To the Graduate Council:

I am submitting herewith a thesis written by Melissa Dyanne Mayfield entitled "Effects of Dairy Manure Applications on Selected Soil Biochemical Properties". 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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EFFECTS OF DAIRY MANURE APPLICATIONS ON SELECTED SOIL BIOCHEMICAL PROPERTIES

A Thesis

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ABSTRACT

Additions of organic wastes to soils have been shown to induce changes in soil biological, chemical and physical properties. This study was conducted to observe effects various rates of manure may have on biochemical properties. The study was conducted on eighteen plots at the Martin Experiment station. The soil type of these plots is a Loring silt loam (fine-silty, mixed, thermic, Typic Fragiudalf). These eighteen plots received 126, 252, 378, or 504 kg manure-N ha^{-1} or 218 kg N ha^{-1} as ammonium nitrate each year since May 1991 while the control plots received 0. Soil samples were taken over four sampling dates (May 4, May 25, June 13, and August 5), at depths of 0-5, 5-10, and 10-20 cm. These samples were analyzed for pH, total nitrogen, organic carbon, Mehlich I extractable cations (Mg, N, K, and P), total bacterial counts as well as fluorescent Psuedomonads, fungal counts using the Rose Bengal media as well as for one date, cellulose degraders. The results of these enumerations showed higher plates counts in the 0 to 5 cm depth. All the sampling dates were analyzed for various enzymes as well. The enzymes activities analyzed for were acid and alkaline phosphatase, arylsulfatase, L-asparaginase, and β-glucosidase. Enzyme activities was highest in the 0 to 5 cm depth, with lower enyzme activity in the 5 to 10 cm and 10 to 20 cm soil depths. Enyzme activities were generally highest in the manure treated soils. This corresponds with generally higher % organic C, % total N, lower C:N ratios and pH in these soils. The pH of the ammonium nitrate treated soils were more acidic than the other treatments, and this resulted in higher C:N ratios, lower bacterial numbers, fungal counts, and lower

activities for some enzymes, particularly arylsulfatase and L-asparaginase. Arylsulfatase was negatively affected by the decrease in pH, particularly in the inorganic treatment. Moreover ^{b}-glucosidase appeared to be positively affected by the organic carbon contents. In short the overall results of this experiment seemed to indicate that the use of organic wastes, such manure, induce positive changes in soil biochemical properties. However, the manure had little effect on the exchangeable cations, (Mg, P, and K), in the beginning of the experiment, however, toward the end of the experiment there was a slight increase in availability.

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PART 1: INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Scientists in the field of agriculture have long had the responsibility of finding the most cost effective means for farmers to attain the greatest possible yields. In order to provide quality information to the farmers, agricultural scientists must integrate the latest information on amendments and their effects on crops with the latest farming techniques. To this end, research has been done with organic and inorganic amendments.

The introduction of inorganic fertilizers came over a hundred years ago (Tisdale, 1985). In 1993, 812,285 tons of inorganic fertilizer were used in the state of Tennessee alone. The large amounts of inorganic fertilizers used resulted in the expenditure of 124.3 million dollars in 1992 in the state of Tennessee (USDA- Statistics, 1994). However, the use of inorganic fertilizers can have negative impacts on soils. Dick (1992), found that continued use of inorganic nitrogen over a long period of time reduces microbial activity in soil by inhibiting the production of certain soil enzymes involved in nutrient cycling. Also, inorganic fertilizer additions have been found to lower the soil pH much faster than manure additions, particularly in the top two or three inches of the soil (Jacobsen and Westerman, 1991). This is a problem because the pH of a soil has a major impact on the availability of many nutrients to the crops.

Having this information, farmers and scientists have been reevaluating the use of organic amendments. In the mid 1980's, livestock and poultry raised by farmers in the United States produce over 100 million metric tons (dry weight) of manure a year

(Westerman et al, 1985), presenting both a resource opportunity and waste handling problem.

In the state of Tennessee, the use of organic amendments, such as animal manures, is an important issue. Tennessee produces large amounts of such organic wastes through its many dairy, swine and poultry farms. The USDA-Tennessee Statistics bulletin (1994) reported that in 1993 there were 470,000 heads of pigs and 1.59 million chickens (excluding broilers). Tennessee ranks 14th in the nation for dairy cattle operations. In 1993, there were 32,000 dairy cattle operations with approximately 175,000 head of dairy cows (USDA - Tennessee Statistics, 1994). The use of manure provides nutrients for crop growth, but may also contribute to water quality deterioration. This is especially true when the rate of manure application exceeds the rate of its disposal. Manure accumulates around milking facilities and near confinement feeding operations. This manure is usually stored in storage pits and lagoons and must be disposed of at some point. Land application has been the method of choice for economic and environmental reasons. Economically, it represents an on-farm input for cropping. Also, when compared to inorganic fertilizer, land application of manure is good because it is relatively inexpensive and reduces the amount of accumulating animal wastes (Patni and Culley, 1989; Cooper et al., 1984; Culley et al., 1981). If 90% of the manure produced each year were returned to the soil, it would have a value equivalent to $5 billion worth of N-P-K fertilizers (Miller and Donahue, 1990). Manure does not always meet all plant nutrient requirements, so farmers often use manure in conjunction with

inorganic fertilizers. Farmers can appreciate a cost savings by using a combination of inorganic fertilizers and manure, as opposed to just using the inorganic fertilizers. A farmer may not know how much nitrogen or phosphorus he has applied as manure, and may then apply excessive amounts of fertilizers (Elliott et al., 1990).

Land application provides an environmentally sound way to dispose of the manure. There are several methods of application, including: 1). spreading of solid material when weather, soil, and crop conditions permit; 2). injecting a slurry of water and manure into the soil or spraying it on the surface; or 3). spraying the slurry onto land with an irrigation system (Tisdale, 1985). The method used depends on the equipment available, the land resource and the cropping system used.

LITERATURE REVIEW

Animal manure can be used by farmers as plant fertilizer, so from a farming perspective, manure is not a resource to be wasted. This natural product of livestock contains many nutrients essential to plant health and growth. A USDA-Tennessee Statistics publication (1978) estimated that cattle manure has (dry basis) an average of 3% nitrogen, 0.8 % phosphorus (1.8 % P_2O_5), 2 % potassium (2.4 % K_2O), 25 % organic carbon, plus other assorted elements in various amounts. Westerman et al. (1985) observed that 61% and 54% of the total nitrogen in dairy slurry was estimated to be available for plant uptake when applied to sand and sandy loam soils, respectively. This percent estimation was taken from an article by Chesheir et al. (1984) using a percentage of organic N mineralization and some adjustments for the difference between NH_4-N and "Nitrogen Meter" value (which was introduced in Sweden) and then multiplying the number obtained by the difference between TKN and the "Nitrogen Meter" value. The "Nitrogen Meter" measures gas pressure resulting from mixing manure with a strong oxidizing agent (calcium hypochlorite, Ca(OCL)2, 30-37% available chlorine). This "nitrogen meter" was developed to estimate the amount of nitrogen that would be available for plant uptake. The amount of estimated nitrogen found by the "nitrogen meter" in the study discussed above was supported in a study done by Van Horne et al. (1994). The amount of total N they found was between 104-123 kg yr^{-1} $animal^{-1}$, of total P was 18 kg yr^{-1} $animal^{-1}$ and total K was 41 kg yr^{-1} $animal^{-1}$. These numbers were calculated for an average (635 kg) Holstein cow over an annual manure production.

Aside from providing essential elements to the plant, manure use provides other benefits as well. Manure may be beneficial in erosion control due to an increase in the rate of infiltration (Mitchell and Gunther, 1976). Culley et al. (1981), found that manure

applied at an annual rate of 31.2 t ha.$^{-1}$ for five years to a sandy clay loam increased organic C by 0.9% ($P<0.01$) in the 0 to 15-cm layer. Organic C increases soil moisture retention, soil structure, and the pH buffering capacity (Tisdale, 1985). The soil pH is extremely important for a number of reasons. The soil pH will indirectly affect plant growth by affecting the activity of beneficial microorganisms (Miller and Donahue, 1990). For most agricultural crops the soil pH for greatest plant availability of most nutrients is around 6.5 (Miller and Donahue, 1990). The use of manure can improve the soil chemical and physical properties and that improvement in soil properties could result in increased grain and crop yields (Magdoff and Amadon, 1980; Mathers and Stewart., 1974). Miller and Donahue (1990) stated that manure enhances CO_2 availability for the plant canopy, availability of P and other micronutrients and increases complexation of Al^{3+}.

For some farmers, land application of manure is the method of choice. They apply the animal waste solids to their fields with heavy tankers or fertilizer wagons. However, Patni and Culley (1989) observed that using heavy tankers to spread manure could lead to compaction of the soil and thereby depress crop yields. Another method of application is to inject the animal waste liquids below the soil surface (Tisdale, 1985). Researchers have found subsurface incorporation of manure to be a favorable practice because it minimizes losses of manure nutrients due to soil runoff and NH_3-volatilization. Kamprath et al. (1958) and Obenshain and Gish (1941) reported higher organic matter contents in soils that had green manures incorporated into them than those that had none. Although this study is not on green manure, studies have shown that manure applications result in higher organic matter. Other researchers have found that using below-surface incorporation of animal liquid manure provided a closer association between the soil organisms and the manure substrates (Patni and Culley, 1989). In addition, below-

surface incorporation of manure generally maximizes manure nutrient use efficiency. However, sub-surface incorporation results in increased soil erosion due to surface disruption if used on highly erodible soils, such as those in Western Tennessee (Mullen et al., 1992).

The last few decades have led to the acceptance of no-tillage as a common cultivation practice. Conventional tillage involves plowing the top 6-8 inches of soil, whereas no-tillage involves no soil disruption. Conventional tillage disrupts soil structure by tearing up aggregates, whereas no-tillage does not (Beare et al., 1994). Doran (1980) found that the microbial populations were greater in the top 0 to 7.5 cm soil layer of the no-tillage system than in the same layers of conventional tillage soil. Several researchers have also reported higher organic matter contents in no-tilled soils when compared to conventional tilled soils (Blevins et al., 1977; Lal, 1976; Moschler et al., 1972). Moreover, no-tillage provides from 47 to 100 % erosion control compared to conventional tillage (Carter and Berg, 1991). The no-tillage system allows the residues left on the soil surface from a previous crop to provide protection against soil erosion (Harold et al., 1967; Johnson et al., 1979) and to reduce evaporation of soil water (Blevins et al., 1971; Jones et al., 1969; Shanholtz and Lillard, 1969; Thomas et al., 1973). These and other benefits of the no-till method accounts for its favor with farmers.

One of the most helpful benefits of no-till is the dramatic erosion control characteristic, which is especially important to farmers with highly erodible land. Erosion is common in Tennessee, a state with a mountainous eastern section which is characterized by steep slopes, and a western section that borders the Mississippi River. This western section is characterized by its highly erodible soils that are heavily farmed. Farmers in west Tennessee produce the most corn, wheat, cotton, soybeans, and sorghum state-wide (USDA-Tennessee Agriculture, 1994). Unfortunately, West Tennessee is

where the worst soil erosion occurs due to the very silty soil textures. As a result, the University of Tennessee and the National Resources Conservation Service have encouraged the use of no-tillage methods for Western Tennessee farmers.

The physical and chemical properties of soils managed under these systems are well documented. Frye et al. (1994) stated that soil organic carbon and nitrogen; exchangeable P; exchangeable Ca, Mg, and K; were significantly higher with no-till than conventional till in the 0 to 5 cm depths. Hoyt et al. (1980) reported higher ratios of soil NO_3^- to NH_4^+ in conventional than in no-till.

Although this is valuable information, there is little documented about the biochemical properties affected by no-till and fewer still on manure effects in no-till. Franzleubbers et al. (1994), found higher microbial populations in no-tillage plots than in conventional tilled plots. Kirchner et al., (1993) found that the biomass carbon amounts were higher in no-tilled plots when compared to the conventional tilled plots. Bolten et al. (1985) showed that the incorporation of a leguminous green manure increased the soil microbial biomass and enzyme activities when compared to soil that were treated with inorganic fertilizers. Kanazawa et al., (1988) found that microbial numbers (bacteria, spore-forming bacteria, actinomycetes, and fungi) were the highest in soils receiving farm-yard manure, followed by a fertilizer treatment, and were lowest in the no-fertilizer plots; moreover, he found that soil microbial biomass carbon was the highest in the farmyard manure plot.

The use of manure may satisfy fertilizer needs, but an over-application of manure may create pollution problems in surface waters and groundwaters (Mullen et al., 1993). The constituents of manure that are contaminants of subsurface waters are nitrogen, organic C and soluble inorganic P. More specifically, nitrate-N, negatively charged

soluble organic carbon compounds, and soluble forms of phosphorus can leach through to the water table from surface applied manure.

