AN ABSTRACT OF THE THESIS OF

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in Crop and Soil Science presented on 21 May 1992 .

Title: Soil Biological Indices and Nitrogen Availability During a Simulated Transition from Inorganic to Organic Sources of Nitrogen

Abstract approved: Redacted for Privacy

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Long-term cultivation has significantly decreased organic matter and biological activity in most soils. This may partially explain why producers interested in a transition from inorganic to organic nitrogen (N) sources initially experience short-term reduction in yields related to N availability. The Residue Utilization Plots (RUP) at the Columbia Basin Research Center, OR have been managed with either organic or inorganic N sources since 1931. As a result, the RUP soils vary widely in organic matter content and biological activity. These soil were used in a greenhouse study conducted to determine the short-term effects of recent organic residue and decreasing inorganic N amendments on plant dry matter yield (DMY) and N uptake, soil microbial biomass N and carbon (MB_N and MB_C, respectively), and soil enzyme activities (protease, histidase, and β-glucosidase) during a simulated transition. Four successive crops of *Zea mays* L. were grown. Treatments were arranged as a complete factorial and included the following factors: four RUP soils (beef manure, pea vine residue, 0 kg N or 90 kg N ha^{-1}, each applied biennially to a wheat-fallow system); four greenhouse organic residues (pea vine, beef manure, poultry manure, or control); and four rates of N fertilizer (0-1600 mg N 2 kg^{-1} soil as NH_4NO_3).

In the absence of organic residue or N fertilizer, DMY and N uptake were greater in soil from the manure RUP than soil from the other field plots. Nitrogen uptake in the beef manure and control residue treatments was the same for each N rate and was directly proportional to the amount of inorganic N applied. Poultry manure and pea vine amendments both increased plant N uptake. Poultry manure was mineralized more quickly than pea vine, however pea vine provide N for plant uptake over a longer period of time than did the poultry manure.

The MB_C and MB_N in the soil from the manure RUP treatment was higher than in soil from the other RUP treatments both with or without greenhouse inorganic or organic N amendments. After the fourth crop, soil amended in the greenhouse with pea vine, beef manure, or poultry manure had 400, 210, and 80% greater MB_C and 280, 140, and 50% greater MB_N, respectively, than the unamended soil (averaged across field history and N treatment), and at the high inorganic N rate, MB_C was smaller and MB_N was larger than in soil from other N treatments (averaged across field history and organic residue treatment).

Soil that received long-term organic inputs had higher enzyme activity than soil from the 0 kg N or 90 kg N plots within each greenhouse organic residue treatment. Each greenhouse organic amendment increased activity relative to the control. Pea vine, added in the greenhouse, produced the greatest increase in activity. Nitrogen fertilizer treatment had little or no significant effect in enzyme activity.

Enzyme activity response to soil additions was similar to that observed for microbial biomass. Only in the absence of inorganic N and in the control residue treatment, were differences in plant N uptake resulting from long-term soil management reflected in the biological parameters measured. When an organic residue was added in the greenhouse, biological and plant parameter responses were not the same. Poultry manure residue treatment provided the most plant available N, but did not increase soil biological activity as much as pea vine residue. Beef manure added in the greenhouse increased biological activity measurements without

increasing N uptake or DMY. In the short-term, pea vine residue was the best organic N source studied for increasing soil biological activity while maintaining plant productivity.

Soil Biological Indices and Nitrogen Availability During a Simulated Transition from Inorganic to Organic Sources of Nitrogen

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed 21 May 1992

Commencement June 1993

APPROVED:

Redacted for Privacy

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Date thesis is presented 21 May 1992

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TABLE OF CONTENTS

TABLE OF CONTENTS, continued

LIST OF FIGURES

LIST OF FIGURES, continued

LIST OF TABLES

LIST OF TABLES, continued

Soil Biological Indices and Nitrogen Availability During a Simulated Transition from Inorganic to Organic Sources of Nitrogen

INTRODUCTION

Long-term cultivation has significantly decreased organic matter and biological activity in most soils (Rasmussen et al, 1989; Bolton et al., 1985; McGill et al., 1986; Stevenson, 1982). This may partially explain why producers interested in a transition from inorganic to organic N sources initially experience short-term reduction in yields related to N availability. Carbon (C) in organic material is a source of energy for the microbial population that mediates the breakdown and release of nutrients contained within the organic material itself. Since microbial biomass increases when C is added to the soil (Frankenburger and Dick, 1983; Nannipieri et al., 1979; Powlson et al., 1987), it is believed that plant N deficiencies are caused, in part, from N immobilization while microbial populations increase (Janzen and Radder, 1989, Doran et al., 1985).

When use of synthetic N fertilizers is reduced or eliminated, N availability is dependent on the soil's biological system. Therefore, the re-establishment of an active microbial community is important during a transition (Culik, 1983). Many studies have shown that soil managed with organic N sources have greater microbial populations and enzyme activity than soils managed with mineral fertilizers (Bolton et al., 1985; Martynuik and Wagner, 1978; Anwarzay et al., 1990; Alef et al., 1988; Dick et al., 1988; McGill et al., 1986), but few studies have monitored microbial biomass and enzyme activity dynamics during the transition from mineral to organic sources of N.

The Residue Utilization Plots (RUP) at the Columbia Basin Research Center, OR have been managed with either organic or inorganic N sources since 1931. As a result, soils from the RUP vary widely in organic matter content and

biological activity (Rasmussen et al., 1980, 1989; Dick et al., 1988). Various combinations of organic amendments and decreasing inorganic N rates were added to these soils in the greenhouse, and four consecutive crops of maize (*Zea mays* L.) were grown to simulate a transition from inorganic to organic sources of N. Treatments were arranged as a complete factorial and included the following factors: four RUP soils (beef manure, pea vine residue, 0 kg N, or 90 kg N ha^{-1}, each applied biennially to a wheat-fallow system); four greenhouse organic residues (pea vine, beef manure, poultry manure, or control); and four rates of N fertilizer (0-1600 mg N 2 kg^{-1} soil as NH_4NO_3). Plant dry matter yield and N uptake, soil microbial biomass, and soil enzyme activities were measured and will be discussed in Chapters 2, 3, and 4, respectively.

CHAPTER 1

LITERATURE REVIEW

Prior to the 1940's farmers recognized that crop yields were dependent on the ability of the soil to supply nitrogen (N). Sound agronomic practices such as crop rotations, use of green manure crops, animal manure application, and return of crop residues to the soil were used to maintain soil fertility and disrupt the life cycle of crop pests. Since N fertilizers became readily available in the 1950's farmers have become increasingly dependent on manufactured N fertilizer.

Manufacturing of N fertilizer is the most energy intensive component of production agriculture today (Keeney, 1982), and as nonrenewable fossil fuels become scarce, the cost of manufactured N fertilizers will rise. The relatively large pool of inorganic N in the soil, that results from inorganic N fertilizer application, is both readily available to plants and vulnerable to losses. Nitrogen in surface water reduces water quality, and nitrates that leach into the groundwater pollute drinking water. Organic sources of N can also cause pollution when applied in excess of crop needs. An estimated 82% of the nonpoint source N pollution comes from agriculture in the U.S.A. (Keeney, 1982).

The development and adoption of economical and efficient N management strategies that conserve natural resources while minimizing adverse environmental impacts is a major challenge to agriculturists today (Hargrove, 1988).

NITROGEN DYNAMICS AND SOIL AMENDMENTS

Nitrogen Cycle

The transformation of N from various chemical and biological pools in the soil to the atmosphere and back to the soil is termed the N cycle. Central to the soil N cycle are the mineralization and immobilization processes of heterotrophic microorganisms. Nitrogen mineralization, the transformation of organic N into the inorganic NH_4^+ form, occurs when heterotrophic microbes consume organic substrates. Nitrogen in organic substrates is assimilated by microbes and used for cell maintenance, growth, and reproduction. Nitrogen in excess of microbial requirements is released into the soil as NH_4^+. Immobilization is the transformation of inorganic N into an organic form. The term is usually reserved for the

assimilation of inorganic N (NH_4^+ and NO_3^-) into microbial tissue, plant uptake and N_2 fixation are excluded (Jansson and Persson, 1982).

Mineralization is responsible for making organically-bound nutrients available to plants, and plant residues are ultimately the substrate for soil microbes. When carbon (C) sources lack sufficient N to meet the requirements of the microbes consuming them, N from the inorganic N pool is immobilized (Jansson and Persson, 1982). Microbes, therefore, can both produce and compete for inorganic N in the soil.

Mineralization and immobilization are integrated processes. They are often thought of as concurrent processes, yet locations in the soil where mineralization occurs can be physically and or temporally separated from sites of immobilization (Drury et al., 1991). The net difference between these two processes ultimately determines the amount of N available for plants. From an ecological point of view, net mineralization (or immobilization) does not accurately reflect the rate of biological activity. A small net effect may be the result of low overall biological activity, or high activity in which the processes work in opposite directions (Jansson and Persson, 1982). The stable isotope, ^{15}N, and simple models can be used together to estimate gross rates of immobilization and mineralization (Paul and Juma, 1981; Nason and Myrold, 1991).

Native soil fertility

Soil organic matter (SOM) is a large reservoir of plant nutrients. Cycling of nutrients through organic and inorganic pools is essential in providing nutrients to growing plants and thus maintaining productivity. The main plant nutrients that occur predominantly in organic forms in surface soil are N, phosphorus (P), and sulfur (S). Nitrogen is a critical component of production agriculture because it is the nutrient required in the highest amount by grain crops (Olson and Kurtz, 1982). Since over 90% of the N in surface soils is in an organic form (Stevenson, 1982), practices that affect SOM effect N fertility.

Native soil fertility and SOM decline rapidly during the first decades of

cultivation, but eventually reach equilibrium values that are a function of the climate, soil type, cropping system, and management practices (Stevenson, 1982; Rasmussen et al., 1980; Black, 1973). The benefits of SOM in maintaining soil fertility and productivity have long been known. It is the source of energy for microbial populations that metabolize organically-bound nutrients (N, P, and S) thus making them plant available. The SOM also promotes desirable physical properties such as tilth, aggregate stability, increased water holding capacity, and improved water infiltration. It is often difficult to distinguish the benefits of SOM through enhanced fertility from those of improved physical structure.

In normal agricultural operations, N is removed from the system with the harvested product. If soil fertility is to be maintained, N must be added back to the system. Nitrogen fixation by microorganisms and additions of synthetic N fertilizer are the primary means by which N is added to the soil. Nitrogen from atmospheric precipitation and application of organic substances produced off site are additional pathways of N addition. Nitrogen increases yields and creates more residues per crop. Residues returned to the soil maintain SOM and fertility and enhance productivity. The beneficial effect of adequate N fertility is both cyclic and cumulative (Oveson, 1966; Larson, 1972; Rasmussen et al., 1980).

LONG-TERM STUDIES

Nitrogen Fertility and Soil Productivity

Additions of either inorganic or organic N tend to increase yield, total N, and organic C (or SOM) in soil when compared with soil that does not receive any additional N source (Campbell et al., 1986, 1991; Rasmussen et al., 1980, 1989; Bonde et al., 1988; Jenkinson and Johnson, 1977). When comparing between inorganic and organic sources of fertilizer differences in yield, total N, and organic C will depend on the relative amount and availability of the N in the organic source. The cumulative effect of the amount and quality of crop residue produced and returned to the soil as a result of the N source will also influence differences. Organic N sources also provide C to soil microorganisms.

Many studies have investigated the effects of quantity and quality of N and C in soil amendments on soil chemical properties. Black (1973) found organic matter, total N, and yields had increased with the quantity of wheat straw added after four wheat-fallow cycles in Montana. All residues produced were removed to eliminate the effects of adding residues of different quality. Nitrogen fertilizer did not affect changes in SOM or total soil N, but did increase grain and straw yields. After 36 years of a fallow-wheat-wheat rotation in Saskatchewan, soil N was not affected by the source of N fertilizer (monoammonium phosphate or barnyard manure), but manure increased organic C and the net rate of N mineralization (Campbell et al., 1986). Data from 30 years of a rotation study, where the wheat straw was incorporated, showed yields and soil N in fertilized rotations were greater than in similar rotations that were not fertilized (Campbell et al., 1991). Rotations that included hay, green manure crops, or reduced fallow frequency had higher yields and greater soil N levels than rotations that returned less C to the soil.

In the long term, large additions of carbonaceous residues help maintain SOM and total N and improve crop yields. In a 12 year study in Iowa, Larson et al. (1972) found a linear relationship between amount of C applied and change in SOM. The type of residue applied, cornstalks supplemented with N fertilizer or alfalfa, did not affect the change in SOM, but available NH_4^+-N did increase with the amount of residue applied. Rasmussen et al. (1980) also found that soil C was correlated with the amount of C added regardless of the type of residue applied (wheat straw, manure, or pea vine) in a wheat fallow experiment initiated in 1931 in a semi-arid region of Oregon. In this case crop residues and animal manure were compared, but only crop residues were compared in the Iowa study. In both studies, C additions of 5 Mg residue ha^{-1} or 2000 kg C ha^{-1} per year were needed to maintain zero change in SOM levels.

In another study where all residues were removed, Bonde et al. (1988) also found that soil C and N were unaffected by different types of residues added over a 50-year period. Soil C and N in plots that received 80 kg N ha^{-1} yr^{-1} as $Ca(NO_3)_2$ and 1800 kg C ha^{-1} yr^{-1} as straw were the same as in plots that received 80 kg N

and 1800 kg C ha^{-1} yr^{-1} as farmyard manure. Soil that received 80 kg N ha^{-1} yr^{-1} as $Ca(NO_3)_2$ but no C had significantly lower soil C and N levels than soil that received C inputs (wheat straw or manure). Nitrogen alone did not cause significant increases in soil C and N levels when compared with unfertilized soil.

Although some studies have shown that type of organic residue is not important (Rasmussen et al., 1980; Bonde et al., 1988), the long-term benefits from organic amendments generally depend on the degree of humification of the residue (Tate, 1987). As plants age their protein and N content decrease while the amount of hemicellulose, cellulose, and lignin increase. Young green manure crops would, therefore, be most easily degraded followed by straw and then animal manures. Animal manures are mostly cellulose or lignin fibers, although some modification of the lignin to humic substances has occurred (Stevenson, 1986). Lignin persists in the soil because it is relatively resistant to microbial decomposition and the probability of an organic substance being incorporated into humus increases with residence time in the soil. For 30 years, a sandy loam in Alabama in a corn-cotton rotation was fertilized with commercial N fertilizer, dry horse manure (11.1 Mg ha^{-1}), or a vetch green manure crop (Cope et al., 1958). Five years after the N treatments were terminated there was no residual yield advantage of the vetch over the N fertilizer treatment. The manure treatment still produced higher yields and had increased soil C and N levels by 33 and 62% of the original levels, respectively. Vetch and N fertilizer failed to maintain C and N levels, but slowed the rate of loss.

The most dramatic example of the benefits of manure comes from the Rothamsted experiments (Jenkinson and Johnson, 1977). In the Hoosfield continuous barley experiment, the soil from plots that received annual applications of manure (35 Mg ha^{-1} yr^{-1}) between 1852 and 1871 and none thereafter still contained more soil organic C and total N than the unmanured plots. Yields on the unmanured plots were similar to those produced on the previously manured plots when no inorganic N was applied, yet when inorganic N was applied the manured plots yielded more than the unmanured plots. Since the late 1800's there has been

little change in soil C and N in the plots fertilized with NPK. The levels of C and N did not differ between the NPK and unfertilized plots even though the fertilized plots produced an average of 1.8 Mg ha^{-1} more straw per crop than the unfertilized plots.

SHORT-TERM NITROGEN AVAILABILITY

A thorough understanding of the factors that affect both the amount and timing of mineral N release is needed for efficient management of organic residues. The short term availability of N from organic substances depends on the rate and extent of decomposition, therefore, microbial activity and population dynamics will influence the net result. The net difference between mineralization and immobilization depends on the energy supply (readily metabolized C source) to the microbes. Given equal C availability, a residue with a low C:N ratio will mineralize N more quickly than a residue with a high C:N ratio. There may be very little immobilization of high C:N ratio residues if the C is a poor energy source (Jansson and Persson, 1982).

Considerable attention has been devoted to predicting potential N availability from organic N sources. Methods of investigation, including lab incubation and leaching studies, short-term greenhouse and field experiments, and prediction models, have met with varying success (Stanford, 1982).

Plant Residues

In general, plant material with less than 1.5 to 1.7% N will cause immobilization during initial decomposition. This is equivalent to a C:N ratio of 25-30 for crop residues since most average 40% C by weight. Higher N concentrations usually result in net mineralization. Besides moisture, temperature, pH, oxygen, and microorganisms, mineralization rate depends on particle size and the chemical composition of the substrate, especially the lignin concentration (Parr and Papendick, 1978).

Vigil and Kissel (1991) compiled mineralization results from eight

experiments (six from the literature and two from their own work) on mineral soil to which crop residues had been added. They found that 75% of the variability in the measured amounts of net N mineralization could be explained by the C:N ratio of the crop residue added. By adding N concentration and lignin:N ratio of the crop residue, 80% of the observed variability could be explained. Müller et al. (1988) concluded lignin concentration was a better parameter than N concentration or C:N ratio for predicting the amount of N mineralized. Of 10 chemical characteristics measured, only lignin correlated with N release from different ^{15}N-labeled plant materials (N concentration ranging from 1.2 - 5.0% N and C/N ratio from 11-46) buried within mesh bags in the soil for 10 months.

