations of P become mobile. At DES, where the soil above the water table is shallow, the possibility of groundwater contamination exists.

Advancements in soil testing for P have occurred over the past several decades to accurately predict the amount of soil P that is available for plant growth during a growing season. Many strong acid and alkaline solutions and various organic and inorganic complexing agents have been experimented with and found suitable for evaluating the bioavailability of P with certain crops and soils (Menon and Chien, 1995). Mehlich I and Olsen extracts are among the most widely used soil P tests. In these tests, as with many others, the extracting solutions react with soil and dissolve P from soil components. These tests have been shown to provide good indications of soil AP, however their extraction processes may not accurately reflect those occurring in natural soil systems.

Initial approaches to determine available P included the fraction of labile P most likely to be mineralized during a growing season. (Bowman and Cole, 1976; Abbott, 1978; Menon and Chien, 1995). Because the rate at which P_o becomes available for plant uptake is largely dependent upon the rate of organic matter decomposition and is a function of microbial activities, Thien and Myers (1992) developed a method for measuring AP that includes microbial biomass P. The BAP method of Thien and Myers (1992) utilizes stimulation of soil microbial activity as a bioextractant to predict AP.

The results presented here are not in agreement with the findings of other researchers. Incubation failed to increase P_o concentrations. Changes in P_o would have been indicative of soil P dynamics and reinforced the need for its inclusion in soil P tests. Failure to observe significant increases in P_o following incubation could have resulted from the use of Olsen's extraction procedure for non-calcareous soils or the failure to include Cowell's modifications. Another possible explanation is that the duration of the incubation period was long enough for the P released by cellular turnover to be adsorbed to soil surfaces or to form precipitates with Al in soil solution. The latter would subsequently remove phosphorus from labile pools. This would result in decreases in the soil AP concentration.

Tennessee soil test P concentrations are determined colorimetrically with Mehlich I extract. Mehlich I extract is used because Al complexation reactions predominantly control orthophosphate concentrations in the soil solution. Olsen 0.5 *M* $NaHCO_3$ extracts were initially developed for use in calcareous soils where Ca complexation reactions control orthophosphate concentrations in soil solution. Cowell's modifications of Olsen's extractable AP procedure allowed Olsens extracts to be applied to a wider range

of soils with varying chemical properties. Failure to use Mehlich I or Cowell's modifications of the Olsen procedure probably contributed to lower AP concentrations for these soils.

The AP results show that the BAP method may not be suitable for soils where P availability is dominated by Al complexation reactions. This is evidenced by the high incidence of negative P recovery rates of the bioextracted P pool. The negative values are thought to be the result of Al complexation of the P released following lysis of soil microbes.

Strong correlations existed among the concentrations of AP extracted by each method compared in this study (Appendix). This revealed that P availability was probably a function of higher P inputs rather than stimulated microbial activity. The failure to obtain positive P_m concentrations demonstrates that the Thein and Myers (1992) biological extraction may be limited when making AP determinations in diverse soils.

Finally, yield data and AP methods had significant correlations in the following order Mehlich I > Olsen > BAP. This indicates that for these soils Mehlich I AP concentrations may best reflect AP concentrations in the soil.

Mehlich-I extracts analyzed by the ICAP typically result in higher AP concentrations than when the same extracts were evaluated colorimetrically because ICAP values includes both organic and inorganic P (Kuo, 1996). Significantly higher concentrations of AP in Mehlich I extracts of the 0-7.5 cm depth in 1997 at both locations indicate that analysis of Mehlich I extracts on the ICAP may be inappropriate because of the possibility that the extracts include residual P_o. However, as an indication of bioavailability, the use of ICAP does approximate the BAP method in that extractable P_i

and P_o are being measured. Soil test labs generally determine P concentrations in Mehlich-I extracts colorimetrically. Thangudu, et al. (1981) reported that Mehlich I extract analyzed colorimetrically produced valid estimates of AP in Tennessee soils receiving manure amendments.

Despite earlier findings that these AP soil tests are appropriate tools to predict fertilizer P to minimize loss each method has its limitations. Two limitations that appear to be universal among P tests are their abilities to predict P in soils with widely differing properties and they were specifically developed to aid fertilizer recommendations in soils fertilized with soluble fertilizers (Menon and Chien, 1995). The ideal soil P test would be insensitive to soil type and predict P requirements equally well in soils receiving nonconventional fertilizers such as manure (Menon, et al., 1988).

Further research needs to be conducted to evaluate AP soil test methods. Future studies should incorporate Cowell's modifications to Olsen extractable P_i and P_o because these modifications account for effects of soil P buffering capacity on P extractability. Using Mehlich I extract to determine P_i, P_o, and P_m is a possibility for modifying Thien and Myers (1992) BAP method to more accurately predict AP in Tennessee soils. Recovery of P_m in the BAP procedure may be higher using these modifications. Available phosphorus levels in samples incubated at various temperatures to simulate the different seasons, showed that there was no change in AP during the cooler winter like months, but that with the onset of warmer temperatures $NaHCO_3$ extractable P declined rapidly (Habib and Hayfa, 1994). Given the latter, the effects of sampling time on the extractability of AP needs to be examined. Finally, Mehlich I extractable AP

concentrations need to be evaluated colorimetrically for comparison with ICAP and possibly BAP data.