There are other problems associated with manure use such as the effects manure have on ground water quality. Reports have shown that health threatening diseases such as methemoglobinemia (blue-baby syndrome) can be a problem due to excess nitrate levels in water supplies (Bouwer, 1990). Fecal bacteria, which are a manure constituent, also pose an environmental health risk. Culley and Phillips (1982) studied the bacteriological quality of surface and subsurface runoff from sandy clay loam soil receiving manure. They suggested that manure be applied and plowed under in the fall before the ground freezes to minimize microbial densities in runoff during the following spring, although this method would not work for all regions. Results of an experiment to determine the fecal coliform contamination in runoff from stimulated rain in five, ten, and fifteen minute intervals indicated that cattle at a nearby stream channel can have a detrimental impact on surface water quality (Thelin and Gifford, 1983), The measurement of total coliforms not only indicates the fecal contamination present in the water, but it also includes a varying proportion of organisms that are of limited sanitary significance and capable of excessive regrowth in nutrient rich water (Geldrich, 1970). Thus, fecal coliforms can be used as an indicator of pathogenic organisms that are found in ground water. Fecal coliform organisms were chosen as indicators based primarily on their ability to survive outside the intestinal tract of cattle. This is important because this organism can adapt and survive for a long time in a new environment. Also, they are used because there is little difficulty in culture isolation and identification of these organisms (Thelin and Gifford, 1983). In support of the theory that the organism can survive for a long time, Marsh and Campling (1970) found that fecal organisms in manure deposits have remained on soil and plant surfaces for up to 1.5 years, depending

on climate, insects, and biological activity. Clemm (1977) found that both fecal coliforms and fecal streptococci can survive for more than a year in bovine feces. The Environmental Protection Agency requires that the fecal coliform (FC) count not exceed 200 FC/100 ml for bathing water (US-EPA, 1976), and that the fecal coliform count in drinking water not exceed 3 FC/100 ml (Bedminster, 1993).

Direct contamination of water causes researchers to raise questions about the indirect impact of manure as a fertilizer on crop fields. Would the answers to questions on indirect impacts of manure lead to a limitation on the quantity of manure spread per field or a change in the application method? Another area of interest to soil scientists are the impacts of manure on soil biological and biochemical properties (Buchanan and King, 1992). In Tennessee, the use of manure in no-till is also of interest with winter covers receiving various rates of manure and inorganic fertilizer.

Microbial numbers give some insight into the interactions going on in the soil. At pH values below 5.5, nutrients like calcium are not available in large amounts and such a nutrient is needed in many microbial metabolic processes (Miller and Donahue, 1990; Tabatabai, 1992). If that nutrient is not available, then the microbes will not be able to break down other nutrients for plant uptake. Microbes are also affected by water potential. In dry soils fungi dominate, but in water logged soils, such as a wetland soil or a rice paddy, bacteria can survive and are dominant. Since microbes are sensitive to water potential it is important for plant growth that there is adequate water in the soil. Also important to plant growth is carbon and nitrogen. With an adequate supply of carbon and nitrogen microbial activity can be enhanced. For these and many other reasons, it is important to this study to observe the interactions and effects manure treatments, (as opposed to inorganic fertilizer treatments), have on microbial numbers like bacteria and fungi.

Yet another area of concern and importance is enzymatic activity. Most biological processes require a means to reduce activation energy so that the process can occur under natural conditions. Enzymes lower the activation energy for biochemical reactions, catalyzing the reaction (Miller and Donahue, 1990). As a catalyst, these enzymes are not consumed and they do not change the outcome of a reaction, but they do accelerate the rate of reactions (Dick and Tabatabai, 1989). Enzymes activities are pH dependent (Dick and Tabatabai, 1989). Enzymes can be denatured at pH values above or below their optimal, and each enzyme is specific for different types of chemical reactions. Nearly all biological processes require enzymes and there are many different types including: cellulases, ureases, phosphatases, sulfatases, proteases, and others (Tabatabai, 1994). For this study the enzymes observed were: arylsulfatase, L-asparaginase, β-glucosidase and acid and alkaline phosphatase.

Generally speaking, the significance in studying arylsulfatase is that sulfatases are classified according to the sulfate esters they hydrolyze (Tabatabai, 1992). More specifically, arylsulfatase is the enzyme that catalyzes the hydrolysis of an arylsulfate anion by fission of the O-S bond (Tabatabai, 1992). Al-Khafaji and Tabatabai (1979) suggested that sulfatases play a key role in the process of organic soil sulfur mineralization. If manure is applied to the soil as an amendment (fertilizer) it is important to know if there is a stimulation or inhibition of the mineralization potential of organic sulfur. Arylsulfatase activity provides a measure of this potential.

Another enzyme that has significance to this study is acid phosphatase. The mode of action of phosphatases is the hydrolysis of phosphate ester bonds to produce humus-OH and $H_2PO_4^-$, making phosphorus available to plants (Speir and Ross, 1978). Phosphatases have very broad specificity ranges and do not require cofactors or coenzymes (Gianfreda and Bollag, 1994). Phosphatase mediates the release of inorganic phosphorus from organically-bound phosphorus that has been returned to the soil in a form plants can not use. The intensity of phosphatase is important in that it affects the rate of phosphorus cycling (Dhruva Kumar Jha et al., 1992). Also, phosphatase activity in the soil has been observed to be associated with intact cell walls of plant tissue, with cell wall fragments, and with amorphous organic material (Ladd, 1978). Therefore it makes perfectly good sense that the type of amendment applied to the soil would affect enzyme activity. Manure has been shown to positively affect enzymatic activity (Dick et al., 1988). However, the amount at which maximum benefits can be seen is not known. Noting this, Dick and Tabatabai, (1992) hypothesized that an adequate level of pH for crop growth could be defined as a pH that has an adequate acid/alkaline phosphatase activity ratio.

Manure can positively affect enzymatic activity through the use of the β-glucosidase enzyme. Beta-glucosidases are believed to be the most common group among soil enzymes (Yoshikura et al., 1980). Hydrolysis products of β-glucosides, glucose, are an important energy source for microorganisms in the soil (Tabatabai, 1994).

L-asparaginase is the last enzyme that was analyzed for this a study. The mode of action for this enzyme is to cleave the asparagine amino acid. Asparagine is important in transporting nitrogen through the plant. It is well suited for this job because among other things, asparagine has a high C:N ratio and effectively transport NH_3 in a non-toxic form (Gomonova et al., 1984). L-asparaginase is important in the release of NH_4^+ from proteins. This enzyme was chosen as an indicator of potential nitrogen mineralization activity in the soils of the current study.

With the information found and the questions presented earlier, research plot were established in 1991 to evaluate different rates of dairy manure in no-till corn silage production. The impacts of these manure applications on soil biochemical and chemical properties are discussed here.

The project site for this study was located in Northwest Tennessee, on the Martin Agricultural Experiment Station. The soils in that region are formed in loess or loess over coastal plain sediments, and are highly erodible. The experimental site is an area of Loring silt loam soils (fine, silty, mixed, thermic Typic Fragiudalf) with 4-6% slopes.

This project was formulated with the overall objective of characterizing some basic biological properties of soils receiving various rates of nitrogen as dairy manure. More specifically, the project objectives were:

1. To evaluate the impact of manure on

    a. soil total C contents
    b. soil total N contents
    c. other soil chemical properties

2. To evaluate the impact of manure on

    a. soil microbial biomass carbon and nitrogen
    b. total populations of soil bacteria and fungi in the surface soil, and
    c. population of various other microbes

3. To evaluate the impact of manuring on specific soil enzyme activities

With these objectives the null hypothesis was that there are no differences in soil biochemical properties due to different rates of dairy manure or inorganic nitrogen fertilization in a no-till system.

PART 2: MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Experimental Plots

There were eighteen no-till corn (*Zea mays L.*) plots used in this study, located on the Martin Agricultural Experiment Station in West Tennessee. The soil on these plots was a Loring silt loam (fine-silty, mixed thermic Typic Fragiudalf) with a 5% slope. After corn harvest in the fall, a forage crop of an annual ryegrass (*Lolium motriflorm*) and crimson clover (*Trifolium incarnatum L.*) was seeded. The cover was harvested for hay in the spring. Remaining winter cover was desiccated by spraying with glyphosate followed by planting corn for silage. The plots were also sprayed with atrazine for grass weed control.

This experiment had a completely randomized design with six treatments and three replications. The treatments consisted of four manure-N rates, an inorganic-N treatment and a 0-N control (Table 1). The inorganic rate of 168 kg ha^{-1}, was at the high end of the recommended rates for silage corn in Tennessee. The manure-N rates corresponded to 0.5, 1, 1.5, and 2 times the recommended rate, respectively.

Soil and Mulch Sampling

The soil profile was sampled in each plot at six locations with two samples taken in the row, halfway between the rows (48 cm), and two 24 cm from the row. Soil samples at each location were taken to depths of 0-5, 5-10, and 10-20 cm. Six cores were taken from each plot. The soil from the six locations were combined by depth. This translates to 54 soil samples per sampling date. Soil samples were kept on ice during transport to minimize microbial activity. Once at the laboratory, the soils were stored at

4° C until analyses were performed. All wet analyses were done within 10 to 14 days after sampling date. The sampling dates were May 4, 1994, May 25, 1994, June 13, 1994 and Aug. 5, 1994.

Table 1. Treatment identification.

		N Rate Applied in:	
Treatment ID	**N Source**	**Spring**	**Fall**
		----------kg N ha^{-1}----------	
ON	None	0	0
AN	Ammonium Nitrate	168	50
0.5X	Manure	84	42
1X	Manure	168	84
1.5X	Manure	252	126
2X	Manure	336	168

Mulch samples were taken by scraping the surface between rows within a 12 cm x 12 cm area. After four randomly selected mulch samples were taken per plot, they were bagged and labeled appropriately. The mulch sampling dates were the same as for the soil with the except that no samples were taken on May 25, 1994.

Soil samples were air-dried and sieved to less than 2 mm. The mulch samples were dried and ground to less than 60 mesh using a cyclone grinder. All soil samples were analyzed for pH, total carbon, total nitrogen, inorganic nitrogen, total bacteria, microbial biomass carbon and nitrogen, calcium, copper, iron, potassium, magnesium, manganese, sodium, phosphorus, zinc and selected enzymes. The enzymes investigated were β-glucosidase, alkaline and acid phosphatase, L-asparaginase and arylsulfatase. Various types of microbes were enumerated on the appropriate medium for sampling

dates 6/13/94 and 8/5/94. Total heterotrophic bacteria were enumerated on tryptic soy agar, fungi on Rose Bengal agar, and fluorescent pseudomonads on fluorescent pseudomonad agar (Wollum, 1994). Fecal coliforms were enumerated on BBL KF Strepoccocal agar and cellulose degrading fungi were enumerated on cellulose degrader agar (Wollum, 1994). Rose Bengal, Fluorescent Psuedomonads, fecal bacteria, and cellulose degraders were only done on the last two sampling dates. Microbial counts were done on moist soil, but total nitrogen, total carbon , pH, and extractable nutrient analyses procedures were done on air-dried soil samples. Each sample was analyzed for total carbon and total nitrogen.

Soil and Mulch Properties

Soil pH was determined by glass electrode as outlined in by McClean (1982). Two grams of soil were placed in a cup and 20 ml of deionized water was added. The suspension was stirred and allowed to equilibrate for 20 minutes.

The total carbon content was determined by dry combustion (Nelson and Sommers, 1982). This was done in a LECO CR-12 total Carbon Analyzer with one gram soil samples. The soil was burnt in a medium-temperature resistance furnace where purified oxygen is present. The unit was calibrated with $CaCO_3$ every 10th sample. The mulch layer was sampled in much the same way, except that the amount of subsample required was much less, only 0.2 grams of mulch sample was required. To keep the machine calibrated nicotinic acid ($C_6H_5NO_2$) was used every 10th sample, in a replication of five calibration samples.

To assess total nitrogen, the Carlo Erba Strumentazione NA1500 nitrogen analyzer was used. The procedure required that a 20 mg sample be wrapped in a tin

combustion boat and then heated at a high temperature. Before the nitrogen was converted it was collected in a nitrometer and the volume of gas is measured as percent nitrogen. The column inside the nitrogen analyzer has a carbon dioxide trap that catches carbon dioxide and allows the other gases to continue to flow through to a manganese anhydride column that removed the gases with the water vapors. The mulch layer was done it the same way, except the amount of the subsample was approximately 7 mg .

The Mehlich I procedure was used to obtain plant available phosphorus, potassium and exchangeable base concentrations (Nelson et al., 1953). The extractant was 0.05 M HCl in 0.025 M H_2SO_4. Five grams of soil were extracted with 20 ml of the extractant for one hour, allowed to settle, and filtered through a Whatman No. 2 filter paper. This extract was then analyzed by inductively-coupled argon plasma spectrophotometry on a Thermo Jarrel Ash Inductively Coupled Argon Plasma Atomic Emission Spectrophotometer model number 61.

Microbial Enumerations

Total bacteria, fungi, cellulose degraders, florescent pseudomonades, and fecal bacteria were tabulated by dilution plate count method (Wollum, 1982). Total bacteria was grown on tryptic soy agar (Martin, 1975). The total fungi were grown on Martin's Rose Bengal media, cellulose degraders were cultured on cellulose media, and fluorescent psuedomonads were grown on florescent psuedomonad agar (Wollum, 1994). The fecal bacteria were cultured on a pre-mixed medium called BBL KF Strepoccoal agar.