^{15}N-labeled crop residues have been used in many field studies to find out how much N from the residue is taken up by the subsequent crop (Vigil et al., 1991; Ladd and Amato, 1986; Wagger et al., 1985). Although the net amount of residue N taken up by the plant indicates how much became plant available, it fails to predict the relative contributions of residue derived N and native soil N mineralized by the microbial population in the presence of a fresh substrate. Few ^{15}N studies take mineralization-immobilization reactions into account (Nason and Myrold, 1991; Jansson and Persson, 1982). Vigil et al. (1991) used a mineralization-immobilization model based on the composition of the ^{15}N-labeled sorghum residues applied to predict net N mineralization. After adjusting model parameters, the model adequately predicted net mineralization.

Animal Manures

Whereas most predictions of mineral N production from crop residues include the C:N ratio or other descriptors of the chemical composition of the residue, the amount of N mineralized from manures is usually based on the NH_4^+ concentration and some fraction of the remaining organic N (Beauchamp, 1986; Beauchamp and Paul, 1989).

Net immobilization of animal manures with very low C:N ratios has been reported (Castellanos and Pratt, 1981; Beauchamp, 1986; Sims, 1986). Poultry

manure is mineralized very rapidly (Castellanos and Pratt, 1981; Sims, 1986; Yadvinder-Singh et al., 1988) whereas dairy and cattle manure tend to cause net immobilization soon after incorporation followed by net mineralization (Castellanos and Pratt, 1981; Beauchamp, 1986; Vadvinder-Singh et al., 1988). Castellanos and Pratt (1981) found composted chicken manure had much less N than fresh chicken manure and that N was only about one half as available as the N in the fresh manure. Few studies have investigated N mineralization from both crop residues and manures.

SOIL BIOLOGICAL INDICES AND NITROGEN IN RELATION TO SOIL MANAGEMENT

Microbial Biomass

The microbial biomass (MB) is recognized as both a source and a sink for plant nutrients (N, P, and S) and an active participant in nutrient cycling. Most of the biochemical reactions in the N cycle are mediated by microbial populations. Mineralization of organic substrates or immobilization of mineral nutrients by the MB depends on the growth dynamics of microorganisms. The C and N in the MB (MB_C and MB_N, respectively) turn over rapidly and reflect changes resulting from different management practices long before changes in total soil C and N are detectable (Powlson et al., 1987). The C and N in the MB typically represent 2-3% of soil organic C and 3-5% of total N in the surface of agricultural soils (McGill et al., 1986).

Soil MB can be estimated by plate counts, direct observation, or by relating the flush of mineralization after fumigation to an original amount of biomass. The chloroform fumigation-incubation method (Jenkinson and Powlson, 1976) is the most common method of analysis. The fumigation-extraction method is gaining popularity and is probably better suited for analysis of freshly amended soils (Brookes et al., 1985; Vance et al., 1987). The MB estimate does not give any indication of the activity of the microbial population. Respiration measurements, and ATP, dehydrogenase, and other soil enzymes are among the various assays

used to estimate microbial activity.

Many research groups have found that long-term additions of organic residues increase microbial population and biomass. Plate counts of soil from field plots under long-term management systems showed low populations in untreated soil, intermediate numbers in soil treated with inorganic fertilizers, and highest population in soil that received annual manure applications (Martynuik and Wagner, 1978). In a comparison of MB in soil from adjoining farms, Bolton et al. (1985) found soil on the farm that had never received inorganic N fertilizer and relied on green manures and native soil fertility for N had a larger and more active MB than soil that had received N, P, and S at the recommended rates since 1948. After 50 years of cropping to two rotations, McGill et al. (1986) found a 5-yr rotation that included forages had 117% more MB_N than did a 2-yr, wheat-fallow rotation. Manured treatments contained twice as much MB_N as did NPKS or control plots. Soil from the Broadbalk Plots at the Rothamsted experiment station that has received manure annually since the mid-1800's has higher MBc than soil from the NPK or control treated plots (Jenkinson and Powlson, 1976).

Recent additions of organic residues also increase MB (Ocio et al., 1991b; Ocio and Brookes, 1990; Perucci, 1990). Field incorporation of 10 Mg ha^{-1} wheat straw (C:N = 48) with and without 100 kg NH_4NO_3-N ha^{-1} caused both MBc and MB_N to double within seven days after incorporation (Ocio et al., 1991a). After seven days the increase in MB_N was the same with and without inorganic N. Since all the added inorganic N was still present, the MB_N formed was probably from the N in the straw itself. After 12 months, MBc and MB_N were still 20 and 18% greater, respectively, than in the untreated soil. The amount of MB in a soil reflects past inputs over many years, but is also influenced by a single input.

It is expected that organic residue additions will increase MB since C in the residues is a source of energy for the microbes. Insam et al. (1991) present data that support the concept that C is the limiting factor for microbes in agricultural soil and that the effect of fertilization on MB is an indirect one resulting from

increased C input. Bottner et al. (1988) also concluded that MB is controlled by C inputs.

On plots initiated in 1956 in Sweden, soil that received either straw and inorganic N or farmyard manure had higher MB than soil from either unfertilized or N fertilized plots (Schnürer et al., 1985). Although the straw plus N and the farmyard manure treatments received the same amount of C and N on an annual basis, MB in the manure treated soil tended to be lower than in the straw plus N treated soil. The authors postulated that the difference resulted from differences in availability of the C and N added. The C in the manure had been digested once prior to land application and, therefore, was not as easily decomposed as the straw. Although the N fertilized plot did not get any C addition other than root biomass (since all aboveground biomass produced was removed from these plots), the MBc and MB_N in soil from this plot was greater than the unfertilized plots. In a 40-week aerobic incubation with soil from these same plots (Bonde et al., 1988), the amount of N mineralized followed the same pattern as the MB; N mineralization was highest in soil supporting a large MB. In an aerobic-leaching incubation study, Robertson et al. (1988) investigated the change in MB in relation to C and N mineralization in soil from the straw plus N plot. Decline in the MBc and MB_N during the 12-week incubation could account for 19 and 40% of the measured amounts of C and N mineralized, respectively.

Microbial biomass estimates give no indication of the activity of soil microorganisms. Soil respiration is often used to estimate microbial activity, however, it is not capable of adequately evaluating changes in activity among the various processes performed by the microbes. The amount of CO_2 produced per unit biomass will depend on the substrate and the efficiency of the organisms involved. Efficiency depends on physical access, and substrate availability, nutrient status, age of organisms, and edaphic factors such as temperature, pH, and O_2 concentration. The microbial respiration to biomass ratio is referred to as biomass specific respiration. Other names such as specific respiratory activity (Schnürer et al., 1985; Insam, 1990; Insam et al., 1991; Šantrůčková and Straškraba, 1991) and

metabolic quotient (Anderson and Domsch, 1985) are also found in the literature. High biomass specific respiration may indicate a more metabolically active biomass that uses C at a faster rate (Ocio and Brookes, 1990). Others believe it is related to soil development and decreases with ecological succession (Insam et al., 1991). In agricultural soils, high biomass specific respiration means nutrient turnover is accomplished at high C expense.

Soil Enzymes

Most of the biochemical reactions involved in soil nutrient cycling are catalyzed by enzymes. Soil microorganisms are believed to be the primary source of enzymes in the soil (Skujins, 1978), and MB has often been correlated with enzyme activity (Alef et al., 1988; Bolton et al., 1985; Dick et al., 1988; Frankenberger and Dick, 1983; Nannipieri et al., 1978, 1979; Perucci, 1990). Because microbes produce enzymes, it is not surprising that factors that affect MB also affect enzyme activity.

Soil enzymes have been grouped into the following 10 distinct categories based on their location by Burns (1982):

1. intracellular
2. in the periplasmic space of Gram negative bacteria
3. bound to the exterior of living cells
4. extracellular
5. within non-proliferating cells
6. attached to dead cells or cell debris
7. released from lysed cells
8. temporarily in an enzyme-substrate complex
9. adsorbed to clay minerals
10. associated with humic colloids

Enzymes in categories 1-3 are under biological control. Those in categories 4-10 have been coined abiontic; they are enzymes acting independently of their biological source. The enzymes in categories 9 and 10 are referred to as bound or immobilized. All enzymes are proteins and thus vulnerable to degradation by proteolytic enzymes in soil. Free enzymes in the soil solution are subject to attack, whereas those absorbed to clay minerals or associated with humic colloids

presumably would be protected. Free enzymes are generally expected to have relatively high turnover rates in soils, and in the absence of renewed synthesis, decline (Ladd, 1972). Bound enzymes may or may not retain their catalytic capacity, substrates may not diffuse to bound enzymes, or steric restrictions may limit access to active sites. Many researchers have investigated the relative activity of free and bound enzymes (Ladd, 1972; Griffith and Thomas, 1979; Ladd and Jackson, 1982), and the persistence or stability of abiontic enzymes in soil (Burton and McGill, 1989).

Soil enzyme activity is believed to be relatively stabile (Burns, 1982). Long-term additions of organic residues result in higher enzyme potential in soil, and recent additions can dramatically increase activity. Differences in enzyme activity that result from changes in management practices may indicate the direction of change before other parameters are measurable.

Protease

The large group of enzymes responsible for hydrolysis of proteins is called proteases (peptide hydrolases, EC 3.4). Over 90% of the N in the soil surface is in an organic form, but only one-third to one-half of the organic N has been adequately characterized. Approximately half of the identified organic N compounds are amino acid-N that is derived from hydrolysis of peptides and proteins (Stevenson, 1982).

Most non-nitrogen fixing organisms must decompose nitrogenous substrates into low molecular weight compounds before N is assimilated. Many soil microorganisms have been shown to produce proteases (Ladd and Jackson, 1982). Niskanen and Eklund (1986) found 68 out of 240 strains of actinomycetes and bacteria isolated from soil had protease activity. Microorganisms can produce intracellular, membrane bound (Lagutina, 1988), and true extracellular proteases (Leake and Reed, 1990). Because of their high molecular weight, proteins are presumed to be hydrolyzed by extracellular enzymes only. Extracellular enzymes are believed to be important because the endproduct is released into the soil

environment. Leake and Reed (1990) found proteins to be the sole N source for *Ericoid*, an ectomycorrhizal fungi, and because these microbes produce extracellular proteases they are believed to be directly involved in mobilization of N from organic matter. Specific proteases produced by soil microorganisms (Ladd and Jackson, 1982; Tsujibo et al., 1990) and extracted from soil (Hayano et al., 1987) have been purified and characterized.

Comparing results of soil protease studies is difficult because choice of substrates and assay procedures are often different (Ladd and Jackson, 1982). Protein substrates tested include haemoglobin, gelatine, ovalbumin, and casein. Dipeptide derivatives such as benzyloxycarbonyl-phenylalanyl leucine (ZPL) or substituted amides such as N-benzoyl-L-arginine amide (BAA) have also been used (Ladd, 1972; Ladd and Butler, 1972). Low molecular weight substrates may be hydrolyzed by both proteases and peptidases, and these may be endo- or exocellular enzymes. Soil assays with casein substrate measure potential activity of a range of proteases and peptidases, and hence these enzymes are often called casein-hydrolyzing enzymes. Throughout this thesis all references to protease activity represent activity measured with casein as the substrate unless specifically noted.

Soil protease activity correlates with organic C , total N (Niskanen and Eklund, 1986; Bonmati et al., 1991; Speir et al., 1980), and arginine ammonification (Alef et al., 1988). Alef et al. (1988) compared biological properties in agricultural soils with different long-term management histories. Soils that received organic fertilizers, no pesticides, and that included legumes in the crop rotation had higher protease activity, biomass respiration, and N mineralization rates than soils that received mineral fertilizer, pesticides, and did not include legumes in the rotation. They also found a highly significant correlation ($r > 0.95$) between protease activity and arginine ammonification. This reflects the close relationship between the hydrolysis of proteins and the ammonification of amino acids in soil.

Carbon additions stimulate an increase in protease activity. In soil incubated with glucose and sodium nitrate, Nannipieri et al. (1979) found that extractable amino acid concentrations rose after a peak in CO_2 production, and that protease

activity increased soon after the amino acids appeared. The increase in protease activity coincided with a decline in the viable bacteria population. The newly synthesized amino acids and protease activity were both short lived, protease activity declined after seven days of incubation. In soil incubated with glucose and sodium nitrate, Ladd and Paul (1973) also found protease activity coincided with death of viable bacteria and was short lived. Four to five days after the incubation was begun, activity had increased twelve-fold, but after four weeks activity was less than 20% of the maximum. During incubation of chloroform fumigated soil, protease activity increased dramatically after a 2- to 3-day lag, and activity appeared to still be increasing at the conclusion of the 10 day incubation (Amato and Ladd, 1988). Dead microorganisms are a large and readily available source of proteins, and it is presumed that the surviving population respond by producing proteolytic enzymes. In a Histosol, Tate (1984) found protease activity increased with the availability of metabolizable organic matter. Two days after addition of mature sugarcane leaves to soil (0.1 g leaves/ 10 g soil), protease activity was twice that of the control and after 15 days, activity in soil amended with sugarcane remained 65% higher than that of the control. In soil incubated with a more complex substrate, municipal solid-waste compost, protease activity increased as the microbial biomass declined (Perucci, 1990). After one month of incubation, biomass C peaked and then declined rapidly until stabilizing by the third month. Protease activity increased steadily until the third month and then began to decline, but after 12 months, activity in the sludge-amended soil was still 3.5 times greater than the control.

Histidase

L-Histidine ammonia lyase (EC 4.3.1.3) catalyses the deamination of histidine to urocanate and NH_3. Histidine is widely distributed in nature and can account for up to 8% of the amino acids found in animal, plant, and microbial proteins (Frankenburger and Johanson, 1981). It makes up approximately 20% of the basic amino acids found in acid soil hydrolysates (6 *M* HCl) (Stevenson, 1982).

The concentration of free amino acids in soil is very low and rarely exceeds 2 μg^{-1} g^{-1} soil. Since amino acids are readily decomposed by microorganisms, the amount present in the soil solution represents a balance between synthesis and destruction (Stevenson, 1982).

During the degradation of proteins histidine is released. Deamination of amino acids is an important source of NH_4^+ in soil. Many microorganisms isolated from soil produce histidase in pure culture (Lessie and Neidhart, 1967). The enzymes responsible for the deamination of amino acids in soil are intracellular, associated with proliferating microorganisms (Kiss et al., 1975), but because histidase does not require an interenzymatic redox carrier, unlike most deaminase enzymes, there is the potential for abiontic histidase activity. The contribution of abiontic deaminase activity to soil N mineralization remains unsolved (Ladd and Jackson, 1982), but Burton and McGill (1989) have shown that histidase has limited abiontic activity in soil. There are at least two enzymatic components of histidase activity: a labile component of recent biological origin and a more stable component with half lives of 3 and 77 hours, respectively. In the absence of microbial growth, histidase activity declined. The decline in activity was not attributable to either substrate limitation or end product inhibition.

Burton and McGill (1991) thoroughly examined C and N control of histidase activity in a black Chernozemic soil. Potential soil histidase activity increased 3-4 times after soil was incubated for 3 days with 100 μg g^{-1} of either histidine-C or urocanate-C. Enzyme synthesis was induced by both histidine and urocanate; the increase in activity was not an artifact of an increased microbial population. Induction of histidase is repressed by the presence of glucose-C at concentrations of 4000 μg g^{-1} soil. Glucose is a "superior" catabolite. Its presence represses synthesis of enzymes that degrade less favorable catabolites. Neither glucose nor NH_4^+ suppressed non-induced histidase activity.

Regulatory control of histidase synthesis occurs at high C concentrations that are uncommon in soil, except in microsites surrounding organic substrates. The regulatory control of histidase synthesis in an oligotrophic environment, such as

soil, is probably of minor importance because enzyme activity in soil is limited by substrate availability rather than the amount of enzyme (Tateno, 1988). Burton (1989) concluded that the majority of soil histidase is the result of constitutive synthesis.

Frankenburger and Johanson (1983) found that histidase activity was correlated with organic C and total N content in the topsoil of 20 diverse soil samples from California and activity decreased with depth and with air drying. In a black Chernozemic soil cropped with barley, Burton (1989) found histidase and protease activities did not correlate with net mineral-N production. Biomass C, which integrates enzymatic potential and substrate supply, provided a better indication of net mineral-N production.

β-Glucosidase

β-D-Glucosidase is one of the three enzymes responsible for cellulose decomposition. Many enzymes are probably involved, but endo-1,4-β-D-glucanase, exo-1,4-β-D-glucanase, and β-D-glucosidase are considered to be primarily responsible (Hope and Burns, 1987). This paper will focus on glucosidase activity. β-D-Glucosidase (EC 3.2.1.21) catalyzes the hydrolysis of terminal non-reducing β-D-glucoside residues with the release of β-D-glucose. It is widely distributed in nature (Eivazi and Tabatabai, 1988), has been isolated and purified from pure cultures of microorganisms isolated from soil (Banerjee, 1990; Bagga et al., 1990; Khandke et al., 1989), and has also been extracted and purified directly from soil (Batistic et al., 1980; Hayano and Katami, 1977).