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SOUTHWORTH
PARCHMENT DEED
100% COTTON

APPENDIX

Appendix

Pearson's Correlation Coefficients

A1. MAES 1991

	P_i	P_o	C	BAP	N	MIN-N	WSOC
Mehlich P	0.51†	0.06	0.56†	0.73†††	0.61††	0.58††	0.73†††
P_i	1.00	-0.25	0.55†	0.38	0.54†	0.37	0.29
P_o	-0.25	1.00	0.17	0.08	0.19	0.10	0.50†
C	0.55†	0.17	1.00	0.36	0.99†††	0.68††	0.61††
BAP	0.38	0.08	0.36	1.00	0.40	0.45	0.53†
N	0.54†	0.19	0.99†††	0.40	1.00	-0.27	0.29
MIN-N	0.37	0.10	0.68††	0.45	-0.27	1.00	0.45
WSOC	0.29	0.19	0.61††	0.53†	0.29	0.45	1.00

A2. MAES 1996

Mehlich P	0.99†††	0.19	0.83†††	0.90†††	0.77†††	0.53†	0.36
P_i	1.00	0.17	0.82†††	0.90†††	0.78†††	0.57††	0.32
P_o	0.17	1.00	0.48†	0.32	0.57††	0.45	0.20
C	0.82†††	0.48†	1.00	0.94†††	0.97†††	0.72†††	0.59†
BAP	0.90†††	0.32	0.94†††	1.00	0.91†††	0.75†††	0.54†
N	0.78†††	0.57††	0.97†††	0.91†††	1.00	0.77†††	0.47†
MIN-N	0.57††	0.45	0.72†††	0.75†††	0.77†††	1.00	0.26
WSOC	0.32	0.20	0.59†	0.54†	0.47†	0.26	1.00
Yield	0.74†††	0.10	0.65††	0.68††	0.55†	0.30	0.40

A3. MAES 1997

Mehlich P	0.96†††	0.32	0.78†††	0.87†††	0.59††	0.30	-0.44
P_i	1.00	0.17	0.73†††	0.87†††	0.54†	0.27	-0.48†
P_o	0.17	1.00	0.14	0.41	0.07	0.44	0.47†
C	0.73†††	0.14	1.00	0.75†††	0.89†††	0.40	-0.39
BAP	0.87†††	0.41	0.75†††	1.00	0.63††	0.44	-0.22
N	0.54†	0.07	0.89†††	0.63††	1.00	0.60††	-0.20
MIN-N	0.27	0.44	0.40	0.44	0.60††	1.00	0.41
WSOC	-0.48†	0.47†	-0.39	-0.22	-0.20	0.41	1.00

A4. DES 1996

Mehlich P	0.95†††	-0.45	0.62††	0.86†††	0.61†	0.84†††	0.72††
P_i	1.00	-0.35	0.64††	0.78†††	0.57†	0.87†††	0.62††
P_o	-0.35	1.00	-0.12	-0.52	-0.27	-0.14	-0.58†
C	0.64††	-0.12	1.00	0.81†††	0.97†††	0.62††	0.53†
BAP	0.78†††	-0.52	0.81†††	1.00	0.80†††	0.64††	0.82†††
N	0.57†	-0.27	0.97†††	0.80†††	1.00	0.55†	0.61†
MIN-N	0.87†††	-0.14	0.62††	0.64††	0.55†	1.00	0.71††
WSOC	0.62††	-0.58†	0.53†	0.82†††	0.61†	0.71††	1.00

A5. DES 1997

	P_i	P_o	C	BAP	N	MIN-N	WSOC
Mehlich P	0.81†††	-0.21	0.15	0.45	0.41	0.10	0.47
P_i	1.00	-0.19	0.58†	0.46	0.68††	0.17	0.36
P_o	-0.19	1.00	0.21	-0.29	0.44	0.29	0.15
C	0.58	0.21	1.00	0.14	0.84†††	0.54†	0.30
BAP	0.46	-0.29	0.14	1.00	0.23	-0.21	0.12
N	0.68††	0.44	0.84†††	0.23	1.00	0.51†	0.43
MIN-N	0.17	0.29	0.54†	-0.21	0.51†	1.00	0.66††
WSOC	0.36	0.15	0.37	0.12	0.43	0.66††	1.00

† Significant at a = 0.05

†† Significant at a = 0.01

††† Significant at a = 0.001

VITA

Gregory B. Hardison was born in Jacksonville, North Carolina on March 10, 1970. He graduated from Franklin Co. High School in May 1988. He entered Motlow State Community College, Lynchburg, TN in August, 1988, where in May, 1991 he received an Associate of Arts degree in Psychology. In January 1991 he entered the undergraduate Psychology program at the University of Tennessee, Knoxville. He withdrew from the University in May 1992 and did not return to his studies until May 1993. During that time he was employed at Peninsula Psychiatric Hospital where he provided care to dually diagnosed adolescent patients. Upon returning to the University Gregory studied Plant and Soil Science with an Environmental Science and Natural Resources concentration. While completing his degree he maintained full time employment at Peninsula Psychiatric Hospital through December 1996 at which time he worked part time as a Student Research Assistant under Dr. Michael Mullen. Gregory received his Bachelor of Science degree in May 1996. In August 1996, he returned to the University of Tennessee to pursue a Master of Science degree in Plant and Soil Science on a Graduate Research Assistantship. Gregory received the Masters degree in August 1999.

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