The overall procedure for plating was to place 10 grams of sieved soil into 90 ml of sterile distilled water. The aliquot obtained was then shaken for twenty minutes. After shaking, a 1 ml aliquot was transferred into 9 ml of sterile distilled water. This procedure

was continued until the desired dilution was obtained. A Spiral Biotech Autoplater model 3000 plater was used to spread the diluted sample on the agar surface. This instrument applies 52 ml of diluted sample to the plate. For each soil sample there were two replications. At this point, the plates were turned upside down (agar side up) and incubated at 30 degrees C for a defined period of time as determined by the methodology used for the organism in question. After incubation, the number of colonies on each plate was counted. The colony forming units (CFU) were calculated by multiplying the number of colonies counted by the dilution factor and dividing by the grams of soil used (Germida, 1993). The log CFU/g soil was averaged over the two replications for each soil sample (Germida, 1993). The dry weights were taken from a 10 gram subsample of the freshly sieved soil that was dried at 105° C and left.

Microbial Biomass Carbon and Nitrogen

Microbial biomass carbon and nitrogen were determined by the chloroform fumigation method as described by Voroney et al. (1993). This method was chosen because of its accuracy over a wide range of pH and soil types (Kaiser et al., 1992). Three, 12 gram subsamples to be taken from each soil sample. To one sample, 30 ml of 0.5 M K_2SO_4 were added to extract background levels of soluble C and N. The other two samples were placed in a vacuum desiccator with a beaker containing 40 ml of chloroform. The dessicator was then evacuated, resulting in the boiling of the chloroform. This saturated the soil with $CHCl_3$ causing the lysis of microbial cells in the soil. The soils were incubated in the $CHCl_3$ atmosphere for 24 hours. After fumigation, the $CHCl_3$ was removed and the dessicator was evacuated four times in order to remove all $CHCl_3$ from the soil. Each subsample was then extracted with 0.5 M K_2SO_4. Both the

fumigated and unfumigated samples were shaken for one hour followed by filtration of the suspension through No. 2 Whatman filter paper. Filtrates were analyzed for soluble carbon on a Dohrmann Organic Carbon analyzer. Another subsample was used to determine the water content by oven-drying it at 105° C for 24 hours. Filtrates were stored at 4° C until they could be analyzed.

Microbial biomass N was also determined using the 0.5 M K_2SO_4 extracts of the fumigated and the unfumigated soils. Nitrogen in the extract was oxidized to NO_3-N by an alkaline persulfate method (Cabrera and Beare, 1993). A 3-ml volume of K_2SO_4 extract was mixed with a 3 ml volume of a solution made up of 15g H_3BO_4, 50 ml of 3.75 M NaOH, 25g $K_2S_2O_8$ and 500 ml deionized water. At this point the weight of the container with the liquid in it was recorded. This mixture was then autoclaved at 121°C for thirty minutes. After the solution had cooled, the weight of the container and liquid was recorded. The NO_3-N concentration in the extracts were determined on an Altech Inorganic Nitrogen analyzer (Carlson et al., 1984).

Soil Enzyme Activities

Five soil enzyme activities were measured in the soils, acid and alkaline phosphatase, β-glucosidase, arylsulfatase, and L-asparaginase. These enzymes mediate key reactions in phosphorus, carbon, sulfur and nitrogen cycling in soils (Tabatabai, 1992). Acid and alkaline phosphatase were studied because they hydrolyze organic phosphates. Acid phosphatase is found predominantly in acidic soils, with low levels of alkaline phosphatase present. Also sensitive to pH, as most enzymes are, β-glucosidase was studied because its hydrolysis product (glucose) is an energy source for soil microorganisms (Eivazi and Tabatabai, 1988). Arylsulfatase was studied because it is

involved in sulfate hydrolysis from organic sulfur in soils (Al-Khafaji and Tabatabai, 1979). Finally, L-asparaginase hydrolyzes the ammonia group from the amino acid asparagine, leaving the amino acid aspartate and free ammonia (Tabatabai, 1992). It can also serve as an indicator of nitrogen cycling.

Acid phosphatase activity was determined by the phosphomonoesterase assay (Tabatabai, 1992). One gram of air-dried soil was placed in a 50 ml flask with 0.2 ml of toluene, four ml Modified Universal Buffer (MUB) pH 6.5, and one ml of 0.025M *p*-nitrophenylphosphate solution made in the same buffer. The toluene was used to sterilize the soil, inhibiting the production of new enzymes by soil microorganisms. The samples, controls, and standards were then mixed and incubated at 37°C. After one hour of incubation, the samples, controls, and standards were removed from the incubator and one ml of 0.5M $CaCl_2$ and four ml of 0.5M NaOH were added to the samples, controls and standards. The suspensions were then mixed again. All suspensions were filtered and the yellow color intensity, which was generated from the *p*-nitrophenol released, was measured on a Milton Roy Spectrophotometer model 401 at 420 nm. The *p*-nitrophenol concentration was calculated from a standard curve. The controls were treated as above, except that the substrate was added just prior to filtration, after the addition of the NaOH. Alkaline phosphatase activity was determined using the same method except that the suspension was buffered at pH 11 instead of pH 6.5.

Beta-glucosidase activity was determined by the β-glucosidase assay (Tabatabai, 1992). There are two major group within this enzyme class. The general terms are, *glucosidases* or *glycoside hydrolases,* these terms have been used to describe a group of enzymes that catalyze the hydrolysis of different glycosides (Tabatabai, 1992). One gram of air-dried soil was placed in a 50 ml flask with 0.25 ml of toluene, four ml of pH 6.0 MUB buffer, one ml of *p*-nitrophenyl-β-D-glucoside (PNG-substrate), and swirled for a

few seconds. At that point, the samples, controls, and standards were incubated for one hour at 37°C. After incubation, one ml of 0.5M $CaCl_2$ and four ml of 0.1M Tris (Hydroxymethyl) Amino Methane (THAM) buffer at pH 12, was added and the suspension was mixed, filtered, and the color intensity was measured on the Milton Roy Spectrophotometer 401 at 420 nanometers. Controls were treated the same as above except the substrate was not added to the samples before incubation, but after right before the $CaCl_2$ and the NaOH were added.

Sulfatases play a large role in nature. They are classified according to the type of organic sulfate esters they hydrolyze, with the following main groups: arylsulfatases, steroid sulfatases, glucosulfatases, chondrosulfatases, and myrosulfatases (Tabatabai, 1992). Arylsulfatase is the enzyme that catalyzes the hydrolysis of a sulfate group from an O-S bond. The procedure for the arylsulfatase assay was essentially the same as the procedure for the acid and alkaline phosphatase (Tabatabai, 1992). One gram of soil aliquot was treated with 0.2 ml toluene, four ml acetate buffer (0.5M) at pH 5.8, and one ml (0.025M) *p*-nitrophenyl-sulfate solution. The flasks were then swirled and incubated for one hour at 37° C. The reaction was stopped by adding one ml $CaCl_2$ and four ml 0.5M NaOH, and mixing. The suspensions were filtered and the yellow color was determined on the Milton Roy Spectrometer at 420 nm.

L-asparaginase activity was determined by the method as outlined by Tabatabai (1992). Five grams of air-dried soil were placed in a 50 ml flask with 0.2 ml of toluene, nine ml of pH 10, 0.1M THAM buffer, and 0.5M L-asparaginase solution as the substrate. The flask was swirled to mix the ingredients and then incubated for two hours at 37° C. After incubation, 100 ppm of KCL-silver-sulfate (2.5M KCL) was added to stop the reaction. The volume was adjusted to 50 ml with the KCL-silver-sulfate solution. The ammonium released by the enzymatic activity was measured with the

Altech Inorganic Nitrogen Analyzer. Controls were done in the exact same way except the L-asparaginase solution (substrate) was added after the KCL-silver-sulfate solution.

Statistics

Analyses of variance (ANOVA) was used to determine the significance of the treatment effects. Any significant effects were separated by the Least Significant Difference (LSD) test, unless otherwise mentioned, at $P = 0.05$.

The SAS statistical package was used for all statistical analyses (SAS Institute, 1995).

PART 3: RESULTS AND DISCUSSION

Soil and Mulch Properties

Total Soil Carbon

Applications of manure since 1991 have resulted in changes in total soil carbon (Table 2). Soil carbon generally increased as the manure rate increased. Over all sampling dates, the amount of carbon in the soil receiving 504 kg manure-N ha-1 was significantly higher than in the control treatment. The same was observed in the results for the inorganic fertilizer treatment. The general trend was for total carbon to increase ass manure rates increased. This would be expected due to the increase in the amounts of carbon that was added to the soil as manure rates increased.

In depth one on sampling date 5/4/94, treatment 1X was significantly higher than the ON. It was expected in that the organic amendments applied added large amounts of carbon to soil. The carbon contents decreased over sampling dates 5/4/94, 5/27/94 and 6/13/94, but an increase in soil carbon amounts on 8/12/94 (Table 2). A similar trend was seen on sampling date three. The difference was that there was no significant difference among sub-surface levels. The activity seen for sampling date one is typical when considering the need for carbon by microbes.

Treatments 1.5X and 2X had significantly higher soil carbon than the inorganic treatment (AN) and the control for sampling date 5/25/94. These results are consistent with what was expected, there was more carbon available for use in the soils that were amended with manure.

As stated before, sampling date 6/13/94, showed similar results to sampling date two. The difference here, was that only the control was significantly less than the manure and inorganic treatments.

Table 2. Effects of manuring on soil carbon, nitrogen and C:N ratios in the 0 - 5 cm depth.

	MAY 4 1994			MAY 25 1994		
	-----%-----			-----%-----		
Treatments	C	N	C:N	C	N	C:N
ON	1.44b*	0.17a	8.39a	1.36c	0.14c	9.84a
AN	1.62ab	0.18a	9.05a	1.61bc	0.21ab	7.75b
0.5X	1.68ab	0.18a	9.39a	1.49bc	0.18bc	8.41ab
1X	2.05a	0.18a	12.57a	1.77ab	0.20ab	8.83ab
1.5X	1.76ab	0.21a	8.63a	1.92a	0.22a	8.57ab
2X	1.99a	0.20a	10.28a	2.04a	0.21ab	9.73a
	JUNE 13 1994			**AUGUST 5 1994**		
	-----%-----			-----%-----		
Treatments	C	N	C:N	C	N	C:N
ON	1.13b	0.14b	8.08b	1.37b	0.15a	9.11a
AN	1.56a	0.17ab	9.26ab	1.39b	0.16a	9.12a
0.5X	1.49a	0.14b	11.19a	1.42b	0.16a	8.77a
1X	1.45a	0.17ab	8.51ab	1.56b	0.17a	9.55a
1.5X	1.67a	0.20a	8.5ab	1.56ab	0.18a	8.87a
2X	1.68a	0.21a	7.92b	1.71a	0.18a	9.60a

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

As for sampling date four (8/5/94), the results showed treatment 2x to be significantly higher than any other treatment. There was generally a treatment effect on total soil carbon. This trend was most evident in the higher manure treatments. These findings were consistent with the findings of Long (1979), Weil and Kroonje (1979), and Mathers and Stewart (1974). Who found that the more manure used, the more total carbon will be available.

Overall the carbon data was as expected. The manure treatments had more carbon than the inorganic treatment. The carbon amounts began to decrease over time, which would suggest the use of this substrate by microbes.

Total Soil Nitrogen

Total soil nitrogen amounts were consistent across all dates. The amounts ranged from 0.15 (control from sampling date 8/5/94) to 0.22 (treatment 1.5X of sampling date 5/25/94). The only significant differences were observed on May 25 and June 13 (Table 2). Total nitrogen tended to increase with increasing manure rates, and on both dates, 1.5X and 2X treatments had higher nitrogen than the control. This is due to the addition of nitrogen in the manure. Depth distribution showed much of the nitrogen was in the 0 to 5 cm depths. The overall means by depth were 0.165, 0.142, and 0.075 %N for depths 1 (0 to 5 cm), 2 (5 to 10 cm) and 3 (10 to 20 cm), respectively.

There was no significant difference seen among treatments for the May 4 and Aug. 5 sampling dates. There were no significant differences in the depths, either.

Reporting very parallel to the results of the total soil carbon, sampling dates two (5/25/94) and 3 (6/13/94) definite treatment effects. On sampling date two, treatments 1X, 1.5X, and 2X was significantly greater than the control (Table 2). Sampling date

three (6/13/94) showed that treatment 1.5X and 2X were significantly greater than treatments AN or ON for depth one. Again these results were expected since manure-N had been applied to the soils after sampling date one.

The total nitrogen data was consistent with the carbon data, in that there was a decrease over time, suggesting the plant uptake and microbial use. The manure had more nitrogen over most dates and the inorganic treatment followed numerically.