The assay for β-glucosidase is based on colorimetric determination of the ρ-nitrophenol concentration when soil is incubated with buffered ρ-nitrophenol-β-D-glucopyranoside (PNG) solution and toluene (Eivazi and Tabatabai, 1988). The substrate, PNG, is artificial. Hayano and Katami (1977) found that β-glucosidase is more active with natural substrates than with PNG. Air drying of field moist soil has been reported to both increase (Eivazi and Tabatabai, 1990) and decrease (Hope and Burns, 1987) β-glucosidase activity. Addition of trace elements during the

assay inhibits activity, and steam sterilization for one hour completely destroys activity. Inorganic N also inhibits β-glucosidase activity when added during the assay (Eivazi and Tabatabai, 1990).

Microorganisms produce extracellular β-glucosidase and many researchers believe the enzyme becomes stabilized in humic-enzyme polymers during humus synthesis (Hope and Burns, 1987; Sarker and Burns, 1984; Sinsabaugh and Linkins, 1989). Although crude humic-enzyme complexes have been extracted from soil, subsequent purification and analysis of these fractions has not revealed the relationship between the enzyme and its polyaromatic support (Sarker and Burns, 1984). Researchers have artificially produced enzyme complexes bound to organic material or phenolic polymers to investigate the activity and stability of immobilized or bound β-glucosidase. Sarker and Burns (1984) found glucosidase-phenolic copolymers had higher K_m values and lower V_{max} values than soluble enzymes. Resistance to commercial protease increased when the copolymer was fixed to clay, but V_{max} decreased even more meaning that the bound enzyme was more stable, but less active.

Sinsabaugh and Linkins (1989) subjected artificially prepared organic matter-cellulase complexes to 10 repeated freeze-thaw or wet-dry cycles and measured total cellulase, endoglucanase, and glucosidase activity (exoglucanase is obtained by the difference between total cellulase and endoglucanase plus glucosidase activity). Both soluble and insoluble enzyme complexes were prepared. Uncomplexed enzymes retained less activity after the freeze-thaw cycles than soluble complexed enzymes. Of the insoluble complexes, only the β-glucosidase complex retained more activity than its uncomplexed control. After the wet-dry cycles β-glucosidase retained 55-105% of the original activity while the exoglucanase retained less than 60% and the endoglucanase retained less than 5%. Of these "bound" cellulases, β-glucosidase activity was the most stable. Hope and Burns (1987) concluded that β-glucosidase was bound to and protected by soil colloids. Their explanation for this conclusion was based on the effect of air drying. Ten percent of the original β-glucosidase activity was accounted for in the

soil extract. Air drying decreased activity by 10-15%. Since air drying decreased total cellulase activity by 64%, they concluded that glucanases were free in solution or associated with microbes that were not resistant to air drying.

The origin of β-glucosidase in soil has also been investigated. Hayano and Tubaki (1985) studied β-glucosidase activity in an Andisol supporting a tomato monoculture under greenhouse conditions. They found that the soil passing through a 2-mm sieve had greater than 50% of the total β-glucosidase activity. The organic debris remaining on the sieve and the greater than 2-mm soil fraction each contributed approximately 20% of the total activity. The organic debris, rice straw, contributed a significant amount on the basis of its dry weight, which was only 1.5% of the total weight. By subjecting the soil to various soil sterilants to inhibit certain groups of microflora, they deduced that mucoraceaous fungi were probably the primary source of β-glucosidase in the soil studied.

β-Glucosidase activity correlated with organic C and decreased with depth in 10 agricultural soils from Iowa (Eivazi and Tabatabai, 1990). Verstraete and Voets (1977) also demonstrated that activity increased with soil organic matter. In a five-year field study, they showed that soil that received green manure plus either farmyard manure or crop residues had higher levels of β-glucosidase, urease, phosphatase, and saccharase activities, respiration and N mineralization than soil that did not receive organic inputs. In an 80-year long-term rotation and fertilizer experiment (Anwarzay et al., 1990), organic fertilizers increased biological activity (β-glucosidase, phosphatase, protease, xylanase, urease, and cellulase activity) more than mineral fertilizers. Lowest activities were found on non-fertilized plots.

Long-term Soil Management Effects on Soil Enzyme Activity

In general, soils that receive higher inputs of organic residues have higher enzyme activity. Soil organic matter is the source of energy for heterotrophs. Therefore, additions of organic residues stimulate microbial activity (Tate, 1984; Nannipieri et al., 1979; Ladd and Paul, 1973), and would be expected to increase enzyme activity as well. A five-year rotation of grains and legumes had

significantly higher dehydrogenase, urease, catalase, phosphatase, and invertase activity than a wheat-fallow rotation after 40 years (Khan, 1970). In the same study, N increased enzyme activity, but soil treated with manure had higher activity than soil fertilized with inorganic N. In the Palouse region of eastern Washington, Bolton et al. (1985) found higher soil enzyme activity (urease, phosphatase, and dehydrogenase) and microbial biomass in a soil managed with leguminous green manures since 1909 than in an adjacent field which has received N, P, and S at recommended rates since 1948. After 55 years of a crop-residue and N-fertilization treatment in a wheat-fallow system, Dick et al. (1988) found soil that received manure or pea vine had higher enzyme activity (phosphatase, sulfatase, β-glucosidase, amidase, and urease) than the control soil. Amidase and urease (enzymes involved in deamination) decreased with increasing inputs of inorganic N, but organic N (manure or pea vine) increased activity.

Transitions from Inorganic to Organic N Sources

Nitrogen deficiency is one of the factors responsible for reduced yield and profit losses during the initial conversion from mineral to organic sources of N. The C in the organic material is a source of energy for the microbial populations that mediate the breakdown and release of nutrients contained in the organic residues (Paul and Clark, 1989). Since MB increases when C is added to the soil (Frankenburger and Dick, 1983; Nannipieri et al., 1979; Powlson et al., 1987), it is believed that N deficiencies are caused, in part, from N immobilization by the increased microbial population (Janzen and Radder, 1989). In a transition study, Doran et al. (1985) found higher microbial biomass and potentially mineralizable N in a legume-grain system than in a conventional grain system. In the second year of the study, N deficiency and lower yields in the legume-grain rotation were associated with less soil NO_3^- and more N in the microbial biomass than in the conventional system.

When synthetic N fertilizers are reduced or eliminated, nutrient availability is dependent on the soil's biological capability. Therefore, the reestablishment of

an active microbial community is important during a conversion (Culik, 1983). Many studies have shown that soil managed with organic N sources has higher microbial populations and enzyme activity than soil managed with mineral fertilizers (Bolton et al., 1985; Martyniuk and Wagner, 1978; Anwarzay et al., 1990; Alef et al., 1988; Dick et al., 1988; McGill et al., 1986), but few studies have monitored microbial biomass and enzyme activity dynamics during the transition from mineral to organic sources of N.

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CHAPTER 2

NITROGEN UPTAKE AND DRY MATTER YIELD

ABSTRACT

Organic N additions can replace inorganic N as a means of conserving natural resources while minimizing adverse environmental impacts. Although long-term organic additions increase soil fertility and biological activity, producers are constrained by the need for short-term returns on their inputs. A successful transition from inorganic to organic N, therefore, depends on short-term N availability from organic N sources to maintain crop productivity. A greenhouse experiment was conducted to determine the effect of different organic amendments and decreasing inorganic N rates on dry matter yield (DMY) and plant N uptake. Treatments were arranged as a complete factorial that included the following factors: four soils obtained from the Residue Utilization Plots (RUP) initiated in 1931 at the Columbia Basin Research Center, Pendleton, OR (beef manure, pea vine residue, 0 kg N or 90 kg N ha^{-1}, each applied biennially to a wheat-fallow system); four greenhouse organic residues (pea vine, beef manure, poultry manure, or control); and four rates of N fertilizer (0-1600 mg N 2 kg^{-1} soil as NH_4NO_3). Four successive crops of *Zea mays* L. were grown over a period of 306 days. In the absence of organic residue or N fertilizer, soil from the manure RUP treatment produced greater DMY and plant N uptake than soil from the other RUP treatments. Plant N uptake from soils in the beef manure and control residue treatments was the same throughout the experiment for each N rate, indicating no net N mineralization from the beef manure added in the greenhouse. In both the beef manure and control residue treatments, N uptake was directly proportional to the amount of inorganic N applied. Both the poultry manure and pea vine amendments increased mineral soil N and plant N uptake. Poultry manure was mineralized more quickly, however, pea vine provided N for plant uptake over a longer period of time.

INTRODUCTION

There is a need to develop and adopt economical and efficient N management strategies that conserve natural resources while minimizing adverse environmental impacts (Hargrove, 1988). Reducing the amount of inorganic N applied to agricultural land is one such strategy. Pollution concern, public criticism, and the desire to cut input costs have prompted some producers to do so. When manufactured N fertilizers are reduced or removed from a production system, alternative N sources are required to maintain crop productivity. Using organic N sources produced on the farm (legumes, green manure, animal manure, and crop residues) is a viable alternative.

In the absence of inorganic fertilizers, N availability depends on the soil's N cycling capacity. Because most of the reactions involved in the N cycle are biologically mediated, the size and activity of soil microbial populations play an important role in N supply. Long-term applications of manufactured N fertilizers result in soils that have lower biological activity relative to soils that have received repeated additions of organic N (Rasmussen et al., 1989; Dick et al., 1988; McGill et al., 1986; Bolton et al., 1985). When making a transition from inorganic to organic N management, re-establishment of an active, functioning microbial community is critical to plant nutrient availability (Culik, 1983).

Although long-term organic additions increase soil fertility and biological activity (Rasmussen et al., 1989; McGill et al., 1986), producers are constrained by the need for short-term returns on their inputs. A successful transition from inorganic to organic N sources, therefore, depends on short-term N availability from the organic N sources to maintain crop productivity.

The Residue Utilization Plots (RUP) at the Columbia Basin Research Center, OR, have been managed with either inorganic or organic (manure or pea vine) N sources since 1931. As a result, soils from the RUP vary widely in organic matter content and biological activity (Rasmussen et al., 1980, 1989; Dick et al., 1988). Soils from the RUP provided a unique opportunity to simulate a transition

from inorganic to organic N sources. The objective was to determine the effect of different organic amendments and decreasing inorganic N rates on dry matter yield and plant N uptake in soil which had been managed with long-term applications of either inorganic or organic N sources.

MATERIALS AND METHODS

Experimental Design

The experimental design was a completely randomized block with three replications. The treatments were arranged as a 4 x 4 x 4 complete factorial that included the following factors: soil field history, organic residue, and N fertilizer (Table 2.1).

Table 2.1. Factorial arrangement of treatments.

Field history	Organic residue	N Fertilizer†
Manure	Pea vine	N_0 (0)
Pea vine	Beef manure	N_{400} (200)
Nitrogen	Poultry manure	N_{800} (400)
Control	Control	N_{1600} (400)

† Subscript number is cumulative mg N applied per pot (2 kg soil) over four successive cropping periods. Number in parentheses is mg N applied as NH_4NO_3 to the first crop.
N_{400} and N_{800} fertilizer levels decreased by 1/3 of the original rate with each successive crop.

Soil

The soil was obtained from the RUP established in 1931 at the Columbia Basin Agricultural Research Center, Pendleton, Oregon. The soil is classified as a Walla Walla silt loam (coarse-silty, mixed, mesic Typic Haploxeroll). Since 1931, the RUP have been cropped with winter wheat in rotation with summer fallow. Plots have received wheat straw plus one of the following treatment on a biennial basis: (1) inorganic N fertilizer (34 kg ha^{-1} from 1931-1966; 90 kg ha^{-1} from 1967-1989), (2) strawy beef manure (22.4 metric tons ha^{-1}), (3) pea vine residue (2.24 metric tons ha^{-1}), and (4) only straw. Manure and pea vine residue were

moldboard-plowed to 20-cm depth in the spring of each fallow year. Nitrogen fertilizer was applied at planting in October. The amount of C and N added to the soil in the RUP are presented in Table 2.2. Detailed descriptions of the experimental conditions and treatment history were reported by Oveson (1966) and Rasmussen et al. (1980).

Table 2.2. The C and N added to the RUP on a biennial basis (1931-1989).

Field history	Wheat straw†	Amendments‡	Total
		C Additions	
		kg C ha^{-1} 2 yr^{-1}	
Manure	2850	1415	4265
Pea vine	2440	790	3230
Nitrogen	2370	–	2370
Control	1875	–	1875
		N Additions	
		kg N ha^{-1} 2 yr^{-1}	
Manure	20	110	130
Pea vine	11	35	46
Nitrogen	13	90 (45)§	103
Control	8	–	8
		C:N Ratio	
Manure	139	13	33
Pea vine	218	23	70
Nitrogen	190	–	23
Control	240	–	240

† Estimated from average straw yield, C and N concentration, 1931-1978.
‡ Estimated from pea vine and manure applied, 1967-1986.
§ N rate changed in 1967, number in parentheses is for 1931-1966.

Surface soil (0-20 cm) was sampled from fallow plots in November 1989. A shovel was used to take six 25 x 25 x 20 cm subsamples of soil from each plot. Soil from each plot was passed through a 15-mm sieve, and any large straw debris discarded. The C and N content of the soil is shown in Table 2.3.

Table 2.3. The C and N content of the soil (0-20 cm) from the RUP prior to the greenhouse experiment.

Field history	Organic C	Total N	C:N Ratio
	——— g kg^{-1} ———		
Manure	14.0	1.21	11.6
Pea vine	11.5	0.92	12.5
Nitrogen	10.5	0.82	12.8
Control	9.8	0.83	11.8

Greenhouse Experiment

Greenhouse pots each held two kg soil (oven dry weight basis) and were lined with double polyethylene bags to prevent leaching. At the beginning of the experiment each pot received a blanket application of 285 mg K, 120 mg S, 350 mg P, and 226 mg Ca in the form of K_2SO_4 and $Ca(H_2PO_4)_2 \cdot H_2O$. Organic amendments were added on a gram total Kjeldahl N (TKN) basis. Chemical characteristics of the organic amendments are shown in Table 2.4. The pea vine residue was the same source applied to the RUP in the field. Beef and poultry manure were composted and commercially available. Soil water content in the pots was maintained at 0.35 kg kg^{-1} throughout the experiment. Temperature in the greenhouse was controlled at 24/18°C, day/night. Days were 16 hours long, and artificial light was provided.

The soil and organic amendments were incubated for 87 days before the first crop was planted. Four maize seeds (*Zea mays* L.) per pot were sown to a depth of 2.5 cm. After seven days plants were thinned to three per pot. The plants were harvested 35 days after planting. Four successive crops were grown. After crop 2 was harvested, a second addition of organic amendments equivalent to 1 g TKN were added followed by a 65 day incubation. All pots received 80 mg S as K_2SO_4 when crop 3 was planted. A schematic diagram of treatment application is presented in Figure 2.1.

At harvest, shoots were cut off at soil level. The roots were removed from

the soil and rinsed clean. Shoot and root tissue were dried at 65°C, weighed, and ground to pass a 0.42-mm sieve. Dry matter yield and plant N uptake values reported include both shoots and roots.

Table 2.4. Chemical characteristics of organic amendments added to the soils in the greenhouse.

Organic amendment	Total C	Total N	Lignin	C:N
	—— g kg^{-1} ——		%	
Pea vine	406	19	5.7	21
Beef manure	365	15	28.4	24
Poultry manure	400	48	19.9	8

Table 2.5. Inorganic N added in the greenhouse N fertilizer treatments.

	Crop				Total†
Nitrogen treatment	1	2	3	4	
	—— mg NH_4NO_3-N pot^{-1} ——				
N_0	0	0	0	0	0
N_{400}	200	133	66	0	400
N_{800}	400	266	134	0	800
N_{1600}	400	400	400	400	1600

† Cumulative inorganic N added per pot (2 kg soil).

Nitrogen, Carbon, and Lignin Determination

Ground shoot and root tissue, organic residues, and soils were analyzed for TKN content as described by Bremner and Mulvaney (1982). Soil inorganic N was extracted with 2 *M* KCl (10:1 extract to soil ratio). Ammonium from the TKN digests and soil extract NH_4^+-N and NO_3-N were determined on an Alpkem autoanalyzer (Alpkem, Clackamas, OR).

Total organic C content of the soil was determined by dry combustion and infrared detection on a Dohrmann C analyzer (Dohrmann, Santa Clara, CA). Carbon in the organic amendments was analyzed with a Leco C analyzer (Leco, St. Josephs, MI).

Lignin concentration of the organic residues was determined as described by Van Soest (1963).

Statistical Analysis

Data were analyzed by standard ANOVA procedures for a completely randomized block design with SAS statistical software package (SAS Institute, Cary, NC). Main effect means were separated with Tukey's at the $p = 0.05$ level.

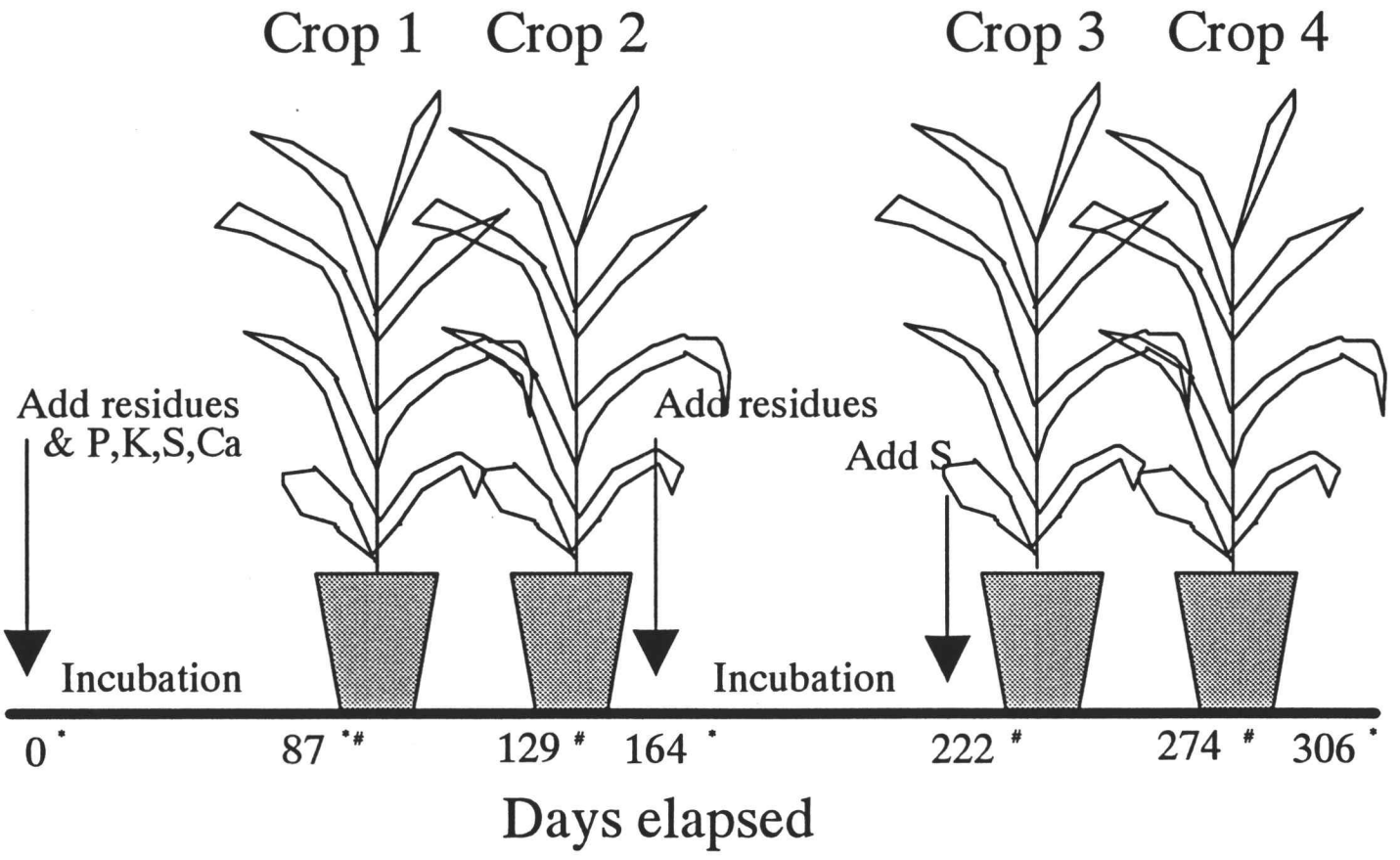

Figure 2.1. Schematic overview of treatments applied during the experiment.

RESULTS AND DISCUSSION

Response to Long-term Field History

In the absence of organic residue or N fertilizer, soil that received long-term manure applications in the field produced greater dry matter yield (DMY) and N uptake than soil from other field treatments at the first crop (Figure 2.2). There was no significant difference in either DMY or plant N uptake from soils with other field histories, although DMY and N uptake were consistently higher in the soil from the pea vine RUP than in soil from either the N or control RUP treatment. Christ and Dick (199x) also found significantly higher ryegrass DMY and N uptake in soil from the manure RUP treatment than soil from the other RUP treatments during a 120-day greenhouse experiment when no inorganic N was added. In their study soil from the pea vine treatment also produced more DMY and N uptake than soil from the other RUP treatments (manure treatment excluded) although, the difference was not significant.

Since no N fertilizer or organic residues were added in the N_0 treatment, N uptake reflects differences in mineral N supplying capacity of the soil resulting from long-term management. Prior to planting crop 1, soil from the manure, pea vine, N, and control field treatments had 42.9, 38.9, 30.1, and 19.2 μg inorganic N g^{-1} soil, respectively. In the first maize crop, N uptake was approximately one-half of this amount for each treatment.

Long-term yields from the RUP indicate that the manure amended soil produces 5% higher wheat yields than the N amended soil (Rasmussen et al., 1989). In the greenhouse, when inorganic N is not added, the manure amended soil supplies more N and produces much higher yields than soil with a long-term history of inorganic N application (Figure 2.2). Yields on unamended soil from the RUP for the first maize crop were 91% higher in the manure amended soil, than in soil from the long-term RUP N treatment, and even the pea vine amended soil produced 24% greater maize yield than the N amended soil.

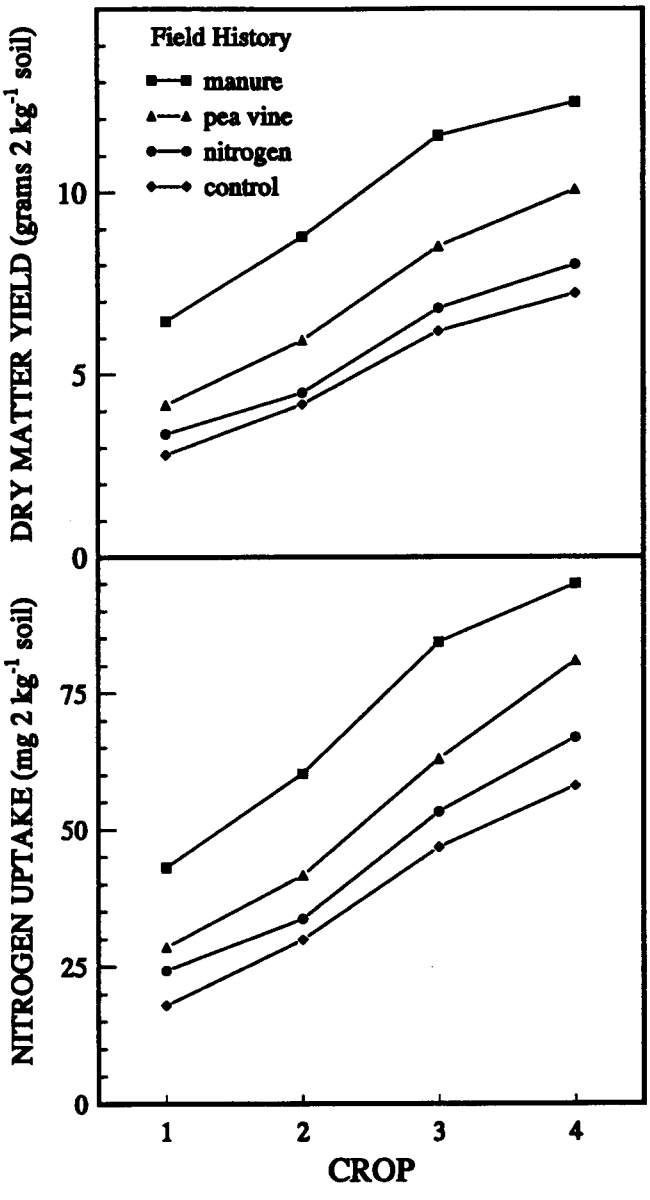

Figure 2.2. Cumulative dry matter yield and N uptake as affected by soil field history in the absence of greenhouse N inputs (n = 3).

In the absence of greenhouse N inputs (organic residue or inorganic N), N uptake and DMY in the first maize crop was correlated with soil organic C, total N, and the amount of C added to soil in the field (r^2 = 0.99, 0.93, and 0.96 for N uptake and 0.99, 0.97, and 0.96 for DMY, respectively). Plant productivity can be maintained with either organic or inorganic N, however, organic N sources improve soil fertility relative to inorganic N. In general, N amendments increase yield and create more residues per crop. Residues returned to the soil maintain soil organic matter and N fertility. The beneficial effect of N fertility is both cyclic and cumulative. If crop residues are removed, however, N fertilizer alone does not maintain soil organic C or total N content (Black, 1973; Bonde et al., 1988).

After the first crop, DMY and N uptake were similar regardless of past management history (Figure 2.2), indicating similar N mineralization rates in these soils. In a previous greenhouse study with soil from the same plots, Christ and Dick (199x) found the soil that received inorganic N had a higher mineralization rate than soil from either the manure or pea vine treatment. Their study also found that although soils from the manure and pea vine treatments had a lower mineralization rate, they had a larger pool of potentially mineralizable N than soil from either the control or N treatment. The pea vine and manure are C as well as N sources, and both have increased the soil organic C and total N levels relative to the N treatment (Table 2.3). Bonde et al. (1988) also found C additions (manure or straw plus N fertilizer) increased N mineralization potential and soil organic C and total N levels. In their study, soil with a history of inorganic N application had higher mineralization rate and lower potentially mineralizable N than soil that received organic matter additions.

Differences in DMY and N uptake resulting from different long-term management practices diminished once inorganic N was added (Table 2.6). Christ and Dick (199x) also found no significant difference in ryegrass N uptake when adequate inorganic N was added, however, manure amended soil produced higher yields in the presence of up to 160 mg N kg^{-1} soil.

Table 2.6. Effect of field history and inorganic N applied in the greenhouse on N uptake and dry matter yield of maize in the control greenhouse residue treatment at crop 1.

	mg N applied 2 kg^{-1} soil		
Field history	0†	200†	400‡
	N uptake		
	mg N 2 kg^{-1} soil		
Manure	43a§	157a	293a
Pea vine	29b	130a	242a
Nitrogen	24b	142a	247a
Control	18b	139a	248a
	Dry matter yield		
	g 2 kg^{-1} soil		
Manure	6.5a	14.6a	18.6a
Pea vine	4.2ab	13.9ab	15.1a
Nitrogen	3.4b	14.4a	16.5a
Control	2.8b	12.6b	15.8a

† $n = 3$.
‡ $n = 6$.
§ Means followed by the same letter within a column are not significantly different (Tukey's, $p = 0.05$).

When organic residues were added in the greenhouse, no differences in DMY resulted from past management history (data not shown). At the N_0 fertilizer rate, trends in N uptake among the soils from the different field histories that received pea vine or beef manure in the greenhouse followed those of the control residue treatment. In the greenhouse poultry manure treatment, N uptake was 32% higher in the soil from the pea vine RUP treatment than soil from other field treatments at crop 1. After crop 1, the N uptake in the poultry manure residue treatment was similar in all soils regardless of field history. There is no explanation for this interaction, however, β-glucosidase activity (Chapter 4)

measured prior to planting was also much higher in this treatment combination.

Field history had little effect on DMY and N uptake relative to the effects of N fertilizer and organic residue treatments, therefore, results presented below were averaged across field history.

Response to Inorganic Nitrogen and Organic Residue Treatments

Dry matter yield - The pea vine and poultry manure treatments had similar dry matter yields as did the beef manure and control (Figure 2.3). At the N_{1600} rate, the DMY was approximately the same for all organic residue treatments indicating N was not limiting DMY at this N rate. Dry matter yield in the N_{800} and N_{1600} treatments were not significantly different in the soil amended with either pea vine or poultry manure throughout the cropping period. The N_{1600} treatment produced significantly greater DMY than the N_{800} treatment in the beef manure and control residue treatments after crop 2 and 3, respectively.

For the first crop, the pea vine and poultry manure provided enough N for maximum DMY regardless of the N fertilizer treatment. In the absence of inorganic N, the beef manure and control residue treatments produced very low DMY, 28% of poultry manure or pea vine treatment at crop 1.

The amount of N added to crop 4 was 0 mg N for all N treatments except the N_{1600}. Crop 4 DMY for the N_0, N_{400}, and N_{800} treatments was the same within each residue treatment. In the beef manure and control treatments the N_{1600} rate produced higher DMY than the other N treatments, but in the poultry manure and pea vine treatments, N rate had no effect on DMY at crop 4.

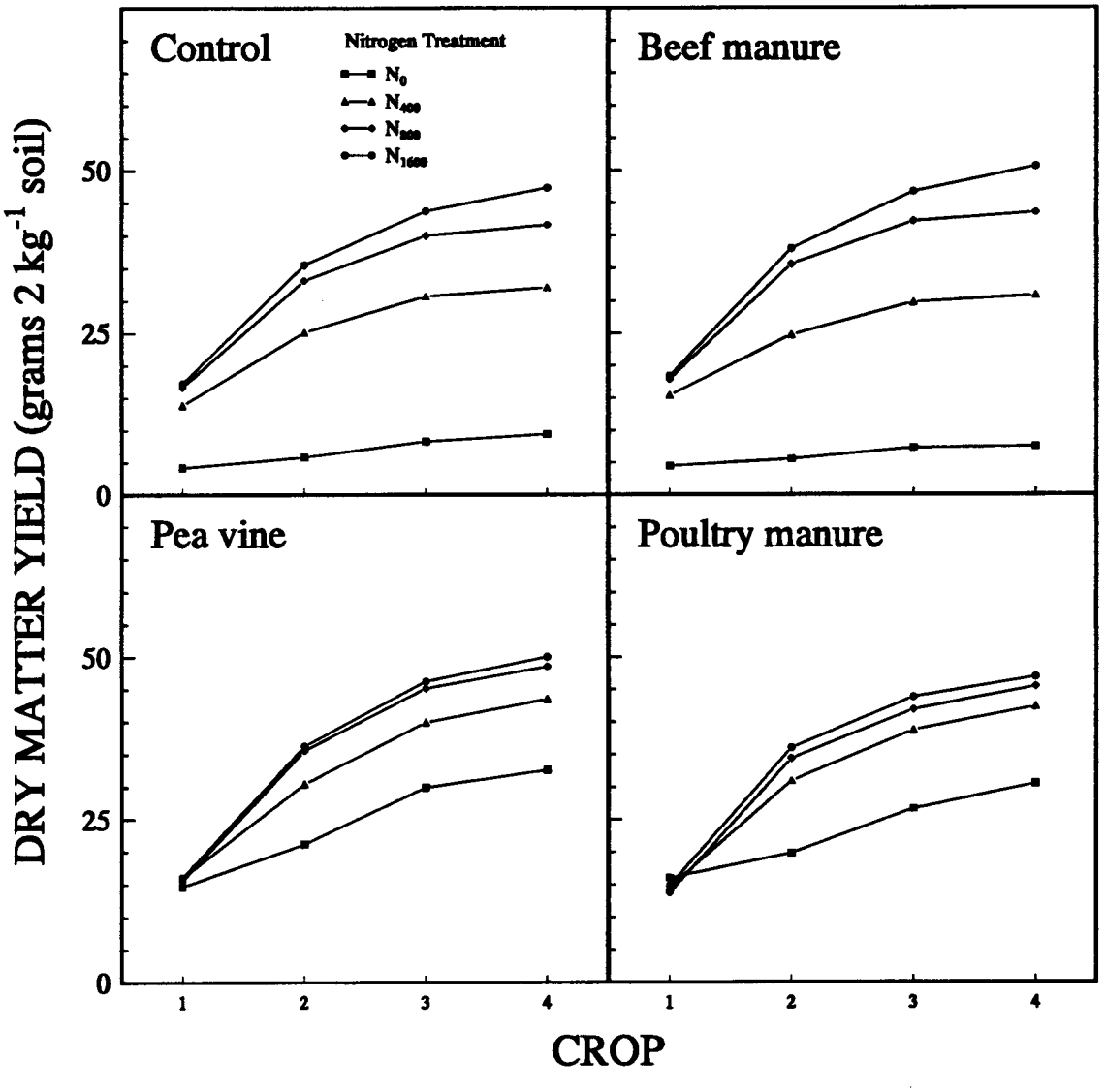

Figure 2.3. Cumulative dry matter yield in the organic residue treatments as affected by N fertilizer treatment, averaged over field history (n = 12).

Nitrogen uptake - Cumulative uptake in the beef manure and control residue treatments was less than that in the poultry manure and pea vine treatments throughout the cropping period (Figure 2.4). In the pea vine, beef manure, and control treatments, N uptake increased with increasing N rates until crop 4 when N uptake was the same within each residue treatment for the N_0, N_{400}, and N_{800} rate. At crop 4, only the N_{1600} treatment received an application of inorganic N. More N was taken up by plants in the N_{1600} treatment at crop 4 than in any other inorganic N treatment with the exception of the poultry manure residue treatment. In the poultry manure treatment, N uptake was influenced by N treatment only at crop 2 when N uptake increased with increasing rates of N applied. Throughout the cropping period for the pea vine, beef manure, and control treatments and after crop 1 for the poultry manure treatment, the difference in cumulative N uptake between the N_0, N_{400}, and N_{800} treatments was proportional to the amount of cumulative N applied.