Total Soil Carbon to Nitrogen Ratios

Over the four sampling dates, the C:N ratios were typical for most soils. There was a strong tendency for the manure treatments to have high carbon rates, low nitrogen rates which resulted in C:N ratios that were in the range of eight to twelve. These results were expected, according to Levi-Minzi et al. (1990), lower C:N ratios are associated with greater respiration rates and will result in greater biological activity. Moreover, the C:N ratios seen in this study could be due to the higher amount of mineralization that occurs with C:N ratios as seen in this study (Wander et al., 1994). Depth one consistently showed significantly higher C:N ratios than any other depth. These results were also expected. This because depth one is where most of the plant roots are and would be expected to be where most of the mineralization would occur. This is also where C:N input, such as manure and plant residues break down occurs. The C:N ratio range for adequate plant growth is eight to ten; and in the current study the AN treatment constantly remained toward the upper end of the range (Table 2). This is probably due to the somewhat higher nitrogen amounts, that were placed in the soil. The manure treatments, on the other hand, were generally around eight.

More specifically, any differences seen in the C:N ratios were seen on May 25 and June 13 dates. This is consistent with the results found for total carbon (Table 2).

On sampling date two (5/25/94) there was a significant treatment effect. Treatment 1.5 X was significantly greater than any other treatment. Also shown on table 2 is sampling date three (6/13/94) which showed treatment 0.5X to be significantly greater than any other treatment. The explanation for this occurrence could be some other environmental interactions.

The most activity was seen on May 25 and June 13 dates. This suggests that the environmental conditions were conducive to microbial activity.

Total Mulch Carbon

Although low, the mulch carbon data for the current study showed significant differences on the May 4 and June 13 dates, while the Aug. date showed none. Generally, the carbon amounts were between the range of 10.1 (the control of June 13) and 23.6 (the control of the May 4 date). The manure treatments, at any date, were mostly numerically lower than the AN treatment and the control (Table 3). The trend seen was one where the numeric values were higher on the May 4 date, decreased on June 13 date and increased again on the August 5 date. The explanation afforded is that this makes sense, considering the soil carbon, nitrogen, and C:N ratios showed most activity during the May 25 and June 13 dates. Yet this trend did have some exceptions.

On sampling date one (5/4/94), the mulch from the control had a significantly higher amount of total mulch carbon than treatments 1.5X and 2X. The fact that the control had a higher carbon amount was probably due to less nitrogen from where the microbes had begun to utilize the carbon. As the mulch was decomposed, the C:N ratio

Table 3. Effects of manure on mulch carbon and nitrogen in the 0 - 5 cm depth.

	MAY 4 1994			JUNE 13 1994			AUGUST 5 1994		
	C	N	C:N	C	N	C:N	C	N	C:N
Treatments	------%----			------%----			------%----		
ON	23.6a*	1.5a	16.2abc	10.1c	0.91c	11.3b	15.1a	1.14a	13.1a
AN	21.9abc	1.2abc	18.2ab	13.7bc	0.88c	15.5a	18.1a	1.25a	14.4a
0.5X	23.1ab	1.1abc	21.3a	10.8c	0.86c	12.6b	16.7a	1.24a	13.5a
1X	15.8cd	0.99c	15.9bc	14.4bc	1.18bc	12.1b	18.7a	1.46a	12.8a
1.5X	12.9d	1.0bc	12.4c	19.1ab	1.61ab	11.9b	16.0a	1.38a	11.6a
2X	16.3bcd	1.4ab	11.8c	22.5a	1.89a	12.1b	19.4a	1.48a	13.7a

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

got smaller which would cause there to be more available at higher manure rates, therefore, allowing carbon to be decomposed more rapidly.

As with the soil total carbon, the mulch total carbon decline came on sampling date three (6/13/94), for most of the treatments. Treatments 1.5X and 2X seemed to have increased in total mulch carbon. This may have been due to some soil having been mixed in with the mulch layer. As expected there was no significant difference in sampling date four total mulch carbon, although, the means of treatments 1.5X and 2X continued to decrease indicating mineralization (Table 3).

Total Mulch Nitrogen

Most likely due to seasonal changes, the amount of mulch nitrogen increased in the higher manure treatments and decreased in the control, generally. The numeric value range for total nitrogen was 0.86 (0.5X of the June 13 date) to 1.89 (2X of the June 13 date). This range was a little higher than that seen in the soil nitrogen.

As seen with mulch carbon in sampling date one (5/4/94), treatment 0N had a significantly greater amount of total mulch nitrogen. The 1X treatment, on the other hand, had a significantly less amount of total mulch nitrogen than nay other treatment. The reason for this occurrence is unknown at this time.

The control, inorganic treatment and the low manure rate of sampling date three (6/13/94) were significantly lower than treatment 2X (Table 3).

On sampling date four (8/5/94) there was no significant treatment effects (Table 3). The larger amounts of mulch nitrogen in the manured treatments were probably due to the increased growth of the cover crop.

Total Mulch Carbon to Nitrogen Ratios

Just as the soil carbon C:N ratios had an adequate range so does the mulch. The adequate range for mulch ratios is between 10 and 20. Overall the highest C:N ratios were found on May 4 date. The trend seen was very slight. The ratios initially decreased and then increased again by the August date. This trend was seen during all the mulch and some of the soil C:N analyses.

When comparing the C: N ratio of the significant differences in total nitrogen on sampling date one (5/4/94) to those of the significant means of sampling date one's carbon, significant ratios result. Treatment 0.5X had a significantly higher C:N than treatment 1.5X and 2X (Table 3). There could be two reasons for this, (1) either there was an operational error causing the reading to come out flawed. Or (2), the 0.5X treatment amount of manure was not applied even thereby causing mineralization.

On (6/13/94), treatment AN was significantly higher than any other treatment. This could be due to the amount of nitrogen left in the soil. Green and Blackmer (1995) suggested that higher rates of fertilization will result in lower C:N ratios and more residual inorganic nitrogen in the soil. Also this can be attributed to the small amounts of carbon left in the soil that would mineralize the remaining nitrogen.

Mulch C:N ratios for sampling date four (8/5/94) results were the same as those of the total soil C:N with no significance differences.

Soil pH

The soil pH over all dates stayed around 6.0 for all the manure treatments and the control (Table 4). In the inorganic fertilizer treatment, the pH ranged from 5.0 to 5.6. In

Table 4. Effect of soil pH over all dates.

	MAY 4 1994	MAY 27 1994	JUNE 13 1994	AUGUST 5 1994
	----------Depth one----------			
Treatments				
ON	5.82b*	5.88a	5.56a	5.45a
AN	5.18c	4.82b	4.96a	5.61a
0.5X	6.00ab	5.91a	5.67a	5.53a
1X	5.98ab	5.97a	5.83a	5.62a
1.5X	6.01ab	6.24a	5.69a	5.73a
2X	6.18a	6.02a	5.82a	5.71a
	----------Depth two----------			
Treatments				
ON	6.20a	6.22a	6.05a	5.59a
AN	5.66b	5.83a	4.79b	5.67a
0.5X	6.29a	6.31a	6.07a	5.56a
1X	6.01ab	6.20a	5.80a	5.55a
1.5X	6.23a	6.19a	6.01a	5.51a
2X	6.13a	6.09a	5.71a	5.45a
	----------Depth three----------			
Treatments				
ON	6.23a	6.25a	6.12a	5.74a
AN	5.64b	5.77a	5.24b	5.82a
0.5X	6.52a	6.40a	6.20a	5.79a
1X	6.17ab	6.27a	5.92ab	5.71a
1.5X	6.12ab	6.24a	5.98a	5.51a
2X	6.22a	6.06a	5.88ab	5.52a

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

the initial stages of the experiment or after sampling date one (5/4/94), there was a slight pH increase in all the manure treatments. An increase in soil pH after the initial addition of manure was observed by Hensler et al. (1970) as well. After this slight increase there was a steady decline in pH in the manured plots. Similar to what was observed by Chang et al. (1991). Fox and Hoffman (1981) concluded that the higher the nitrogen rate the lower the pH. The decline occurred because of the nitrogen mineralization followed by nitrification. The higher pH of the manure treatments was also observed in other studies Kanazawa et al. (1988) and Frye et al. (1994).

As previously stated, sampling dates one (5/4/94) and two (5/25/94) resulted in pH's of all the manure treatments and the control being significantly higher than the ammonium nitrate treatment. In depth one of sampling date one, treatment 2X was significantly different than the inorganic fertilizer treatment. This trend was found in depths two and three (Table 4). There was a depth effect, where depth one had more activity and a treatment effect for sampling date two but the interaction was only slight. In the depths the treatment effect trend was the same for depths two and three.

Similarly, in sampling date three (6/13/94), all the manure treatments were significantly greater than treatment AN. Frye et al. (1994) suggested that the reason for the lower pH values in the inorganic fertilizer treatments was because of nitrification and mineralization of nitrogen in plant residues.

There were no treatment or depth effects by sampling date four (8/5/94), although there was a rise in the pH of the inorganic nitrogen treatment (Table 4). This may be

explained by the fact that when the ammonium is released in the soil the nitrifying bacteria oxidize the ammonium to nitrate, gain energy and release H^+ to the soil which causing acidity. One cane deduce that the cause of the significant pH increase is that the ammonium was all used up and less H^+ is in the soil.

Concentrations of Mehlich I Exchangeable elements

Organic amendments provide other nutrients, such as P, K, and Mg. As mineralization of the amendment occurs, these nutrients are released to the soil where they are then available for microbial and plant use.

This discussion will be solely on phosphorus and potassium, and magnesium. Data for several other elements are shown in Appendix.

EXTRACTABLE PHOSPHORUS

The concentrations of phosphorus were low and generally unaffected by treatments in depth one (Table 5). This trend was observed throughout all sampling dates except sampling date four (8/5/94). This is consistent with the findings Patni and Culley (1989), in that they too found phosphorus to be unaffected by treatment changes. Although it will not be discussed further, overall, depth 3 (10 to 20 cm) appeared to have the highest concentration of phosphorus (data not shown). Depths 1 (0 to 5 cm) and 2 (5 to 10 cm) were around the same amounts; consequently, this was the trend across all dates.

Although on sampling dates one (5/4/94) and two (5/27/94) there was a treatment effect, in depth one of sampling date one, treatment 0.5X was significantly greater than

Table 5. Effects of manure on phosphorus concentrations in the 0 - 5 cm depth.

Treatments	MAY 4 1994	MAY 25 1994	JUNE 13 1994	AUGUST 5 1994
	---------- mg kg^{-1} ----------			
ON	19.61bc*	32.86ab	66.64a	24.50a
AN	14.13c	22.58b	14.08a	23.48a
0.5X	33.25a	40.93a	35.10a	27.90a
1X	26.36ab	32.71ab	30.96a	27.44a
1.5X	21.65bc	31.18ab	19.30a	74.06a
2X	24.42ab	31.27ab	25.98a	20.16a

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

treatments 1.5X, ON, and AN (Table 5). Similarly sampling date two revealed treatment 0.5X to be significantly greater than treatment AN. Also like sampling date one, depth three of sampling date two showed no treatment effects, but depth two did (data not shown). Treatments 2X (0.97) and 0.5X (0.94) were significantly higher than the control (0.52).

As for sampling dates three (6/13/94) and four (8/5/94) of depth one, there were no significant differences.

EXTRACTABLE POTASSIUM

Interestingly, there were no differences found in depth one (0 to 5 cm) for sampling dates one (5/4/94), two (5/25/94) and four (8/5/94) (Table 6). This seems odd considering all other analyses showed that there were differences on the May 25 and June 13 dates. Depth one had fewer significant differences than depths two or three (data not shown). Over all dates the control tended to have lower potassium than the other treatments. Sampling date three (6/13/94) of depth one showed a significant treatment effect. The 1.5X and 2X treatments had significantly more potassium than treatment AN (Table 6). These findings were consistent with the findings of (Culley et al., 1981; Patni and Culley, 1989). They also observed higher concentrations in the first depth (0 to 5 cm).

There was very little to discuss because of few differences. The highest manure treatment had significantly more potassium than the control or the inorganic treatment. The reason for the manure treatment being so high, could have been that the carbon to nitrogen level was lower at this time.

Table 6. Effects of manure on potassium concentrations in the 0 - 5 cm depth.

	MAY 4 1994	MAY 25 1994	JUNE 13 1994	AUGUST 5 1994
	----------	----------	mg kg^{-1}	----------
Treatments				
ON	49.42a*	66.07a	49.76c	59.55a
AN	70.57a	69.02a	47.55c	67.57a
0.5X	68.58a	64.65a	54.09bc	78.47a
1X	52.46a	65.61a	60.24bc	59.84a
1.5X	52.49a	67.67a	71.32ab	64.40a
2X	61.97a	71.78a	83.11a	66.79a

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

EXTRACTABLE MAGNESIUM

A previous study showed that exchangeable calcium and magnesium was closely related to soil pH (Frye et al., 1994). Table 7 shows that sampling date one (5/4/94) and three (6/13/94) showed this trend, where the highest pH value and the highest extractable magnesium concentration occurred in treatment 2X. Treatment 2X was found to have a significantly higher magnesium concentration than treatment AN for all dates but two. Not only was treatment 2X significantly higher than treatment AN but it had a significantly higher concentration of magnesium than treatments 0.5X and 1.5X as well (Table 7). This follows the observation of Frye et al. (1994) who suggested that the reason the pH and the magnesium concentration was lower in the inorganic fertilizer was due to nitrification of NH_4^+ from surface-applied NH_4NO_3 fertilizer and from

mineralization of nitrogen in plant residues. Fine et al. (1989) when on to suggest that another reason the pH and magnesium concentration was lower in the inorganic fertilizer treatment may be due to a decrease in pH and soluble NH_3 concentrations, so the nitrifying bacteria would not be inhibited. They observed that this acidification results in the release of calcium and magnesium to the soil solution through solubilization of carbonates and desorption. This calcium and magnesium can then be lost by leaching.