In the absence of applied inorganic N, differences in plant N uptake between the control residue treatment and the other residue treatments reflect the amount of plant-available N derived from the residues. Nitrogen uptake in the beef manure and control residue treatments was the same throughout the experiment at each N rate, indicating no net N mineralization from the beef manure (Figure 2.4). However, differences in soil inorganic N between days 0 and 87 (the incubation period prior to cropping) suggest that 23 mg N 2 kg^{-1} soil was immobilized in the beef manure treatment (data not shown).

Net immobilization of N contained in beef or dairy manure, in soil incubation experiments, has previously been reported (Yadvinder-Singh et al., 1988; Beauchamp, 1986; Castellanos and Pratt, 1981). In a greenhouse experiment, Castellanos and Pratt (1981) found amending soil with a fresh dairy manure (C:N ratio of 15.9, 2% total N) depressed yields in the first barley crop. Beauchamp (1986) found beef manure (C:N ratio of 15.4, 0.5% total N) addition had no effect on the first crop of maize seedlings grown in the greenhouse, but increased DMY and N uptake in the second crop. Composting stabilizes the N and C in manure

and reduces its value as an N fertilizer (Castellanos and Pratt, 1981). The beef manure added to the soil in the greenhouse in the present study was composted and no short-term benefit to the plants was derived. In both the beef manure (Figure 2.5) and control residue treatments, N uptake was directly proportional to the amount of inorganic N applied. In long-term studies, however, manure has been shown to improve soil fertility, N supplying capacity, and physical parameters (Rasmussen et al., 1989), as well as provide an energy source for soil microorganisms.

Assuming that soil organic matter mineralization was unaffected by the addition of the organic residues, 36% of the organic N originally present in the poultry manure residue was mineralized during the 87 days prior to planting crop 1. Sims (1986) reported 36 and 38% of the organic N from two poultry manures (C:N of 12 and 15, respectively) was mineralized within the first 90 days of a soil incubation experiment conducted at 25°C. Castellanos and Pratt (1981) found approximately 28 and 48% of the total N content of fresh and composted poultry manure (both with a C:N ratio of 6.5), was mineralized in 10 weeks, respectively.

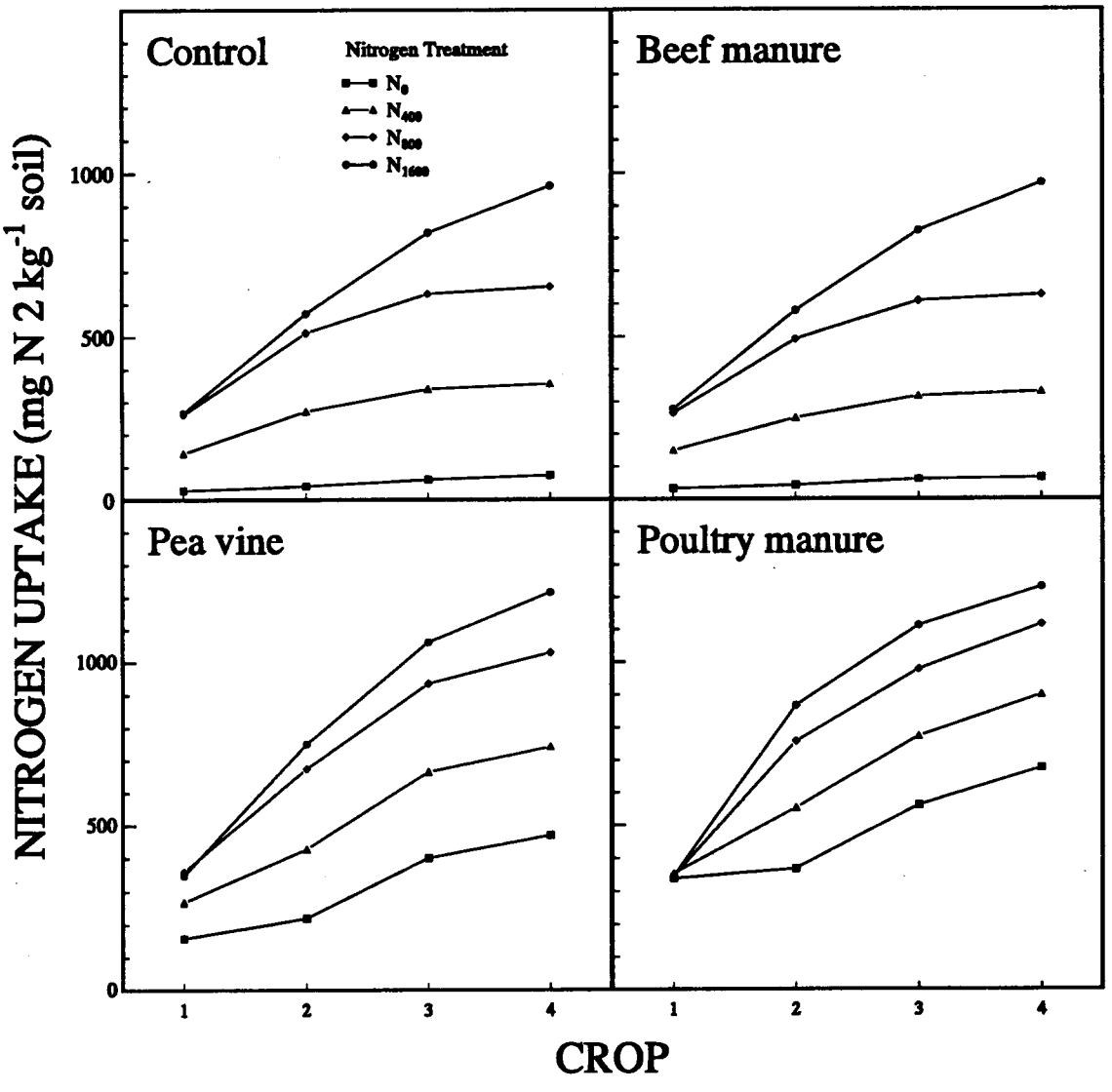

Figure 2.4. Cumulative N uptake in the organic residue treatments as affected by N fertilizer treatment, averaged over field history (n = 12).

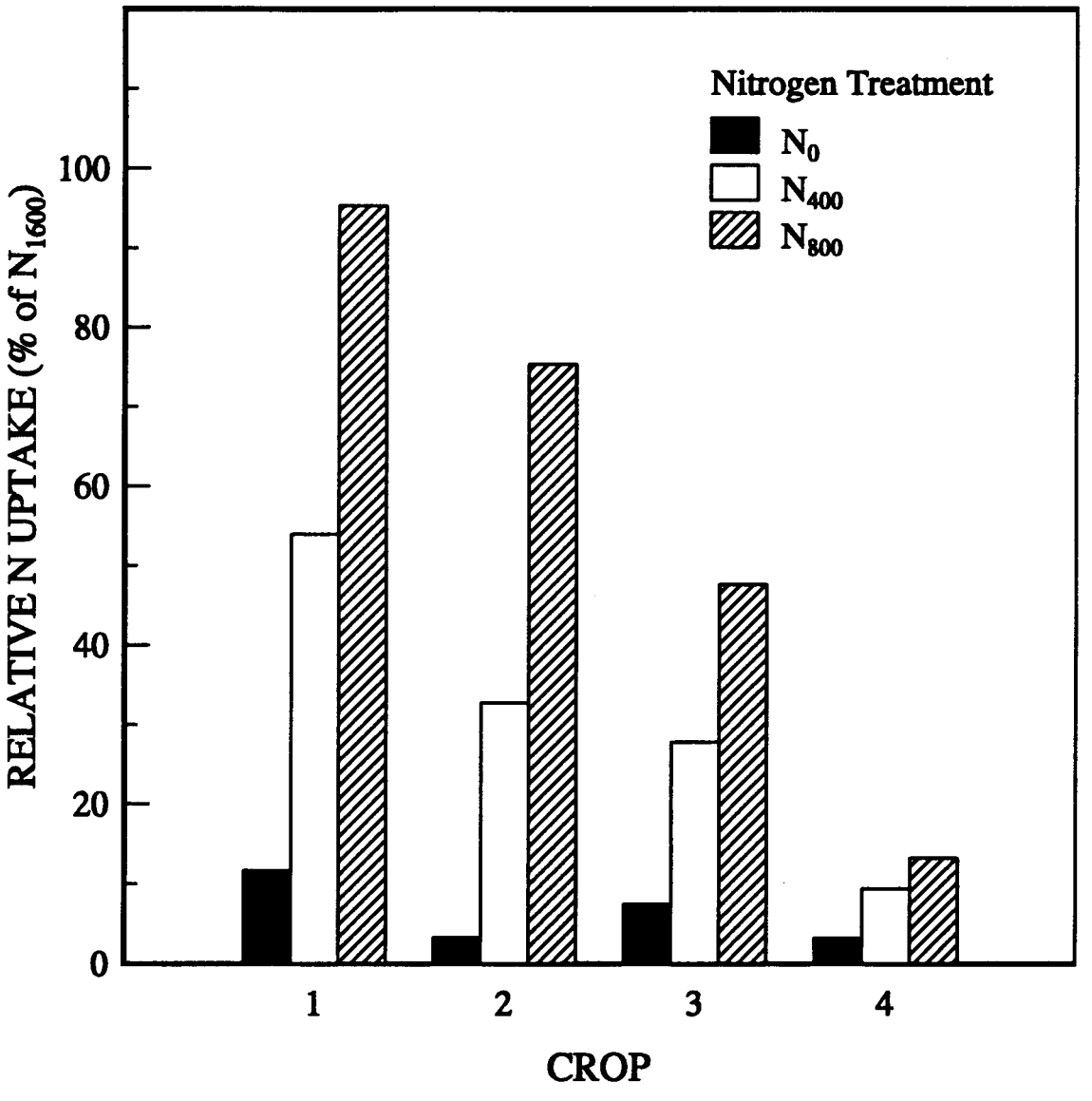

Figure 2.5. Effect of inorganic N treatment relative to the N_{1600} treatment on N uptake in the beef manure residue treatment, averaged over field history (n = 12).

Legumes have historically been used to maintain soil N fertility. Short-term benefit from legumes depends on mineralization of N from the residues after incorporation into soil. During the 87 days prior to planting crop 1, 15% of the organic N originally present in the pea vine residue added to the soil in the greenhouse was mineralized. Yadvinder-Singh et al. (1988) measured rapid mineralization of a young green manure residue, *Sesbania aculeata*, incubated with soil at 30°C. By week 16, all of the residue-N was mineralized. As plants age they become more resistant to decomposition (Parr and Papendick, 1978). The pea vine residue used in the present study consisted of mature pea plants minus the peas and pods. Yaacob and Blair (1980) investigated N uptake from two mature ^{15}N-labeled leguminous residues, soybean (*Glycine max*) and Siratro (*Macroptillium atropurpureum*). After 12 weeks of growth in a greenhouse, Rhodes grass (*Chloris gayana*) recovered 15% of the soybean N and 14-56% of the Siratro N. Greater N recovery from the Siratro residue was believed to be due to its higher N content and greater percentage of leaf material. In a greenhouse experiment, Janzen and Radder (1989) found that 26% of the N from mature ^{15}N-labeled tangier flatpea (*Lathyrus tingitanus* cv. Tinga) residue added to soil was recovered by wheat and canola plants.

Thirty one and 13% of the total N content of the poultry manure and pea vine residues was taken up by the first maize crop as estimated by the difference in N uptake between the control and the residue treatments at the N_0 rate (Table 2.7). An additional 2 and 5% was taken up by the second crop, respectively. It is not possible to distinguish the percent of the N taken up by crops 3 and 4 derived from the first or second addition of organic residues. By the end of the experiment, however, 20% of the total pea vine-N and 30% of the total poultry manure-N was taken up by the four maize crops. Since N was not limiting N uptake at the N_0 rate during crops 3 and 4 in the poultry manure residue treatment, more poultry manure-N may have been available than the amount taken up by the crop suggests. The pea vine-N was not mineralized and subsequently taken up by plants as quickly as the poultry manure-N, but pea vine provided N over a longer period of time than

did poultry manure. In the pea vine residue treatment N uptake at crop 3 was greater than at crop 1, indicating that the pea vine added to the soil before crop 1 was still contributing N for uptake by the third crop. Based on the amount of N taken up at crop 2, it seems likely that the benefit from poultry manure amendment was of limited duration.

Table 2.7. Effect of organic residue added in the greenhouse on N uptake in the N_0 treatment, averaged over field history (n = 12).

	Crop			
Organic residue	1	2	3	4
	mg N 2 kg^{-1}			
Poultry manure	337.6	29.5	193.4	114.5
Pea vine	158.3	60.2	182.4	69.3
Beef manure	32.3	9.7	18.4	4.6
Control	28.4	12.9	20.5	13.4

CONCLUSIONS

Only in the absence of inorganic N additions, do soil differences resulting from long-term management practices influence plant productivity (DMY and N uptake). Soil managed with organic residues (manure or pea vine) are more productive and have higher total N and C levels than soil managed with inorganic or no N fertilizer. In the short-term there was no benefit to the plants from adding composted beef manure to the soil. Both poultry manure and pea vine additions increased DMY and N uptake. Poultry manure-N was mineralized more quickly than pea vine-N, however the pea vine provide N to the plants over a longer period of time.

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CHAPTER 3

MICROBIAL BIOMASS CARBON AND NITROGEN

ABSTRACT

Soils under long-term management practices that rely on organic material and native soil fertility for N support larger microbial biomass (MB) than soils managed with mineral fertilizers. Since soil microbes mediate most biochemical reactions in the N cycle, management practices that influence the size of the MB may affect the ability of soils to cycle and provide N for plant growth. A simulated transition from inorganic to organic N sources was conducted in a greenhouse to determine the long and short-term effects of inorganic and organic N additions to soil on the soil MB. Treatments were arranged as a complete factorial that included the following factors: four soils obtained from the Residue Utilization Plots (RUP) initiated in 1931 at the Columbia Basin Research Center, Pendleton, OR (beef manure, pea vine residue, 0 kg N or 90 kg N ha^{-1}, each applied biennially to a wheat-fallow system); four greenhouse organic residues (pea vine, beef manure, poultry manure, or control); and four rates of N fertilizer (0-1600 mg N 2 kg^{-1} soil as NH_4NO_3). Four successive crops of *Zea mays* L. were grown over a period of 306 days. Microbial biomass carbon and nitrogen (MBc and MB_N, respectively) in soil that received long-term manure applications were significantly higher ($p < 0.05$) than in soil from the other RUP treatments. At the final sampling, soils amended with pea vine, beef manure, or poultry manure in the greenhouse had 400, 210, and 80% greater MBc and 280, 140, and 50% greater MB_N than unamended soil, respectively. Soil from the N RUP treatment respired significantly ($p < 0.05$) more per unit MBc than soil from the other RUP treatments At the high greenhouse inorganic N rate, MBc tended to be lower and MB_N was significantly higher than in soil receiving lower rates of inorganic N. Soil MB did increase with organic amendments in the presence of inorganic N. This suggests that a gradual reduction of inorganic N additions during a transition in the presence of organic amendments does not inhibit an increase in MB.

INTRODUCTION

The soil microbial biomass (MB) is a source and sink for plant nutrients and an active participant in nutrient cycling (McGill et al., 1986). Both the quantity and bioavailability of material added to soil affects the size of the MB. Soils under long-term management practices that rely on organic material and native soil fertility for N support a larger MB than soils managed with mineral fertilizers (Schnürer et al., 1985; McGill et al., 1986; Jenkinson and Powlson, 1976; and Bolton et al., 1985). Recent additions of organic residues also increase the MB in the short-term (Ocio et al., 1991b; Ocio and Brookes, 1990; and Perucci, 1990). The C and N in the MB (MB_C and MB_N, respectively) turn over rapidly and indicate changes resulting from management practices long before changes in total soil C and N are detectable (Powlson et al., 1987). Because most biochemical reactions in the N cycle are mediated by soil microorganaisms, management practices that influence the size and activity of the MB may affect the ability of soils to cycle and provide N for plant growth. In the absence of organic amendments, biological activity declines (Bolton et al., 1985; Dick et al., 1988). This decline reduces the potential of soil to mineralize organic residues. Yield losses that occur as inorganic N fertilizers are replaced by organic inputs probably result from microbial immobilization of nutrients as microbial population size increases in response to organic amendments. In a transition study, Doran et al. (1985) found higher MB and potentially mineralizable N in soil from a legume/grain system than in soil from a conventional grain system. In the second year of the study, N deficiency and lower yields in the legume/grain rotation were associated with lower soil NO_3^- and more N in the MB than in soil from the conventional system. Crop productivity after a transition from inorganic to organic N sources depends on re-establishment of an active microbial community (Culik, 1983).

The Residue Utilization Plots (RUP) at the Columbia Basin Research Center, OR, have been managed with either organic or inorganic N sources since 1931. As a result, soils from the RUP vary widely in organic matter content and biological activity (Rasmussen et al., 1980, 1989; Dick et al., 1988). The objective of this study was to determine the effects of long-term and recent additions of inorganic and organic N sources on the soil MB in soils from the RUP.

MATERIALS AND METHODS

The soil came from the experiment described in Chapter 2. In brief, a simulated transition from inorganic to organic N sources was conducted to determine the effects of organic residues and decreasing N rates on plant and soil-biological parameters. Treatments were arranged as a complete factorial that included the following factors: four soils obtained from the Residue Utilization Plots (RUP) initiated in 1931 at the Columbia Basin Research Center, Pendleton, OR (beef manure, pea vine residue, 0 kg N or 90 kg N ha^{-1}, each applied biennially to a wheat-fallow system); four greenhouse organic residues (pea vine, beef manure, poultry manure, or control); and four rates of N fertilizer (0-1600 mg N 2 kg^{-1} soil as NH_4NO_3). Four successive crops of *Zea mays* L. were grown over a period of 306 days.

Organic amendments were added on an equal N basis (1 g total Kjeldahl-N 2 kg^{-1} soil) before cropping began and again after the second crop was harvested. Inorganic N was applied at a split rate at each planting and at 21 days after planting. The initial rates were 0, 200, 400, or 400 mg N 2 kg^{-1} soil for the N_0, N_{400}, N_{800}, and N_{1600} treatments, respectively (subscript number is the cumulative mg N added per pot over the 4 crops). The rate of N applied in the N_0 and N_{1600} treatments remained constant throughout the experiment. The N_{400} and N_{800} treatments decreased by one-third of the original rate with each successive crop.

Soil MB analysis was performed prior to the greenhouse experiment (day 0), after the soil was incubated with the organic residues but before any crop was grown (day 87), after the second harvest (day 164), and after the fourth harvest (day 306). Soil was passed through a 2-mm sieve and stored moist at 4°C in the dark. Microbial biomass C (MBc) and N (MB_N) were determined by the chloroform-fumigation incubation method. The original procedure of Jenkinson and Powlson (1976) was modified as follows. Twelve grams (fresh weight) of soil was weighed into glass scintillation vials. The vials were placed in a desiccator containing wet paper towels and 50-ml beaker containing 40 ml of ethanol-free

chloroform and a few glass beads. The desiccator was evacuated and the soil exposed to chloroform vapors for 24 hours.

The soil was transferred into polyethylene tubes (21 cm x 22.5 mm diameter) fitted with rubber septa that enabled sampling for gas chromatography. No inoculum was added and soil moisture was not adjusted. Samples were incubated at 24°C for 10 days in the dark. Total CO_2 produced after 10 days was determined with a thermal conductivity gas chromatograph.

After CO_2 sampling, 50 ml of 2 *M* KCl was added to the tubes. The tubes were shaken lengthwise for 1 h and stored at 4°C until filtered. Extracts were filtered through a 25 mm glass fiber filter (Type A/E, Gelman Sciences Inc., Ann Arbor, MI) directly into autoanalyzer vials. The vials were capped and frozen until NH_4^+-N and NO_3^{-1}-N were determined on an Alpkem autoanalyzer (Alpkem, Clackamas, OR).

The MBc and MB_N were calculated with the following formulas:

$MBc = CO_2\text{-}C_f / 0.41$ (Voroney and Paul, 1984),

$MB_N = (NH_4^+\text{-}N_f - NH_4^+\text{-}N_{uf})/0.68$ (Shen et al., 1984),

where *f* and *uf* denote fumigated and unfumigated samples, respectively. The $CO_2\text{-}C_{uf}$ was used to estimate basal respiration. Metabolic specific respiration was expressed as respiration per unit MBc.

All results are expressed on a per g oven dry (105°C, 24 hr) weight basis. The data were analyzed by standard ANOVA techniques for randomized blocks with SAS statistical software package (SAS Institute, Cary, NC). Main effect means were separated with Tukey's at the $p = 0.05$ level.

RESULTS AND DISCUSSION

Field History

Soil from the Residue Utilization Plots (RUP) that received long-term manure applications had significantly higher MBc and MBN than soils with other field histories (Table 3.1). Studies from other long-term plots have shown similar results. Soil from the Broadbalk Plots at the Rothamsted Experiment Station that have received manure annually since the mid-1800's has higher MBc than soil from the NPK or control treated plots (Jenkinson and Powlson, 1976). After 50 years of cropping in Alberta, McGill et al. (1986) found manured soil contained twice the MBN as did NPKS or control plots. All of these studies, however, are confounded by the return of crop residues. Higher biomass measured in manure amended soil may have resulted from either the N or C in the manure itself or indirectly via increased amounts crop residues returned to the soil.

The manure applied to the RUP has increased the amount and quality of the straw residue returned to the plot (2850 kg straw-C ha^{-1} 2 yr^{-1}, C:N = 139 in manure treatment vs. 1875 kg straw-C ha^{-1} 2 yr^{-1}, C:N = 240 in control treatment) and has also supplied additional C and N (1415 kg C and 110 kg N ha^{-1} 2 yr^{-1}, Table 2.2). Initial MBc and MBN measurements were highly correlated with the total amount of C added to the soil in each RUP treatment (r^2 = 0.90 and 0.92, respectively). Neither was correlated with total N added, but both were correlated with the organic N added (r^2 = 0.94 and 0.99, respectively). In a long-term experiment where all residues were removed, Schnürer et al. (1985) found that adding inorganic N alone increased both MBc and MBN, but when both C and N (via straw plus N fertilizer or manure) were added, MBc and MBN increased by more than 50% relative to the inorganic N treated soil. Carbon additions had a greater effect than N supply on MB. Insam et al. (1991) suggested the effect of N fertilization on MBc is an indirect one resulting from increased C input to soil via roots and crop residues in response to improved N nutrition.

Table 3.1. Effect of long-term field history on MBc and MBN, averaged over organic amendment and N fertilizer treatments (n = 48).

	Sampling day			
Field history	0†	87	146	306
	Biomass carbon			
	—— μg C g^{-1} soil ——			
Manure	276a‡	287a	230a	327a
Pea vine	202b	244b	199b	280b
Nitrogen	196b	210c	155c	271bc
Control	168b	227bc	163c	253c
	Biomass nitrogen			
	—— μg N g^{-1} soil ——			
Manure	21a	51a	35a	48a
Pea vine	12b	39b	30b	42b
Nitrogen	8c	32d	21d	39bc
Control	7c	37c	26c	36c

† There was no organic amendment or N treatment at day 0 (n = 8).
‡ Means in each column followed by the same letter are not significantly different (Tukey's, p = 0.05).

Long-term crop yields from the RUP indicate that the manure amended soil has produced 5, 30, and 75% greater wheat yields than the N, pea vine, and control treatments, respectively (Rasmussen et al., 1989). The N and manure treated soils produce similar yields which indicates that 130 kg N ha^{-1} added via manure and wheat straw (110 and 20 kg N ha^{-1}, respectively) in the manure treatment supplies as much plant-available N as the N treatment which supplies 102 kg N ha^{-1} (90 kg NH_4NO_3-N ha^{-1} and 12 kg straw-N ha^{-1}). Organic N in the manure must be mineralized by the MB before becoming plant-available, and as expected, the manure treated soil supports a much larger MB than the soil from the N RUP (Table 3.1).

At the beginning of the experiment, MB_C was 1.9, 1.6, 1.4, and 1.6% of soil organic C and the MB_N was 2.0, 1.5, 1.1, and 1.0% of total soil N in the manure, pea vine, N, and control treated soils, respectively. The RUP amended with manure or pea vine had higher soil C and N levels and more of the C and N tended to be in a biological form. Others have found long-term additions of straw, green, or farmyard manure increase the MB_C : soil C ratio (Insam et al., 1991; Bonde et al., 1988; Anderson and Domsch, 1989) and flush of N to total soil N ratio (McGill et al., 1986). When agricultural practices remain unchanged for long periods, the MB_C to soil C ratio is thought to represent an equilibrium characteristic of the system. Although there may be temporary changes (e.g., increases in MB resulting from recent soil additions) the soil system maintains an apparent equilibrium when measured over time.

Organic Amendments

Recent additions of organic amendments had a larger effect on the MB than did long-term field treatments (Figure 3.1 and Table 3.1). Additions of organic amendment in the greenhouse increased MB_C and MB_N relative to the control at each sampling date. At the 306 day sampling, the soil amended with pea vine, beef manure, or poultry manure had 400, 210, and 80% greater MB_C and 280, 140, and 50% greater MB_N than the unamended soil, respectively. This is consistent with other studies which have shown that recent additions of organic residues have increased soil MB during short term incubations (Ocio and Brookes, 1990; Ocio et al., 1991b). Long-term incubations and field studies have shown rapid increases in MB shortly after the addition of an organic residue followed by a gradual decline. Perucci (1990) found MB_C and MB_N peaked one month after municipal sewage sludge was added to soil. After 12 months of laboratory incubation, both MB_C and MB_N were still two-fold greater than in untreated soil. Ocio et al. (1991a) found that MB_C and MB_N were 20 and 18% greater than in the unamended soil one year after field incorporation of 10 Mg ha^{-1} wheat straw (C:N = 48).

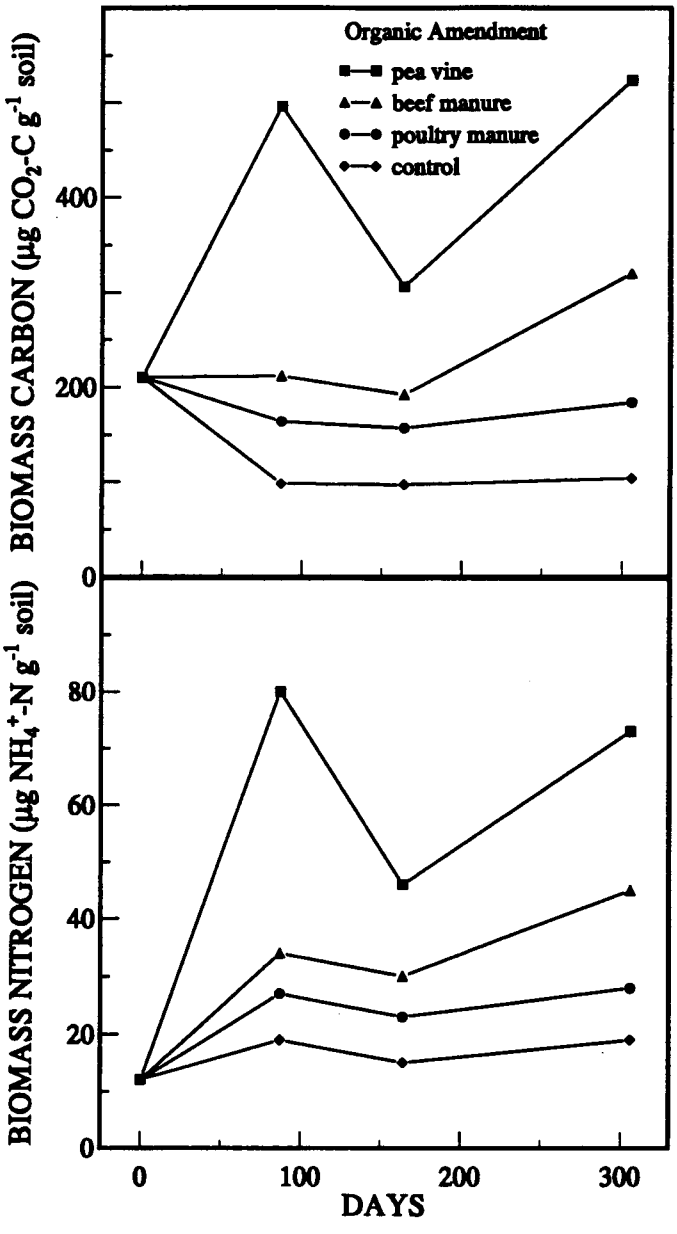

Figure 3.1. Effect of organic amendments added in the greenhouse on MBc and MBN, averaged over field history and N treatment (n = 48).

All residues were added on a equal N basis, but the pea vine and beef manure had similar C:N ratios (21 and 24, respectively) and, therefore, both treatments added approximately the same amount of C. Soil amended with pea vine in the greenhouse supported 60% more MBc and MBN than the beef manure amended soil. Schnürer et al. (1985) found greater MB in straw plus inorganic N treated soil than in manure treated soil even though both were added on an equal C and N basis. They postulated the difference resulted from substrate bioavailability. Because the C in manure was "digested" prior to soil application it is expected to be more resistant to microbial decomposition than crop residues. The lignin concentration in the beef manure was four times greater than that in the pea vine (28 and 6% lignin, respectively, Table 2.4). Because lignin is resistant to microbial degradation, less C would be available for MB incorporation from the beef manure. Both manures used were composted, but the beef manure supported a greater MB than did the poultry manure (Figure 3.1). The amount of C added in the beef manure was three times greater than in the poultry manure (24 and 8 g C 2 kg^{-1} soil, respectively). The MBc and MBN increase in the beef manure amended soil over the control was approximate three times greater than the MBc and MBN increase in the poultry manure amended soil over the control at the 306 day sampling.

Biomass Specific Respiration

Although the functional relationship between MB and microbial respiration is not yet fully understood (Šantrůčková and Straškraba, 1991), high biomass specific respiration in the presence of easily degradable substances is commonly observed (Schnürer et al., 1985; Insam et al., 1991). Soil with a field history of N application had higher biomass specific respiration than soil from other RUP treatments (Table 3.2). The overall C:N ratio of straw residue and N amendment added to the N treated soil was the lowest of the RUP treatments (C:N ratio of 23 vs. C:N ratios of 33, 70, and 240 for the manure, pea vine, and control treatments, respectively), thus, the organic material was probably the easiest to decompose.

The total amount of C added to the soil in the field in the N treatment was less than in the manure and pea vine treatments. Schnürer et al. (1985) and Bonde et al. (1988), however, found that long-term N fertilizer additions had no effect on biomass specific respiration and soil that received C additions (either straw plus N fertilizer or manure) had higher biomass specific respiration than soil that did not receive C.

Table 3.2. Effect of long-term field history on biomass specific respiration, averaged over organic amendment and N fertilizer treatments (n = 48).

	Sampling day			
Field history	0†	87	146	306
	——— $\mu g\ CO_2\text{-}C\ \mu g^{-1}$ biomass-C ———			
Manure	0.12	0.28c§	0.25c	0.30a
Pea vine	0.12	0.32bc	0.27bc	0.34a
Nitrogen	0.18	0.39a	0.41a	0.36a
Control	ND‡	0.33b	0.30b	0.32a

† There was no organic amendment or N treatment at day 0 sampling date (n = 8).
‡ Not determined.
§ Means in each column followed by the same letter are not significantly different (Tukey's, p = 0.05).

Insam et al. (1991) compared soils with various fertilizer treatments and found lower biomass specific respiration in soil receiving full fertilization compared with inadequately fertilized soil. A negative correlation between biomass specific respiration and soybean yield was obtained. In contrast, RUP soil from the long-term N treatment had the highest biomass specific respiration. It also produces higher grain yields than either the pea vine or control plots (Rasmussen et al., 1989). Insam et al. (1991) hypothesized microbes may require more C and energy if they have to compete for nutrients, and hence, will have a higher biomass specific respiration under low nutrient conditions. Results from my study do not support this hypothesis since inorganic N added to otherwise adequately fertilized

soil in the greenhouse had no effect on biomass specific respiration (data not shown).

Soil amended with beef manure in the greenhouse respired more CO_2-C per unit MBc than soil from the other residue treatments (Figure 3.2). Amending soil with either pea vine or poultry manure in the greenhouse, however, did not increase biomass specific respiration relative to the unamended control. Ocio and Brookes (1990) found that biomass specific respiration increased in soil amended with wheat straw (2% w w^{-1}). If high biomass specific respiration truly indicates presence of organic matter that is easy to decompose, soil amended with beef manure (28% lignin) would not be expected to exhibit high biomass specific respiration. The beef manure amended soil not only respired the most CO_2-C per unit MBc, it respired the most CO_2-C per unit soil at the end of the experiment (Table 3.3).

Table 3.3. Effect of recent organic residue amendments on soil respiration, averaged over field history and N rate (n = 48).

	Sampling day		
Organic amendment	87	164	306
	μg CO_2-C g^{-1} soil 10 day^{-1}		
Pea vine	149a†	72a	116b
Beef manure	90b	83a	131a
Poultry manure	46c	43b	54c
Control	27d	24c	33d

† Means in each column followed by the same letter are not significantly different (Tukey's, p = 0.05).