Sampling date two (5/25/94) continued to show the trend where exchangeable magnesium concentrations somewhat follow the pH trend (Table 7). It seemed the more manure applied the higher the pH and Mg concentrations. There were no significant

Table 7. Effect of manure on pH and magnesium concentrations in the 0 - 5 cm depth.

Treatments	MAY 4 1994		MAY 25 1994		JUNE 13 1994		AUGUST 5 1994	
	pH	Mg ($mg\ kg^{-1}$)	pH	Mg ($mg\ kg^{-1}$)	pH	Mg ($mg\ kg^{-1}$)	pH	Mg ($mg\ kg^{-1}$)
ON	5.82b*	148.8ab	5.88a	162.9a	5.56a	142.9ab	5.45a	166.0ab
AN	5.18c	125.5b	4.82b	139.9a	4.96a	129.3b	5.61a	144.8ab
0.5X	6.00ab	148.9ab	5.91a	141.4a	5.67a	138.0ab	5.53a	141.9ab
1X	5.98ab	159.8ab	5.97a	154.9a	5.83a	149.2ab	5.62a	158.5ab
1.5X	6.01ab	136.3ab	6.24a	139.4a	5.69a	126.5b	5.73a	126.1b
2X	6.18a	191.0a	6.02a	183.4a	5.82a	197.6a	5.71a	182.9a

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

differences among treatments, but, one might expect to see this because of the mineralization of the residues. The slight increase in concentration suggests that some other phenomenon in the soil is contributing to the magnesium pool.

Microbial Enumerations

Total Bacteria

Microbial numbers in the surface 0 to 5 cm soil layer were greater across all dates than the subsurface depths. The general trend was for the manured treatments to have higher bacterial populations than the inorganic or control treatments. This trend was also observed by Fraser et al. (1988), who did a study on the soil microbial populations under conventional and organic management. Trends like these can be seen clearly because of the pH effect in the inorganically fertilized plots. Ammonium nitrate undergoes nitrification by nitrifying bacteria resulting in released nitrates and hydrogen ions which increasing soil acidity. The manured soils, on the other hand, would have a lot more nutrients available for the microbes.

There were no significant treatment effects on bacterial numbers (Table 8). As expected, there was a depth effect for both total bacteria and the Fluorescent Psuedomonads (FP) that showed depth one (0 to 5 cm) to have the most bacteria. As for the FP, the numbers counted for the first three dates were to few to get any statistical read out on; therefore, it will not be discussed in great detail. The dilution should have been 10^{-1} or 10^{-2} to get a better presentation of the activity during these dates.

Table 8. Effects of manure on viable heterotrophic bacteria in the 0 - 5 cm depth.

Treatments	MAY 4 1994		MAY 25 1994		JUNE 13 1994		AUGUST 5 1994	
(kg N ha^{-1})	---------- $LOG_{10}CFUg^{-1}$ ----------							
	NA	FP	NA	FP	NA	FP	NA	FP
ON	6.63a*	nd†	6.60a	nd	6.63a	nd	6.89a	4.91a
AN	6.63a	nd	6.68a	nd	6.25a	nd	6.86a	4.94a
0.5X	6.78a	nd	6.68a	nd	7.02a	nd	6.88a	4.71a
1X	6.70a	nd	6.83a	nd	6.97a	nd	6.85a	4.97a
1.5X	6.81a	nd	6.90a	nd	6.98a	nd	6.81a	4.72a
2X	6.74a	nd	6.86a	nd	6.89a	nd	6.59a	4.95a

NA = nutrient agar

FP = fluorescent psuedomonad

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

†no data

Total Fungi

The cellulose degraders were chosen because fungi are key in breaking down cellulosic material like corn stalks (Miller and Donahue, 1990). Rose Bengal medium (Wollum II, 1994), similarly, was used to get a good representation of the total fungi numbers in the soil. Only dates three (6/13/94) and four (8/5/94) will be discussed here as fungi were not enumerated at dates one and two (Table 9). The results showed that the three depths were all significantly different from each other, with depth one (0 to 5 cm) having the highest populations. Over the fungal numbers were somewhat low which probably was the reason for the inconsistency seen in the results.

No data for cellulose degraders were usable for sampling date four (8/5/94) due to unrecognizable characteristics of the colonies for this date. Moreover the plates were left incubating for a longer period of time resulting in the plates being too populated to count.

There was a significant treatment effect on sampling date three (6/13/94) with the fungi plated on the Rose Bengal medium (Wollum II, 1994). Treatments 1.5X and 2X were significantly higher than treatment AN (Table 9). The lower fungal numbers for the inorganic treatment was consistent with the results of the current study on bacteria populations. The cellulose degraders, on the other hand, showed no significance across the overall various treatments. This inconsistency between media could be due to the lower fungal numbers of the cellulose degraders plate counts.

On sampling date three (6/13/94) there were no treatment effects observed for cellulose degraders in depth one (0 to 5 cm) but there was for Rose Bengal (Table 9).

Table 9. Effects of manure treatments on soil fungal and cellulose degraders in the 0-5 cm depth.

Treatments	JUNE 13 1994		AUGUST 5 1994	
	$LOG_{10}CFUg^{-1}$			
	CD	RB	CD	RB
ON	3.45a*	4.88ab	nd†	5.26a
AN	5.10a	4.60b	nd	5.09a
0.5X	3.43a	4.62b	nd	4.84a
1X	1.76a	5.03ab	nd	4.97a
1.5X	5.35a	5.04ab	nd	4.84a
2X	5.09a	5.30a	nd	5.15a

CD = cellulose degraders

RB = rose bengal

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

† no data

Treatment 2X was statistically higher than in fungal numbers than treatments 0.5X and AN. Fraser et al., (1988) concluded that the fungal numbers of manure treated soils were greater than those of an unmanured soil. The fungal numbers were higher in the manure treated soil because the microbes had more substrate.

Results for cellulose degraders and Rose Bengal were similar in that treatment means were around the same amount in both cases for sampling date three (6/13/94). There was no statistical differences for the cellulose degraders (Table 9). The suggested reason for the higher fungal numbers in the manure treatment and not in the inorganic, is that the microbes had slightly more nitrogen and carbon to use in treatment 1.5X than in AN (Table 2).

The fungal numbers of sampling dates three (6/13/94) and four (8/5/94) appeared to show a slight trend. All the manure treatment fungal counts decreased and the inorganic treatment counts increase slightly. As noted by Dhruva Kumar Jha et al. (1992). In their study, the maximum fungi numbers in soils amended with manure were seen in July, and then declined until January. For sampling date four there was no significant differences found among treatments. Depth one showed no treatment effects.

Although there was very little data to compare in this section, some important conclusion could be made. From the Rose Bengal data one can see the adequate amount of fungi. One can also see slight a decrease in populations over the two dates. The manure treatments had around 5 LOG_{10} $CFUg^{-1}$ on the June 13 and 4 LOG_{10} $CFUg^{-1}$ on

August 5. The inorganic treatment did complete opposite, where fungal numbers increased.

Microbial Biomass Carbon and nitrogen

Unfortunately, this section resulted in very little concrete data. The only sampling dates that will be discussed here are 5/4/94 and 5/25/94 (Table 10). The majority of the biomass carbon was found in the first depth for sampling date one (5/4/94). Depth one was significantly higher than depths two or three. The overall means were 263.53, 78.63, and 68.00, for depths one (0 to 5 cm), two (5 to 10 cm), and three (10 to 20 cm), respectively. Results obtained by Kandeler et al. (1994), showed that biomass carbon and nitrogen amounts were highest in the surface depths (0 to 30 cm); Kirchner et al. (1993) noted biomass carbon numbers to be highest in depths (0 to 7.5 cm). This is where we observed the greatest biomass.

In the data obtained for biomass carbon, sampling date one (5/4/94) showed no statistical differences in biomass carbon amounts among treatments. This was further supported by data for biomass nitrogen. There was no significant differences across treatments but it is important to keep in mind that the analysis was done on dry soil. This is essential to note because Zagal et al., (1993) suggested that dry soil enhances mineralization; therefore, the numbers observed here may be higher than the actual numbers for biomass taken straight from the plot. Also the data observed was limited to the first depth because this analysis was done on dry soil.

Buchanan and King (1992) found that microbial biomass numbers were highest in the June/July time period, which is what occurred here.

Sampling date four (8/5/94) showed depth one (0 to 5 cm) to revealed treatment ON to be significantly different from treatment 1X (Table 10). The explanation for this

Table 10. Effects of manure on biomass carbon and nitrogen in the 0 - 5 cm depth.

	MAY 4 1994		MAY 25 1994		JUNE 13 1994		AUGUST 5 1994	
	----------mg biomass kg soil^{-1}----------							
Treatments	C	N	C	N	C	N	C	N
ON	262.42a*	11.43a	91.04ab	25.28a	nd†	20.63a	nd	47.57a
AN	224.56a	12.77a	79.07ab	92.50a	nd	22.72a	nd	28.90ab
0.5X	323.60a	11.23a	80.58ab	58.29a	nd	57.94a	nd	8.43b
1X	219.22a	10.97a	46.62b	21.39a	nd	24.05a	nd	37.17a
1.5X	289.42a	6.33a	112.27a	44.43a	nd	36.09a	nd	29.37ab
2X	261.92a	7.57a	131.21a	40.63a	nd	29.24a	nd	20.67ab

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

† no data

is that the season were changing, and there was less mineralization occurring due to the lack of biomass carbon.

As a result of the inconsistencies and the questionable data, different methodologies should have be considered. The chloroform-fumigation extraction method is good, but the results may have come out better if we used the chloroform-fumigation incubation (CFI) method or the substrate-induced respiration (SIR) method instead. Kaiser et al. (1992) did a study comparing three methods for accurate retrieval of biomass carbon. They concluded that the SIR or the CFI methods would give the most precise quantifying results for biomass carbon.

Soil Enzyme Activities

Arylsulfatase activity

As for all other enzymes studied, arylsulfatase activities across all treatments were significantly higher in the 0 to 5 cm depth than in the 5-10 cm and 10-20 cm depths. This was expected since organic carbon contents were also highest in the 0 to 5 cm depth. On May 4, depth one was significantly different from depth two or three. The overall means were 70.4, 29.4, and 24.1 μg p-nitrophenol g $soil^{-1}$ hr^{-1} , for depths one, two and three respectively. Dick et al. (1988) observed that higher organic carbon amounts were found in the manure treated soil, which was also seen in the current study. They concluded that higher organic matter levels in the manure treated soils may provide a more favorable environment for the accumulation of enzymes in the soil matrix, since soil organic constituents are thought to be important in forming stable complexes with free enzymes (Burns, 1982). Figure 1 illustrates the effect of treatment with depth on June 13

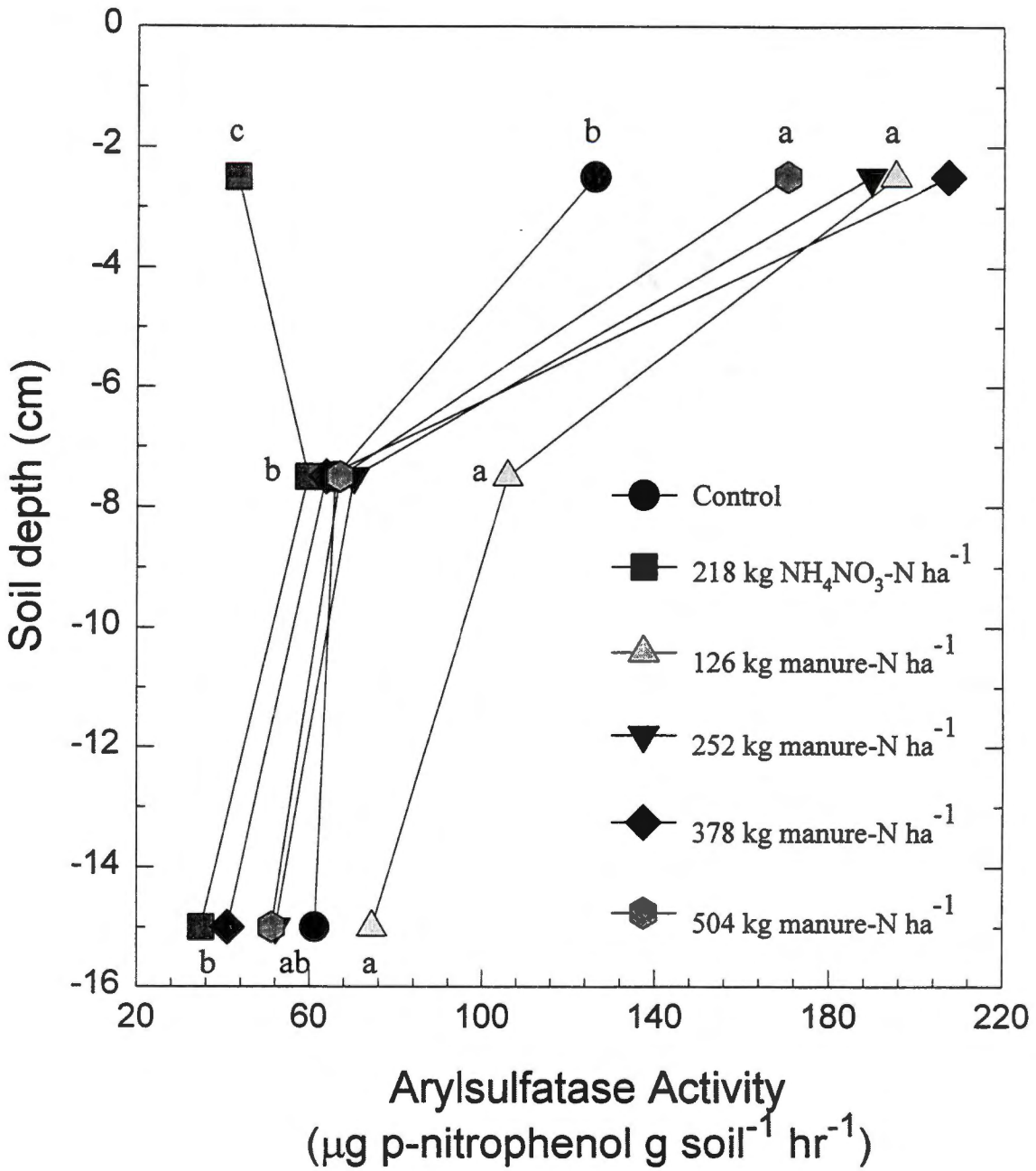

Figure 1. Effect of manure applications on arylsufatase activity in the top 20 cm of soil on June 13, 1994. Means labeled with the same letter at the same depth are not different by LSD at $\alpha = 0.05$.

for arylsulfatase activity in these soils. There were some differences among treatments at each depth, where the 0.5X treatment tended to have higher activities than the other treatments. The trend at each depth was for the AN treatment to have lower overall activities. This may be related to pH effects; pH for this treatment was significantly lower than in the other treatments (data not shown). The data for May 25 and August 5 were similar (data not shown).

Arylsulfatase activities in the 0 to 5 cm depth are shown in Table 11. For May and June sampling dates, the activities were highest in the manure treated soils. The activity in the AN treatments were always significantly lower than in any of the manure treatments. This is most likely due to the lower soil pH in the inorganic nitrogen treated soil on these dates (Table 4). Dick et al. (1988) and Kirchner et al. (1993) found that as soil pH decreases, arylsulfatase activity also decreases. Arylsulfatase has a pH optimum of approximately 6.5 in soil, and as the pH decreases below 6.0, one would expect a decrease in activity. Moreover, Dick et al. (1988) observed results like this and concluded that the negative correlation between arylsulfatase activity and inorganic fertilizers was due to the fact that this enzyme does not play a direct part in nitrogen cycling. On the first three dates, the activities in the control soil were significantly lower than at least one of the manure treatments, and were generally numerically lower than the manure treated soils. Again, the effect of increased organic C in the manured soils resulted in the presence of more organic substrate for enzyme activity. On August 5, the activity in the 2X treatment was greater than all other treatments except for the 0.5X treatment. There were no differences in pH between treatments on this date (Table 4).

In summary, for arylsulfatase, activities were highest in the 0-5 cm depth, and activities were generally highest in soils receiving manure. Decreased soil pH in the AN treatment depressed activity on the first three dates. Organic C contents in the manured

Table 11. Effects of manure on arylsulfatase activity in the 0 - 5 cm depth.

Treatments	MAY 4 1994	MAY 25 1994	JUNE 13 1994	AUGUST 5 1994
	ug p-nitrophenol g soil^{-1} hr^{-1}			
ON	55.60abc*	112.95bc	126.16b	99.05b
AN	34.98c	41.65c	43.07c	99.51b
0.5X	101.20ab	158.11ab	195.09a	156.86ab
1X	89.61abc	187.41ab	189.52a	128.26b
1.5X	39.38bc	207.02a	207.22a	95.40b
2X	101.57a	149.86ab	170.46a	276.73a

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

soils are generally greater than in the control soil, explaining the differences between those treatments.

Acid Phosphatase

Acid phosphatase activities were highest in the 0 to 5 cm. Depth effect for sampling date one (5/4/94) was significantly different for all depths; and there were high levels of activity within the depths (Fig. 2). Depth one showed treatment 1.5X to be significantly higher in activity than the control (Table 12). These findings are confirmed by Kirchner et al. (1993) who found most of the acid phosphatase activity in the 0 to 7.5 cm zone. The high activities seen in this study are related to pH differences for this depth. Dick and Tabatabai (1992) concluded that defining the acid phosphatase /alkaline phosphatase would give the adequate level of pH for crop growth. Such a ratio would be dependent on the needed pH of a particular crop.

There was no significant activity for the overall treatment effect of sampling dates one (5/4/94), two (5/2/94), three (6/13/94), and four (8/5/94). With this being the case the discussion will be on the differences found in depths for the different dates. Most of the activity for the overall depth effect on sampling date three (6/13/94) was found in depth one as depth two and three were not significantly different (Fig 2). Although, when looking at the statistical mean result for the treatment effects within depths, most of the activity was in the second depth.

Over the depths for sampling date two (5/25/94) the activity increased and depth one was significantly greater than depths two and three in phosphatase activity. There were no significant differences in depths two and three; therefore, depth one will be the only depth discussed. In depth one (0 to 5 cm) of sampling date two (5/25/94) there was

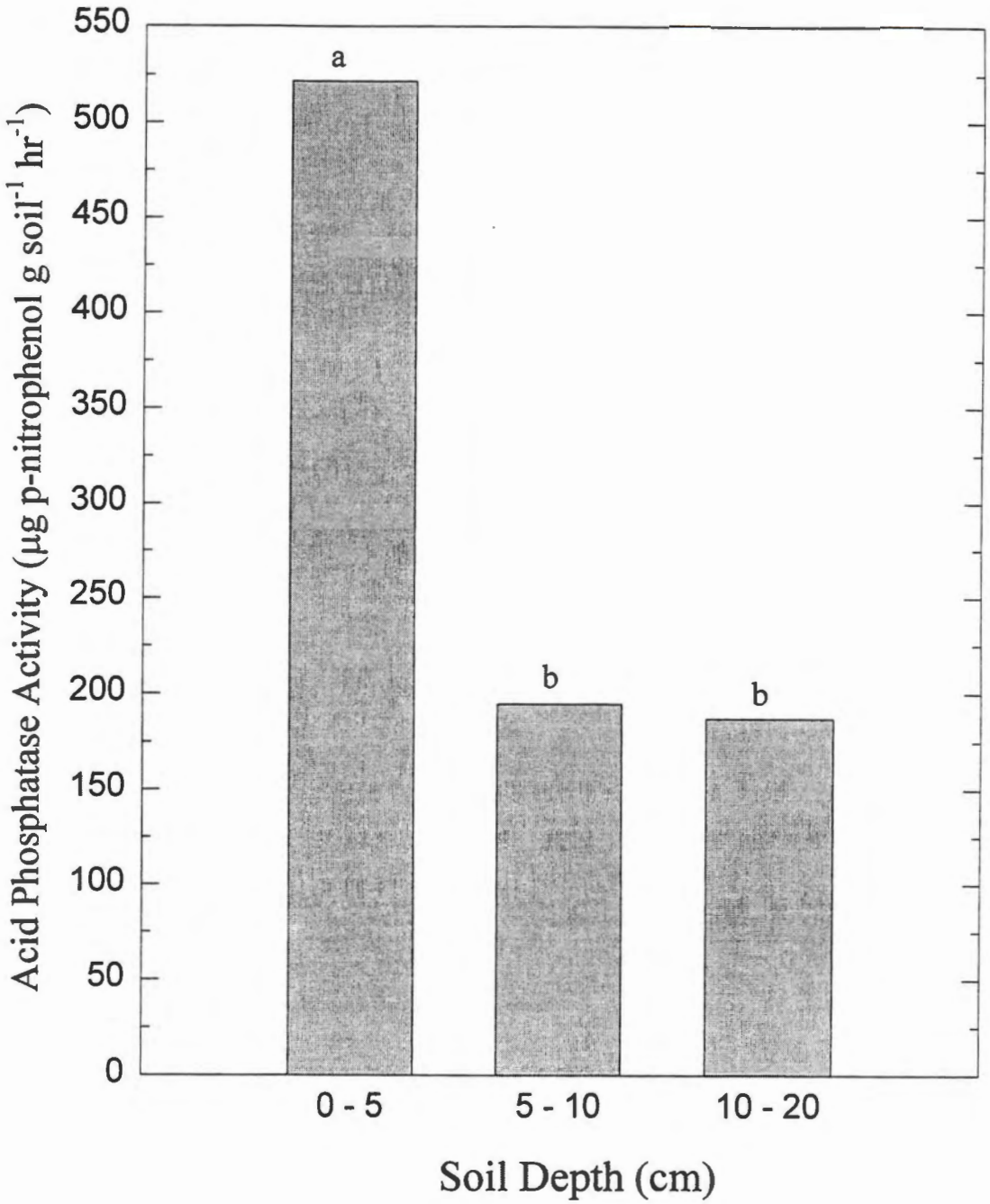

Figure 2. Effect of manure applications on acid phosphatase activity for all depths of soil on June 13, 1994. Means labeled with the same letter are not different by LSD at $\alpha = 0.05$.

Table 12. Effects of manure on acid phosphatase activity in the 0 - 5 cm depth.

Treatments	MAY 4 1994	MAY 25 1994	JUNE 13 1994	AUGUST 5 1994
	----------ug p-nitrophenol g soil^{-1} hr^{-1}----------			
ON	195.72b*	516.74a	457.21a	492.15a
AN	301.58ab	319.25b	443.81a	576.62a
0.5X	297.02ab	504.93ab	563.46a	488.37a
1X	300.96ab	596.61a	585.99a	512.76a
1.5X	398.46a	564.84a	583.63a	482.39a
2X	361.64ab	571.89a	497.27a	513.55a

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

a significant difference between the highest manure treatments and the inorganic (Table 12). The inorganic treatment was lower than the manure treatments probably because of a pH effect and/or due to the fact that the organic carbon contents in the manure treatments were higher. Dick et al. (1988) concluded that the dominant effect of inorganic nitrogen on phosphatase activity may be due to the pH changes associated with inorganic nitrogen rather than direct nitrogen effects on phosphatase activity. There seems to be some relationship occurring with the pH and organic C in the current study.

Alkaline Phosphatase activity

Initially, alkaline phosphatase activity was low. There were no significant differences found for 5/4/94. Moreover, the only trend seen to report are those found across treatments. There was a clear increase in activity around the end of May, the decline was seen in June and August. These results are similar to those of Kirchner et al. (1993) where they saw a peak in June and a decrease in August. There was more activity found in the manure treatments, over all dates (Table 13). There was also a steady decline in the control over time. As with all enzymes the most activity was found in the 0 to 5 cm depth. The May 25 overall means were typical for all dates: 138.76, 63.75, and 62.50 μg p-nitrophenol g $soil^{-1}$ hr^{-1} , for depths one, two, and three, respectively. Figure 3 illustrates the effect of treatment with depth on June 13 date for alkaline phosphatase activity in these soils. The figure shows that less activity was found in the inorganic treatment when compared to the manure treatment. Depth one also shows a separation where the control and the inorganic treatment were together and all the manure treatments were increasing in activity. Such a separation was expected due to the larger amount of substrate that was put out as well as the higher pH's of the manure treatments.