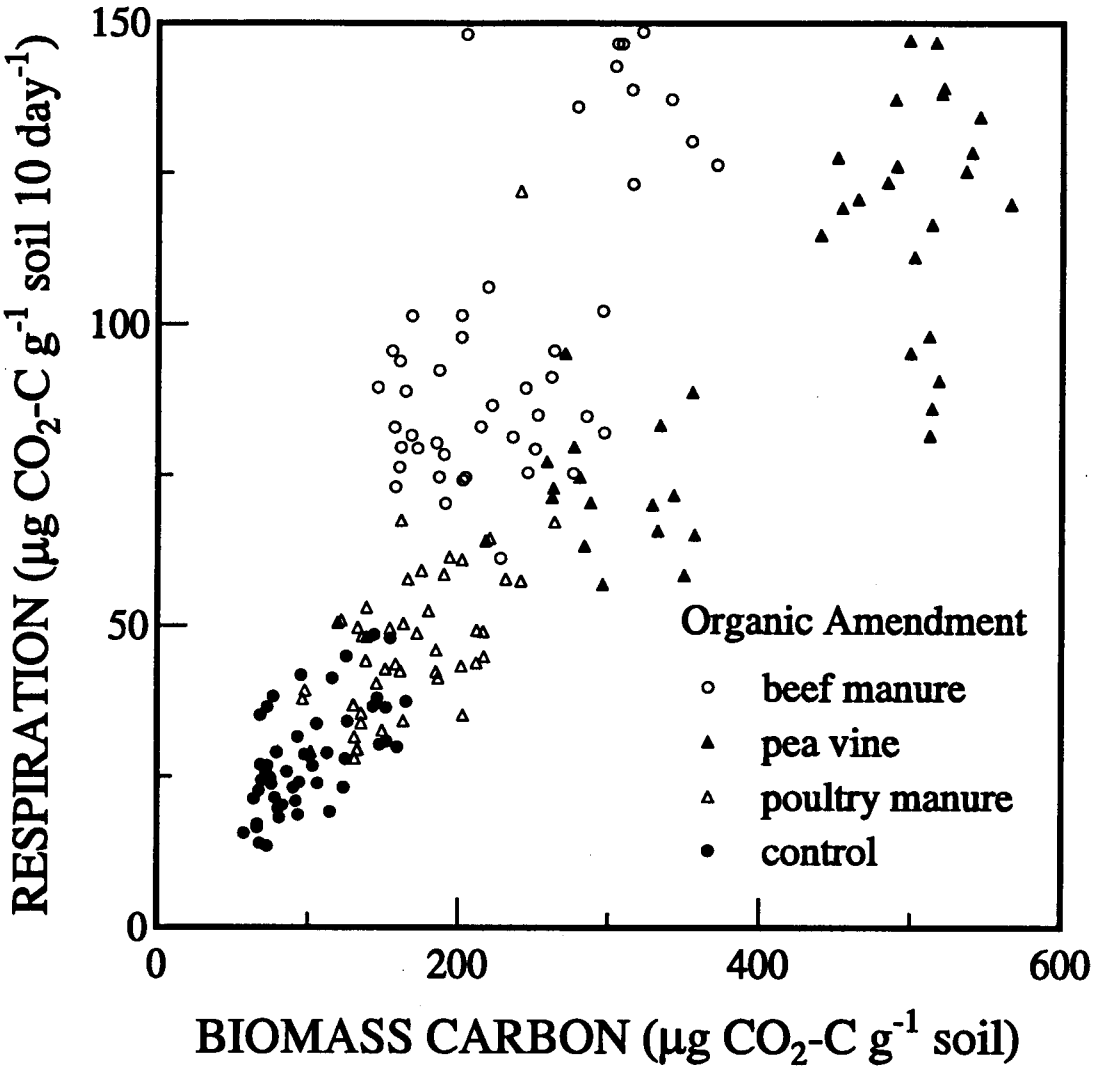

Figure 3.2. Microbial respiration per unit MBc (biomass specific respiration) as affected by organic amendments added in the greenhouse. Each symbols represents the mean of three replicates. Sampling day 0 has been omitted.

Nitrogen Fertilizer

Nitrogen fertilizer treatment began after the 87 day sampling. At the 164 day sampling, MBc and MBN were not affected by N fertilization. At the 306 day sampling, the effect of N rate on MBc was not consistent among the organic residue treatments although the N_{1600} treated soil tended to have the smallest MBc (Table 3.4). The MBN was significantly higher in the N_{1600} treatment than the other N rates which were not different from one another. Only the N_{1600} rate received any inorganic N when crop 4 was grown. Apparently there was no residual effect on MBN from N fertilizer added to the N_{400} and N_{800} treatments during earlier cropping. Drury et al. (1991) found high MBN values in corn plots soon after fertilization. The MBN approximately doubled after N application, but after one month there was no longer any apparent effect. Although field and laboratory studies (Ocio et al., 1991a, 1991b) have shown most of the N required by the MB soon after straw application comes from the N contained in the straw itself, adding inorganic N along with ^{15}N-labeled wheat straw (C:N ratio = 56) increased total biomass N in the laboratory (Ocio et al., 1991b). The inorganic N increased the total amount of MBN, yet decreased the amount of MBN coming from the ^{15}N-labeled wheat straw. If it assumed that the C and N from residues are incorporated proportionally into the MB, this may explain both the slight decrease in MBc and the large increase in MBN in the presence of inorganic N at the 306 day sampling.

Table 3.4. Microbial biomass and respiration as affected by N treatment at the 306 day sampling period, averaged over organic amendment and field history (n = 48).

	Microbial biomass			
Nitrogen treatment	Carbon	Nitrogen	C:N	Respiration
	$\mu g\ C\ g^{-1}$	$\mu g\ N\ g^{-1}$		$\mu g\ CO_2\text{-}C\ g^{-1}$
N_0	285	40	7.4	85
N_{400}	288	38	7.7	88
N_{800}	293	36	8.4	93
N_{1600}	265	51	5.5	67

In the soil with a history of N fertilization, the percentage increase in MB_N in the N_{1600} treatment over the other N treatments was greater than in soil with other field history (Table 3.5). Although the percent increase was greater, the actual amount of MB_N in soil from the N RUP / N_{1600} treatment combination was no greater than in soil from the manure RUP / N_{1600} treatment. Repeated additions of inorganic N over the years may have preconditioned the soil, resulting in more rapid or efficient response to additional inputs of inorganic N than soil that did not have a history of inorganic N additions. Another explanation may be that N was not limiting to microbes in the N_{1600} treatment at crop 4. Insam et al. (1991) found the effect of fertilization on MB_C was more pronounced on low nutrient status soils. This may also explain the greater effect of N fertilization on MB_N in the soil from N treated RUP which has low total N.

Table 3.5. Effect of N treatment on MB_N in soil from each RUP treatment at the 306 day sampling period, averaged across organic amendment (n = 12).

	Field history			
Nitrogen treatment	Manure	Pea vine	Nitrogen	Control
	$\mu g\ N\ g^{-1}$			
N_0	48	41	39	34
N_{400}	44	38	33	35
N_{800}	45	40	32	29
N_{1600}	54 (18)†	50 (26)	54 (56)	45 (38)

† Number in parentheses is percent increase of N_{1600} over the mean of other N levels.

CONCLUSIONS

Soil organic amendments increased the MB in both the short and long-term. In the long-term the amount of organic material correlated with the amount of MB present, while in the short-term response the composition of the material added influenced the response. Inorganic N did not affect the MB in the long-term, but increased the MB_N in the short-term. The MB to soil C and N ratios were higher under management practices that included an additional organic amendment (manure or pea vine) than in soil that received inorganic or no N fertilizer. The MB changed rapidly in response to soil amendments even in the presence of inorganic N application. This suggests that MB can be increase by adding an organic N source at the same time inorganic N is added, thus, allowing for the re-establishment of an active microbial community without severe plant N deficiencies.

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CHAPTER 4

SOIL ENZYME ACTIVITIES: PROTEASE, HISTIDASE, AND β-GLUCOSIDASE

ABSTRACT

Soils that receive only inorganic nutrients may have a low biological potential to cycle material of organic origin. This can cause nutrient availability problems during a transition from conventional practices to alternative agricultural systems that rely on organic amendments. A greenhouse experiment was conducted to investigate the effects of nutrient management on soil enzyme activity (protease, L-histidine NH_3-lyase, and β-glucosidase) during a simulated transition from inorganic to organic sources of N. Treatments were arranged as a complete factorial that included the following factors: four soils obtained from the Residue Utilization Plots (RUP) initiated in 1931 at the Columbia Basin Research Center, Pendleton, OR (beef manure, pea vine residue, 0 kg N, or 90 kg N ha^{-1}, each applied biennially to a wheat-fallow system); four greenhouse organic residues (pea vine, beef manure, poultry manure, or control); and four rates of N fertilizer (0-1600 mg N 2 kg^{-1} soil as NH_4NO_3). Four successive crops of *Zea mays* L. were grown over a period of 306 days. Soil that received long-term organic inputs had higher enzyme activities than the soil from the 0 kg N or 90 kg N plots regardless of greenhouse organic amendment or N fertilizer treatment. Each greenhouse organic amendment increased activity relative to the control. Pea vine, added in the greenhouse, produced the greatest increase in activity. Inorganic N treatments had little or no significant effect on enzyme activity. In the short-term, it is possible to increase soil enzyme activity by adding an organic substrate while continuing to add inorganic N.

INTRODUCTION

Soils managed with organic materials generally have larger and more active soil microbial populations than soils managed with mineral fertilizers (Bolton et al., 1985; McGill et al., 1986; Dick et al., 1988; Alef et al., 1988; Anwarzay et al., 1990). Most of the biochemical reactions involved in the soil N cycle are catalyzed by enzymes, and the primary source of soil enzymes is believed to be soil microorganisms (Skujins, 1978). When synthetic N fertilizers are reduced or eliminated from a production system, crop N availability dependents on the soil's biological capacity. Thus, enzyme-catalyzed reactions that mineralize organic N and release NH_4^+ become increasingly important. Management practices have the potential to influence enzyme activity and, therefore, may affect the ability of soils to cycle and provide N for plant growth.

The Residue Utilization Plots (RUP) at the Columbia Basin Research Center, OR, have been managed with either organic or inorganic N sources since 1931. As a result, soil from the RUP vary widely in organic matter content and biological activity (Rasmussen et al., 1980, 1989; Dick et al., 1988). Dick et al. (1988) found long-term additions of inorganic N have resulted in decreased urease and amidase activities (enzymes involved in N cycling) relative to the control soil, whereas organic N additions (manure or pea vine) have increased activities . Other soil enzyme activities (acid phosphatase, alkaline phosphatase, arylsulfatase, β-glucosidase, amidase,and urease) are also higher in soils managed with organic N sources than in the control soil.

Soil from this long-term study provided a unique opportunity to study enzyme dynamics during a simulated transition from mineral to organic sources of N in the greenhouse. Protease (EC 3.4) and L-histidine NH_3-lyase (histidase, EC 4.3.1.3) activity were measured because they are enzymes involved in N mineralization. Protease is often thought to catalyze the first step in the N mineralization process since most microorganisms must decompose nitrogenous substrates into low molecular weight compounds before N is assimilated. During

the degradation of proteins, amino acids are released, and subsequent deamination of amino acids is a source of ammonium in soil. Histidase deaminates the amino acid, histidine. β-Glucosidase (EC 3.2.1.21) was measured because its hydrolysis product (glucose) is an energy source for soil microorganisms (Eivazi and Tabatabai, 1988). By manipulating organic and inorganic N inputs, both the short and long-term effects of organic amendments and inorganic N on enzyme activity were investigated. The objective of this study was to determine the impact of soil inputs on the activity of enzymes involved with N and C cycling.

MATERIALS AND METHODS

The soil came from the greenhouse experiment previously described in chapter 2. In brief, a simulated transition from inorganic to organic N sources was conducted to determine the effects of organic residues and decreasing N rates on plant and soil-biological parameters. The experimental design was a completely randomized block with three replications. Treatments were arranged as a complete factorial that included the following factors: four soils obtained from the Residue Utilization Plots (RUP) initiated in 1931 at the Columbia Basin Research Center, Pendleton, OR(beef manure, pea vine residue, 0 kg N, or 90 kg N ha^{-1}, each applied biennially to a wheat-fallow system); four greenhouse organic residues (pea vine, beef manure, poultry manure, or control); and four rates of N fertilizer (0-1600 mg N 2 kg^{-1} soil as NH_4NO_3). Four successive crops of *Zea mays* L. were grown over a period of 306 days.

Soil from the Rup was collected in November 1989 for the greenhouse experiment. Organic residues were added on an equal N basis (1 g total Kjeldahl-N 2 kg^{-1} soil) before cropping began and again after the second crop was harvested. Inorganic N was applied at a split rate at planting and at 21 days after planting. The initial rates were 0, 200, 400, or 400 mg N 2 kg^{-1} soil for the N_0, N_{400}, N_{800}, and N_{1600} treatments, respectively (subscript number is the cumulative mg N added per pot over four crops). The rate of N applied in the N_0 and N_{1600} treatments remained constant throughout the experiment. The N_{400} and N_{800} treatments decreased by one-third of the original rate with each consecutive crop.

Soil enzyme analysis was performed prior to the greenhouse experiment (day 0), after the soil was incubated with the organic residues (day 87), after the second harvest but before any crop was grown (day 164), and after the fourth harvest (day 306). The soil was passed through a 2-mm sieve and stored moist at 4°C in the dark. β-Glucosidase activity was assayed as described by Eivazi and Tabatabai (1988). Protease activity was determined as described by Nannipieri et al. (1979) except controls were incubated with Tris buffer, and received 1 ml of

2.5% casein (in Tris) after the reaction was terminated with 17.5% trichloroacetic acid. Samples were incubated in a rotary shaker (160 RPM) at 52°C. The histidase activity assay of Frankenburger and Johanson (1981) was used with modification. Samples were incubated in 125 ml capped specimen cups and terminated with 25 ml of 2.5 *M* potassium chloride - silver sulfate (100 mg L^{-1}) solution. Use of this procedure eliminated the need for disposal of radioactive uranyl acetate.

All results are expressed on a per g oven dry (105°C, 24 h) weight of soil basis. The data were analyzed by standard ANOVA techniques for randomized blocks with SAS statistical software package (SAS Institute, Cary, NC). Significant main effects were separated with Tukey's at the $p = 0.05$ level.

RESULTS AND DISCUSSION

Inorganic N

Inorganic N added in the greenhouse had little effect on soil enzyme activity. Only β-glucosidase at the 164 day sampling was affected by N fertilizer treatment (Figure 4.1), and the response was approximately proportional to the cumulative amount of N added. Eivazi and Tabatabai (1990) found that $(NH_4)_2SO_4$ or KNO_3 added during the assay decreased β-glucosidase activity by 15%. Inorganic N added in the present study, therefore, was likely incorporated into the organic pool prior to enzyme analysis. Under N-limited conditions, inorganic N is expected to stimulate microbial activity. This may explain the observed results.

In this study inorganic N had no effect on protease or histidase activity (data not shown). Burton (1989) found no significant relationship between protease activity and NH_4^+ or NO_3^- content of a Chernozemic soil sampled throughout a cropping season. Labile histidase activity was inversely related to soil NH_4^+ concentration and at low soil NH_4^+ concentration (< 3 $\mu g\ g^{-1}$) labile histidase activity positively correlated with soil NO_3^- content. In a laboratory incubation study, Burton and McGill (1991) found NH_4^+ additions in the absence of glucose had no effect on soil histidase activity, however, some portion of histidase activity was sensitive to feedback inhibition by NH_4^+-N at low concentrations (0-5 $\mu g\ g^{-1}$). Dick et al. (1988) found that urease and amidase activities were inversely related to the amount of inorganic N added to the soil in the RUP. Since NH_4^+ is the reaction product of both enzymes, the authors postulated that repeated additions of NH_4^+ to the soil had inhibited microbial induction of urease and amidase. Bremner and Mulvaney (1978) reported unpublished data from a laboratory study which showed that several NH_4^+ based fertilizers had no effect on soil urease activity. Ammonium feedback inhibition for urease or amidase activity, however, has not been specifically studied.