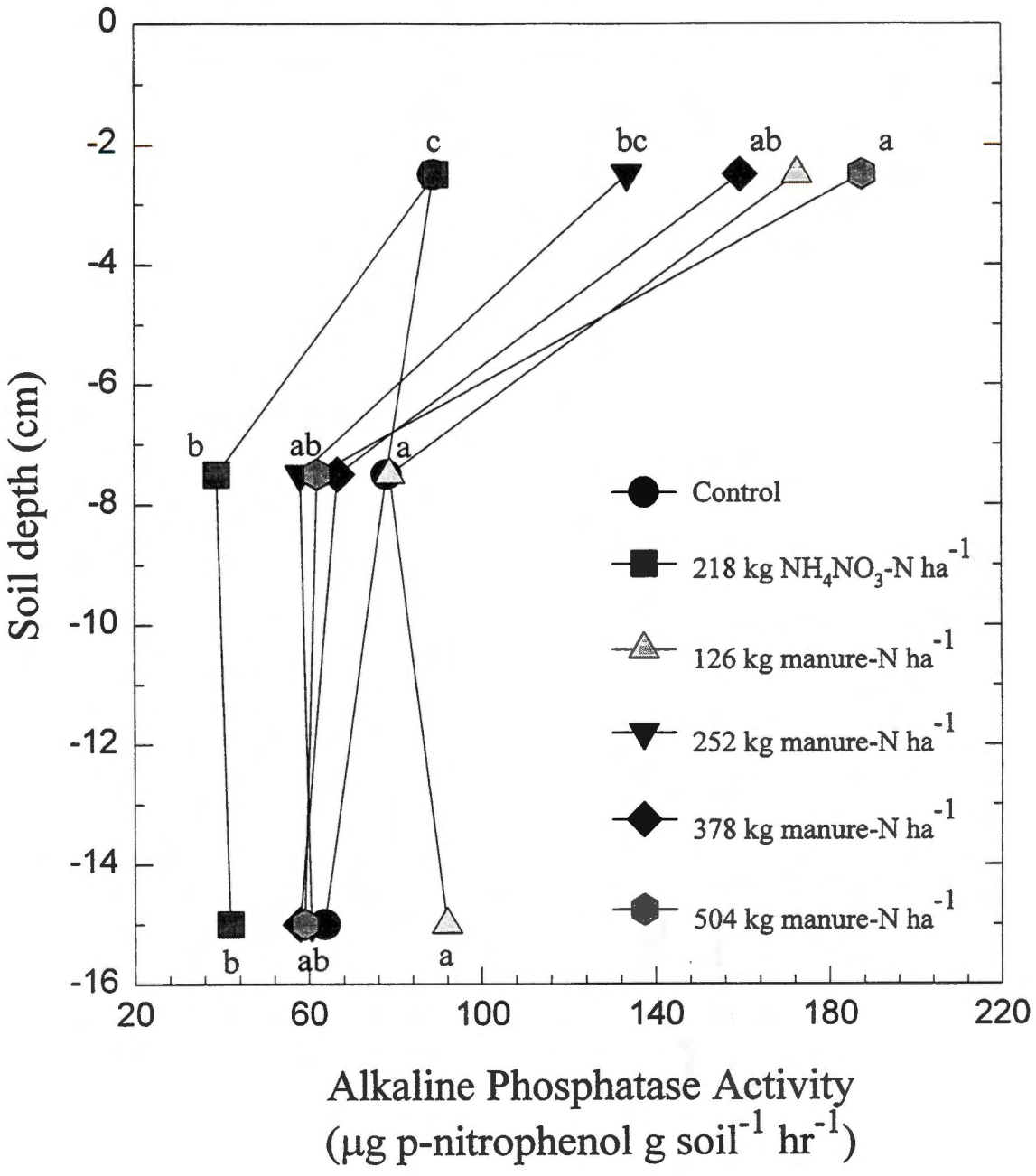

Figure 3. Effect of manure applications on alkaline phosphatase activity in the top 20 cm of soil on June 13, 1994. Means labeled with the same letter at the same depth are not different by LSD at $\alpha = 0.05$.

Table 13. Effects of manure on alkaline phosphatase activity in the 0 - 5 cm depth.

Treatments	MAY 4 1994	MAY 25 1994	JUNE 13 1994	AUGUST 5 1994
	----------ug p-nitrophenol g soil^{-1} hr^{-1}----------			
ON	94.10a*	101.56c	89.01c	81.99a
AN	41.87a	107.60c	89.52c	140.24a
0.5X	83.90a	137.68bc	172.86ab	167.51a
1X	112.87a	132.49c	133.70bc	144.79a
1.5X	86.01a	283.56a	159.77ab	134.29a
2X	100.97a	231.41ab	187.67a	161.51a

** Means in a column followed by the same letter are not signficantly different as determined by LSD at alpha=0.05.*

The results of the May 4 date showed that there were no significant differences among treatments (Table 13). For sampling date two (5/25/94), on the other hand, results showed an increase in activity. Treatment 1.5X was significantly greater than treatments AN, 1X, and ON. This was a trend seen in many of the other enzyme activity results as well. Maybe this is due to a slower release of carbon in this treatment.

Following the usual depth effect trend, sampling dates two (5/25/94), three (6/13/94), and four (8/5/94) showed depth one to be significantly greater in alkaline phosphatase activity than depths two or three (Fig 3). Depth one showed a greater amount of alkaline phosphatase activity than depths two or three, by as much as two times their amounts.

The increased phosphatase activity in the summer month may be attributed to high temperatures and bacterial numbers (Chhonkar and Tarafdar, 1984). This seemed to have occurred for sampling dates two (5/25/94) and three (6/13/94). Sampling date three showed treatment 0.5X to be significantly greater than the AN treatment (Table 13). These results are consistent with other works (Dick et al., 1988 and Kirchner et al., 1993). There was no difference found between the control and ammonium treatments. This is probably due to the very acidic pH of the inorganic treatment or the lack of effects here may be due to lower organic carbon content of the control soil. Total nitrogen appeared to have had an effect on the alkaline phosphatase activity. The lower manure rates may have slowed the activity of this enzyme. This findings were confirmed by the findings of Dick et al., (1988).

Beta-glucosidase activity

Activity was generally greater in the manure treatments than in the inorganic treatments or the control (Table 14). Over time the activity decreased. This occurrence is consistent with the results seen for the organic carbon data (Table 2). The inorganic treatment, on the other hand, had little activity in the May 4 date, increased on May 25 and June 13 dates, and begun to decline on Aug. 5. The overall depth distribution was similar to those of to those of other enzymes seen previously. The means on the May 4 date were 204.44, 45.30 and 33.21 μg p-nitrophenol g $soil^{-1}$ hr^{-1} , for depths one, two and three, respectively. Figure 4 shows the distribution of activity among treatments. The clearest distribution can be seen on the May 25 date. The 2X treatment had the most activity over any other treatment; and the amount of activity decline as the amount of substrate decreased. With the exception of the control on the May 4 date , the activity levels were low in the beginning, increased on May 25 date and declined on the June 13 and August 5 dates. This is similar to what Kirchner et al. (1993) reported in their study.

Clear significance was shown in the depth effect as well. The activity in depth one was almost four times as much as that of depths two or three. In depth one the two highest rates of manure were significantly greater than the control or the inorganic treatment (Fig 4).

On sampling date one (5/4/94) β-glucosidase showed no significant differences in treatments. Depth one had significantly higher activities than depth two.

Depth one consistently had more activity than depth two or three. For sampling date four (8/5/94) depth one had at least twice the amount of activity depth two or three had. Depth one (0 to 5 cm) resulted in treatment 2X being significantly greater than

Table 14. Effects of manure on beta-glucosidase activity in the 0 - 5 cm depth.

Treatments	MAY 4 1994	MAY 25 1994	JUNE 13 1994	AUGUST 5 1994
	----------ug p-nitrophenol g soil^{-1} hr^{-1}----------			
ON	183.79a*	151.73c	114.73c	99.05b
AN	71.17a	151.14c	127.52bc	99.51b
0.5X	79.66a	176.63bc	208.87a	156.86ab
1X	152.38a	227.15ab	182.47ab	128.26b
1.5X	92.64a	254.29a	224.64a	95.40b
2X	135.29a	265.71a	179.08ab	276.73a

** Means in a column followed by the same letter are not signficantly different as determined by LSD at alpha=0.05.*

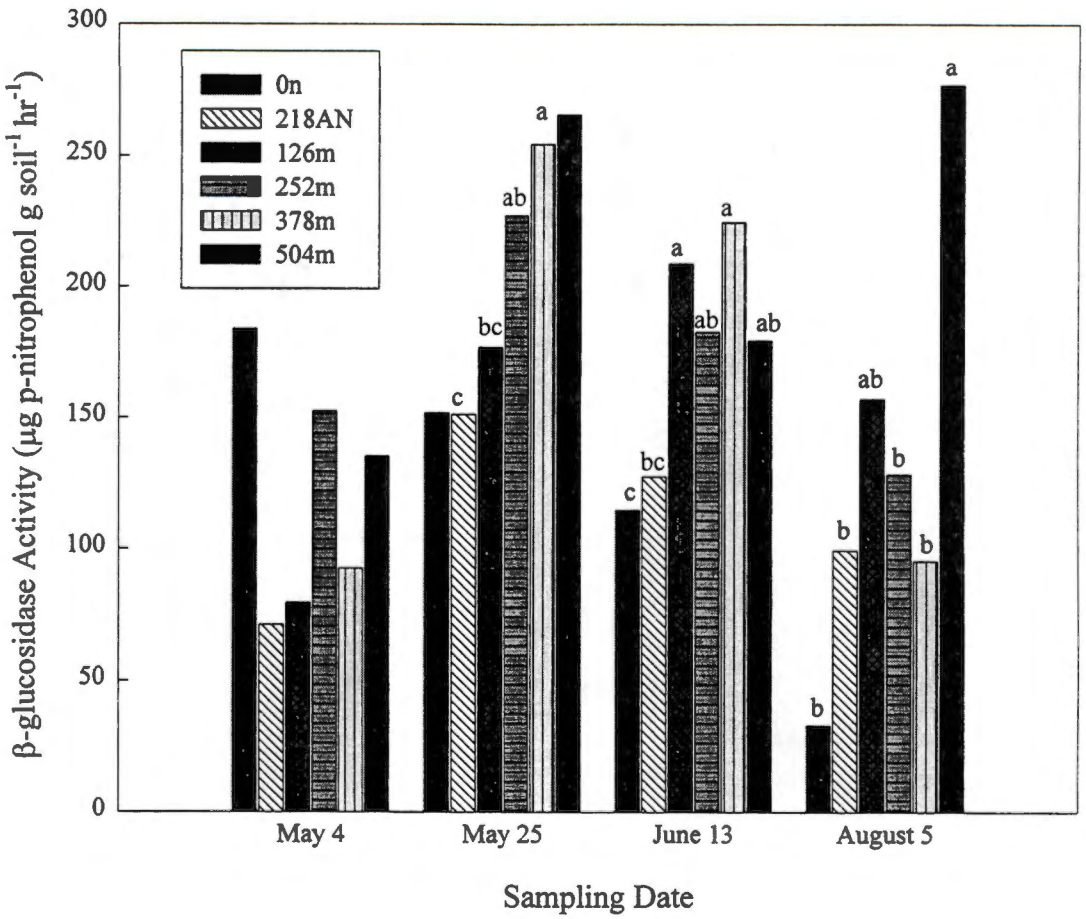

Figure 4. β-glucosidase activities in manure-amended and control soils over four sampling dates in 1994. There were no significant differences on May 4. Bars within a date labelled with the same letter are not significantly different as determined by LSD at the 5% level.

treatments 1X, AN, ON, and 1.5X (Table 14). These results are likely due to the carbon content in the soil which was high.

Sampling date two (5/25/94) showed clear differences in overall treatment effects. Treatment 1.5X was significantly greater than AN (Table 14). Also the two highest manure treatments were significantly different from the inorganic treatment. Interestingly, there seemed to be a parallel in results between treatment effects of β-glucosidase activity and total nitrogen. Fauci and Dick (1994) concluded that β-glucosidase was affected by inorganic N. After 164 days they saw β-glucosidase activity was nearly proportional to the cumulative amount of inorganic N added.

For sampling date three (6/13/94) beta-glucosidase activity seemed to be affected by organic carbon content. From the previous discussion on carbon and nitrogen, it is understandable why treatment 1.5X was significantly different from the control. The activity was highest in treatment 1.5X. Contrary to other studies, β-glucosidase activity in the current study does not seem to be affected by nitrogen. The results for β-glucosidase in a study done by Dick et al. (1988) came out similarly and they concluded that the low activity of β-glucosidase was due to the fact that this enzyme is not directly involved with nitrogen cycling.

On sampling date three (6/13/94), all depths were significantly different from the other. In depth one, treatment 1.5X was significantly higher than the control, but this time treatment 0.5X was higher than the control as well (Table 14). This is clearly due to more nutrients becoming available through the amendments.

On sampling date four, the results showed treatment 2X to be significantly greater in term of activity than treatments 1.5X, AN, and ON. The treatment effect appeared to be affected by the organic carbon content (Table 2). The statistical results show a parallel between the carbon result for this date and the results of this enzyme. The carbon

amounts for this sampling date were relatively low with a mean for treatment 1.5X of 0.92%. The reason for the activity results is probably due to lower carbon contents in the control, inorganic fertilizer and the second highest manure treatment.

Earlier in this discussion the relationship between carbon content and β-glucosidase was made. The carbon contents across dates for depth one are shown on table 2. Using this information in conjunction with the results found here, it is apparent why the 2X treatment for this sampling date had a significantly greater β-glucosidase activity.

L-Asparaginase activity

Higher L-asparaginase activities were seen in treatment 2X, which was significantly different from the control and AN (Table 15). This is logical due to the fact that there is more organic N in the highest manure treatment (2X). Also, the activity of this enzyme was significantly lower in the ammonium treatment. Continued application of manure results in the application and accumulation of organic nitrogen in the soil which may result in increases in L-asparaginase and other amidase enzymes involved in hydrolyzing amide groups from organic molecules. For example, manure treated soils exhibited significantly higher amidase activities than inorganic nitrogen fertilizer treatments in a work done by Dick et al. (1988). All depths were significantly different. The May 25 date shows the typical overall means which were 61.89, 17.38 and 11.36 μg p-nitrophenol g $soil^{-1}$ hr^{-1} , for depth s one, two and three, respectively. Figure 5 illustrates the effect of treatments with depths activities. From this figure one can see that in depth one the pH had a profound affect on the activity. The inorganic treatment had

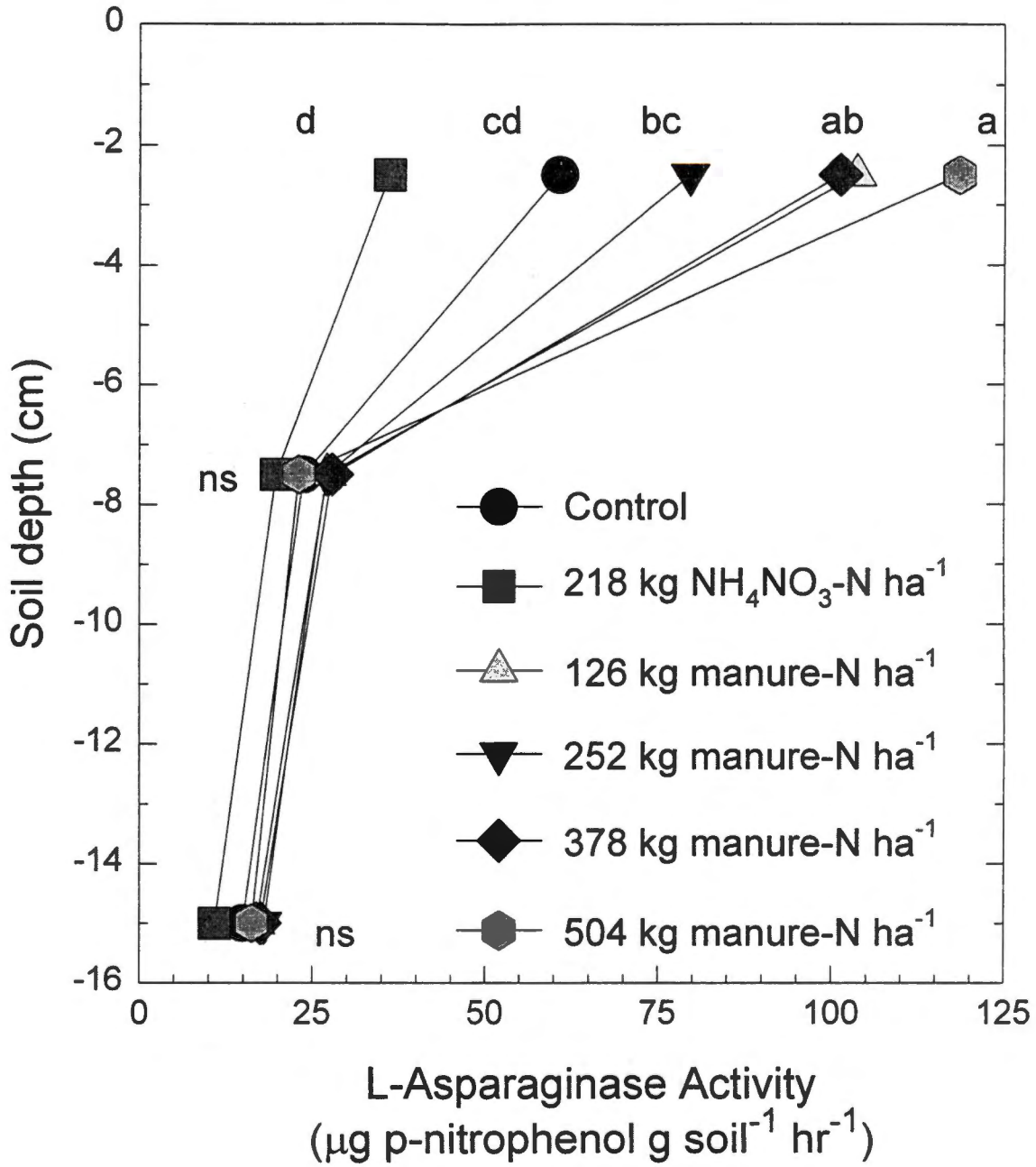

Figure 5. Effect of manure applications on L-asparaginase activity in the top 20 cm of soil. May 4, 1994. Means labeled with the same letter at the same depth are not different by LSD at $\alpha = 0.05$.

Table 15. Effects of manure on L-asparaginase activity in the 0 - 5 cm depth.

Treatments	MAY 4 1994	MAY 25 1994	JUNE 13 1994	AUGUST 5 1994
	----------ug p-nitrophenol g soil^{-1} hr^{-1}----------			
ON	60.88cd*	38.15c	32.24bc	46.92a
AN	36.18d	8.23 d	16.07c	48.80a
0.5X	103.76ab	53.29bc	56.11ab	52.50a
1X	79.71bc	65.89b	64.96ab	56.42a
1.5X	101.37ab	100.21a	86.36a	41.68a
2X	118.49a	105.59a	90.39a	71.14a

** Means in a column followed by the same letter are not signficantly different as determined by LSD at alpha=0.05.*

the least activity, with control having a little more than it. Recalling the results found for pH, one would see that the inorganic treatment had the lowest pH with the control following it. Also in this figure one can see that in depths two and three, there were no significant differences found among treatments.

Depth one (0 to 5 cm) of sampling date one (5/4/94) showed that treatment 2X was significantly higher than the control and the inorganic treatment (Table 15) (Fig 5). This further supports the theory that the larger amounts of substrate being available I ncrease the microbial activity. Treatment 2X and 1.5 X of sampling date two (5/25/94) were significantly higher than the control and the inorganic treatment (Table 15). These results were confirmed by the previous discussion on total nitrogen. Depth one continued to exhibit greater L-asparginase activity in treatment 2X and 1.5X than ON and the inorganic treatment.

Treatment 2X and 1.5 X, and 0.5X were significantly higher than the inorganic treatment for sampling date three (6/13/94), as expected. This activity in this depth shows that there is sufficient amount of carbon and nitrogen. These rates were expected to be higher because of the larger amounts of manure that made substrates available in the soils. For sampling date four (8/5/94), the only thing that was significant was the depth effect.

L-asparaginase showed a clear depth distribution, where depth one had the most activity. This was illustrated in the figure. The trend seen here was for the manure treatments to have more activity than the control or the inorganic treatment, as expected. The inorganic treatment seemed to have a slow start with increasing activity. This was probably due to the form of substrate preferred by the microbes.

PART FOUR: SUMMARY AND CONCLUSIONS

Data from this two year study showed that high rates of manure do have many beneficial properties which supports the findings of other scientists. Generally, as manure rates increased, the concentration of organic carbon and total nitrogen in the soil increased. The pH values for the manure treatments were significantly higher than that of the ammonium nitrate treated soil on May 4. This pH increase continued until around the month of June, and thereafter declined. Also, over all dates the manure treated soils had significantly higher amounts of exchangeable cations, bacterial and fungal counts, as well as biomass nitrogen. This led to increased microbial activities in the manure plots as compared to the inorganic treatment and control.

The highest enzyme activities were seen mostly in the surface layer. Acid phosphatase activities were not greatly affected by treatment. On May 4, the control soil exhibited significantly lower activities than the two highest manure treatments. The alkaline phosphatase showed a lot of activity, particularly on the May 25 date. The two highest manure treatments had LOG CFU's in the 200 to 300 range. Arylsulfatase showed an interesting parallel in results with the carbon amounts. It was also negatively affected by pH as expected. β-glucosidase activity was greatest late in the year on sampling date four, (8/5/94) where treatment 2X had the highest amount of activity and the AN treatment next. β-glucosidase and L-asparaginase activities were low overall but the highest numbers were in the manure treatments. This study showed L-asparaginase activities to be significantly affected by manure applications. Continued application of manure resulted in increased L-asparaginase and other amidase enzymes involved in hydrolyzing amide groups from organic molecules. Organic carbon was also generally higher in these treatments and may explain the increased activities in manure treatments. The most successful part of the study was the enzyme assays. The results corroborated

those found in the literature including that manure applications provide the necessary nutrients to produce increased microbial activity.

Although this study using manure application resulted in inconsistent changes in some analyses, there are still many answers that were provided though this thesis. Before this experiment there was very little known on the biochemical impacts of manure applications, but this information can now be used as a solid foundation for further studies in the area.

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APPENDIX

Appendix A1

Table A1. Effect of manure on concentrations of mehlich I extractable elements at each sampling date and in the 0 - 5 cm depth.

MAY 4 1994											
	Aluminum	Boron	Calcium	Copper	Iron	Potassium	Magnesium	Manganese	Sodium	Phosphorus	Zinc
0N	327.2ab*	0.99a	1508.3a	3.79a	114.4b	49.42a	148.9ab	94.18a	60.69a	19.61bc	1.99 b
AN	293.9b	0.99a	1526.0a	0.93a	87.9b	70.57a	125.5 b	86.36a	59.47a	14.13c	2.83ab
0.5X	377.6a	1.01a	1395.3a	1.42ab	186.6a	68.58a	148.9ab	120.8a	62.34a	33.25a	3.71ab
1X	322.3ab	1.47a	1543.1a	2.43ab	120.1ab	52.46a	159.8ab	98.42a	72.90a	26.36ab	2.75ab
1.5X	360.5a	0.93a	1453.5a	1.05b	133.8ab	52.49a	136.3ab	103.5a	65.40a	21.65bc	4.29a
2X	368.4a	1.31a	1466.5a	1.21b	133.3ab	61.97a	191.0a	94.26a	59.17a	24.42ab	3.59ab
MAY 25 1994											
	Aluminum	Boron	Calcium	Copper	Iron	Potassium	Magnesium	Manganese	Sodium	Phosphorus	Zinc
0N	382.9a	0.85a	1500.1a	1.84a	173.5ab	66.07a	162.9a	126.7ab	69.59ab	32.86ab	3.46a
AN	352.8a	1.48a	1424.2a	1.26a	122.5b	69.02a	139.9a	108.6b	69.37ab	22.58b	3.59a
0.5X	407.5a	1.07a	1418.7a	1.57a	218.1a	64.65a	141.4a	157.8a	78.57a	40.93a	3.11a
1X	382.3a	0.94a	1575.8a	1.37a	152.7b	65.61a	154.9a	129.2ab	68.35ab	32.714ab	4.11a
1.5X	370.9a	0.79a	1370.1a	1.18a	135.0b	67.67a	139.4a	134.7ab	68.39ab	31.18ab	3.61a
2X	352.8a	1.10a	1677.7a	1.89a	123.9b	71.78a	183.4a	114.6b	62.11b	31.27ab	5.41a

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

Table A1. continued

JUNE 13 1994											
	Aluminum	Boron	Calcium	Copper	Iron	Potassium	Magnesium	Manganese	Sodium	Phosphorus	Zinc
0N	266.5c*	0.69a	1448.0a	1.15a	98.72a	49.74c	142.9ab	84.29b	60.98ab	66.64a	1.51a
AN	276.0bc	0.61a	1388.7a	1.49a	91.42a	47.55c	129.3b	93.65ab	57.35 b	14.08a	1.40a
0.5X	353.4a	0.66a	1298.9a	1.35a	125.00a	54.09bc	138.0ab	125.98a	70.43ab	35.10a	1.65a
1X	326.0a	0.83a	1536.0a	1.27a	130.2a	60.24bc	149.2ab	103.65ab	71.61ab	30.96a	1.25a
1.5X	330.1ab	0.86a	1380.7a	1.30a	122.4a	71.32ab	126.5b	129.71a	64.71ab	19.30a	3.95a
2X	335.4ab	0.72a	1455.3a	1.55a	119.5a	83.11a	197.6a	99.83ab	75.03a	25.98a	2.10a
AUGUST 5 1994											
	Aluminum	Boron	Calcium	Copper	Iron	Potassium	Magnesium	Manganese	Sodium	Phosphorus	Zinc
0N	364.9a	0.80ab	1619.1a	1.65a	151.02a	59.85a	166.04ab	95.82a	65.22a	24.50a	2.55a
AN	381.9a	0.89ab	1538.7abc	1.15a	160.76a	67.57a	144.82ab	109.28a	71.31a	23.48a	1.97a
0.5X	354.3a	0.99ab	1310.1c	1.39a	196.18a	78.47a	141.88ab	126.32a	65.48a	27.90a	2.05a
1X	353.8a	1.29a	1608.3ab	1.33a	166.48a	59.84a	158.52ab	100.06a	69.43a	27.44a	2.50a
1.5X	342.0a	0.59b	1369.1c	1.05a	151.54a	64.40a	126.12b	115.18a	61.61a	74.06a	1.75a
2X	308.2a	0.84b	1377.8bc	1.13a	106.74a	66.79a	182.86a	86.92a	66.07a	20.16a	1.52a

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

VITA

On March 5, 1970, Melissa Dyanne Mayfield was born in Philadelphia to Carolyn Hall and Herman Chisholm. Melissa attended John Bartram High School and graduated with honors. She received her Bachelors of Science in Chemistry from the Historically Black Knoxville College and, once again, graduated with honors. While at Knoxville College, Melissa married Daniel Mayfield, and together they have two beautiful children.

Melissa Mayfield enrolled as a graduate student in the Plant and Soil Science Department of the University of Tennessee. She is to graduate in August 1996 with a Masters in Soil Biochemistry. After graduation, Melissa plans to contribute significantly to her neighborhood.

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