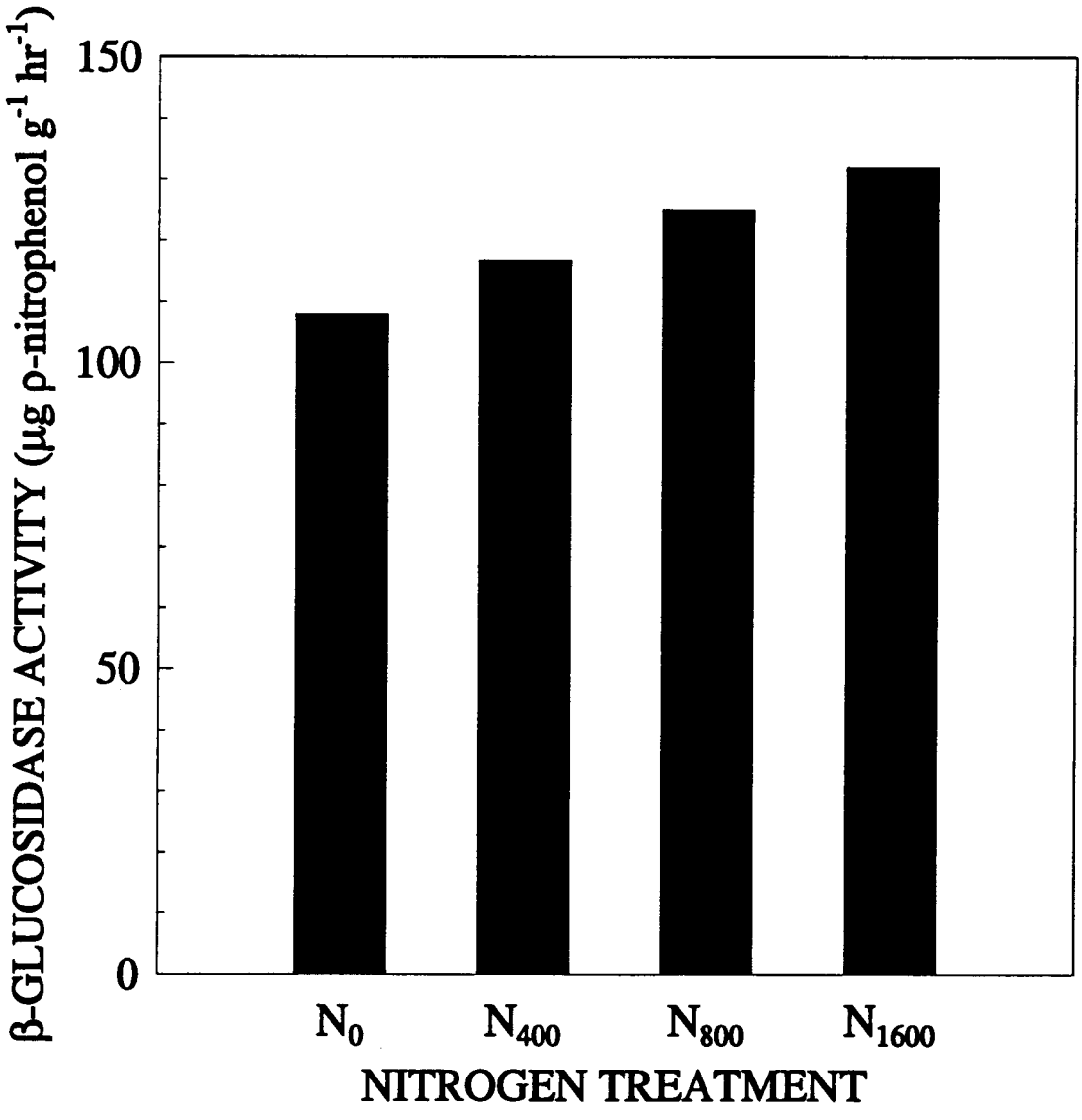

Figure 4.1. β-Glucosidase activity as affected by N treatment at the 164 day sampling, averaged over organic residue and field history treatments (n = 48).

The N fertilizer treatment did not interact with field history or organic residue treatment, therefore, results presented below were averaged over N fertilizer treatment.

Long-term nutrient management practices - field history

The effect of field history varied among the enzymes studied. Protease activity in soil with a long-term history of manure application was significantly greater than in the soil from other RUP treatments (Table 4.1). Soil from the pea vine treatment tended to have higher protease activity than soil from either the inorganic N or control treatments. Alef et al. (1988) found higher protease activity in soil that received organic fertilizers, no pesticides, and included legumes in crop rotations than in soil that received mineral fertilizers.

Table 4.1. Protease activity as affected by long-term management, averaged over organic amendment and N fertilizer treatments (n = 48).

	Sampling day		
Field history	87	164	306
	——— μmol tyrosine g^{-1} soil h^{-1} ———		
Manure	0.98a†	0.86a	0.85a
Pea vine	0.85b	0.67b	0.75b
Nitrogen	0.80b	0.56c	0.61c
Control	0.75b	0.67b	0.61c

† Means followed by the same letter within a column are not significantly different (Tukey's p = 0.05).

Histidase activity was highly variable, especially when an organic residue was added in the greenhouse (coefficient of variation 17-49%, depending on residue treatment). In the control residue treatment, soil that received long-term additions of organic N (manure or pea vine) tended to have higher histidase activity than soil that received inorganic or no N fertilizer (Table 4.2).

Table 4.2. Effect of long-term management on histidase activity in soil from the control residue treatment, averaged over N fertilizer treatment (n = 12).

	Sampling day		
Field history	87	164	306
	$\mu g\ NH_4^+\text{-}N\ g^{-1}$ soil $48\ h^{-1}$		
Manure	270a†	252ab	254a
Pea vine	253ab	278a	250a
Nitrogen	221ab	201bc	242a
Control	204b	182c	242a

† Means followed by the same letter within a column are not significantly different (Tukey's p = 0.05).

β-Glucosidase activity differences among soil from the RUP treatments were similiar to those observed for histidase activity. Although there was some interaction between the organic residue and field history treatments (mainly in the pea vine residue treatment), soil that received either manure or pea vine in the field tended to produce 20 μmol ρ-nitrophenol (PNP) g^{-1} soil hr^{-1} more than soil that received inorganic or no N fertilizer in the field (Figure 4.2). Data from a five year field study showed soil that received green manure plus either farmyard manure or crop residues had higher β-glucosidase activity than soil that did not receive organic inputs (Verstaete and Voets, 1977). In an 80-year crop rotation and fertilizer study, organic fertilizers increased enzyme activities (β-glucosidase, phosphatase, protease, xylanase, urease, and cellulase) more than mineral fertilizers, and lowest enzyme activities were found on non-fertilized plots (Anwarzay et al., 1990).

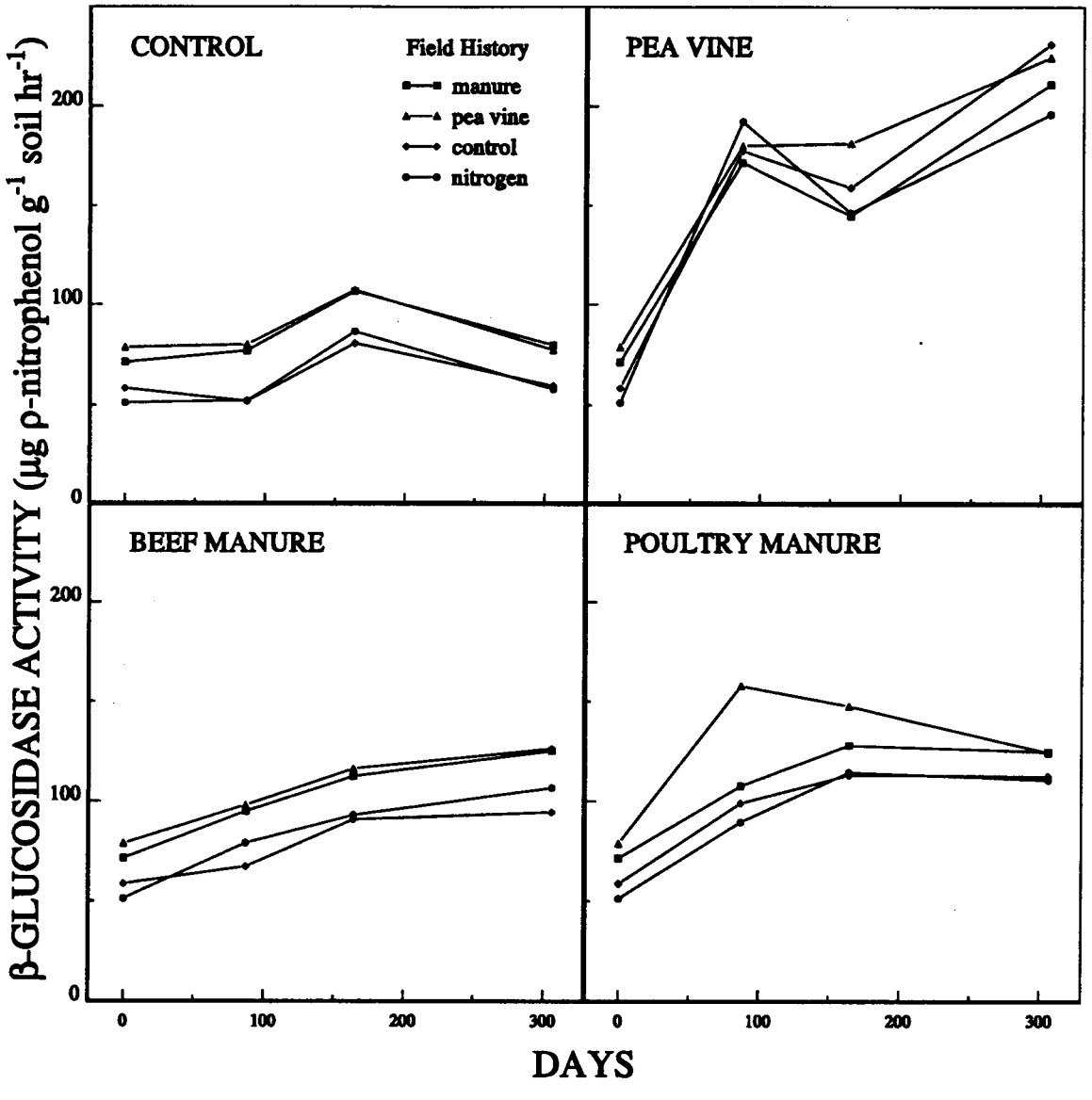

Figure 4.2. β-Glucosidase activity in the organic residue treatments as affected by field history, averaged over N fertilizer treatment (n = 12).

Others have correlated protease, histidase, and β-glucosidase activity with soil organic C and total N content (Niskanen and Eklund, 1986; Speir et al., 1980; Frankenburger and Johanson, 1983; Eivazi and Tabatabai, 1990). In general, enzyme activity measured prior to the greenhouse experiment followed soil organic C and total N levels (Table 4.3).

Table 4.3. Soil enzyme activity, organic C, and total N prior to the greenhouse experiment.

Field History	Protease	Histidase	β-Glucosidase	Organic C	Total N
	μg TYR g^{-1} h^{-1}†	μg NH_4^+-N g^{-1} 48 h^{-1}	μg PNP g^{-1} h^{-1}‡	——— g kg^{-1} ———	
Manure	0.76†	241‡	71.4	14.0	1.21
Pea vine	0.50	228	78.7	11.5	0.92
Nitrogen	0.39	170	51.1	10.5	0.82
Control	0.41	172	58.5	9.8	0.83

† TYR represents tyrosine.
‡ PNP represents ρ-nitrophenol.

Soil that received long-term additions of organic N tended to have higher enzyme activity than soil that received inorganic or no N fertilizer. Organic N provides a C source to heterotrophic microbes and since soil enzymes originate from microbes, proliferation of microbes should increase potential enzyme activity. Less C is returned to the soil in the RUP that receives long-term additions of inorganic N (2370 kg C ha^{-1} 2 yr^{-1}) than in either pea vine or manure amended soil (3230 and 4265 kg C ha^{-1} 2 yr^{-1}, respectively). Nitrogen mineralization rate is greater in soil from the inorganic N RUP (0.0211 mg N kg^{-1} soil d^{-1}) than in soil managed with either manure or pea vine (0.0152, and 0.0156 mg N kg^{-1} soil d^{-1}, respectively; Christ and Dick, 199x). Smaller C additions and more rapid N mineralization may account for the lower microbial biomass (Chapter 3; Rasmussen et al., 1989) and lower enzyme activity found in the soil managed with inorganic N.

Recent additions of organic residues

Recent additions of organic residues had a greater effect on enzyme activities than either field history or N fertilizer treatment. Organic residues added in the greenhouse increased protease and β-glucosidase activity relative to the control (Tables 4.4 and 4.5 and Figures 4.2 and 4.3) response to the different organic residue treatments varied, however, pea vine amended soil had the highest enzyme activity. Adding an organic residue increased histidase activity (Figure 4.4), however, the assay was highly variable which makes interpretation difficult.

Increased potential enzyme activity may be the result of direct addition of enzymes via the residue, or microbial proliferation or microbial induction in response to residue addition. Pea vine was the only residue itself that had appreciable enzyme activity, and only β-glucosidase activity at that (potential contribution at rate added amounts to 88 μg PNP g^{-1} soil hr^{-1}). Pea vine addition increased soil protease activity as well as soil β-glucosidase activity even though pea vine residue did not have any protease activity itself. Increased enzyme activity in response to pea vine addition was, therefore, most likely a result of microbial proliferation or induction. No attempt was made to distinguish between proliferation or induction.

After the initial organic residue treatments were mixed with the soil in the greenhouse, the soil surface of pots amended with the pea vine residue was covered with a thick mat of *Mucor* sp.. This fungal proliferation may have contributed to the tremendous increase in β-glucosidase activity observed in this treatment. By subjecting an Andisol in a greenhouse to various sterilants that inhibit certain groups of microflora, Hayano and Tubaki (1985) concluded that mucoraceaous fungi were the primary source of β-glucosidase in the soil studied.

Table 4.4. Protease activity as affected by greenhouse amendment, averaged over N fertilizer and field history treatment (n = 48).

	Sampling day		
Organic amendment	87	164	306
Pea vine	1.97a†	1.18a	1.37a
Beef manure	0.63b	0.74b	0.73b
Poultry manure	0.44c	0.44c	0.48c
Control	0.33d	0.44c	0.23d

† Means followed by the same letter within a column are not significantly different (Tukey's p = 0.05).

Table 4.5. β-Glucosidase activity as affected by greenhouse amendment, averaged over N fertilizer and field treatment (n = 48).

	Sampling day		
Greenhouse amendment	87	164	306
Pea vine	180.3a†	158.1a	215.7a
Poultry manure	113.5b	126.1b	118.3b
Beef manure	84.7c	101.3c	113.1b
Control	65.3d	95.2d	68.6c

† Means followed by the same letter within a column are not significantly different (Tukey's p = 0.05).

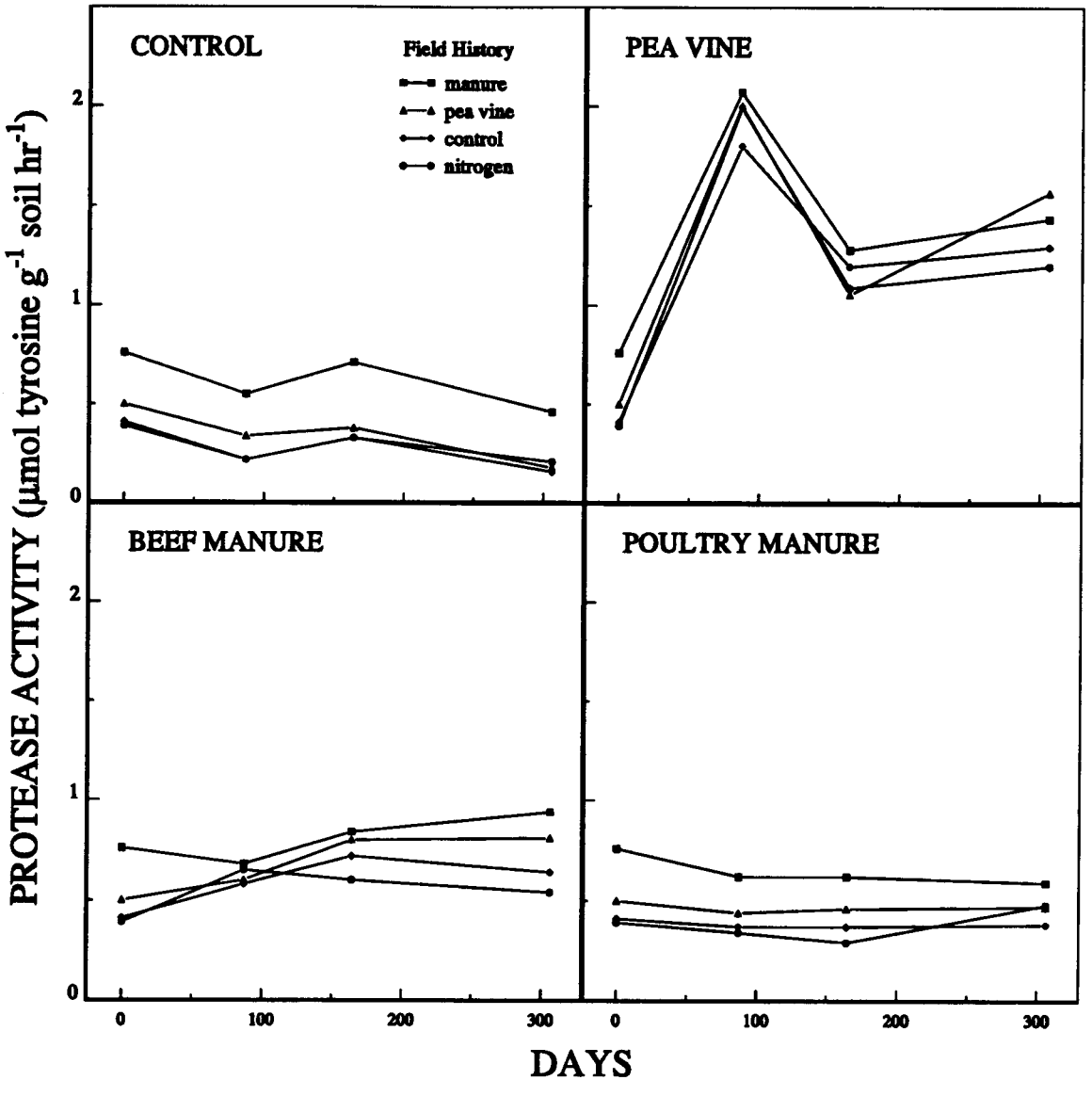

Figure 4.3. Protease activity in the organic residue treatments as affected by field history, averaged over N fertilizer treatment (n = 12).

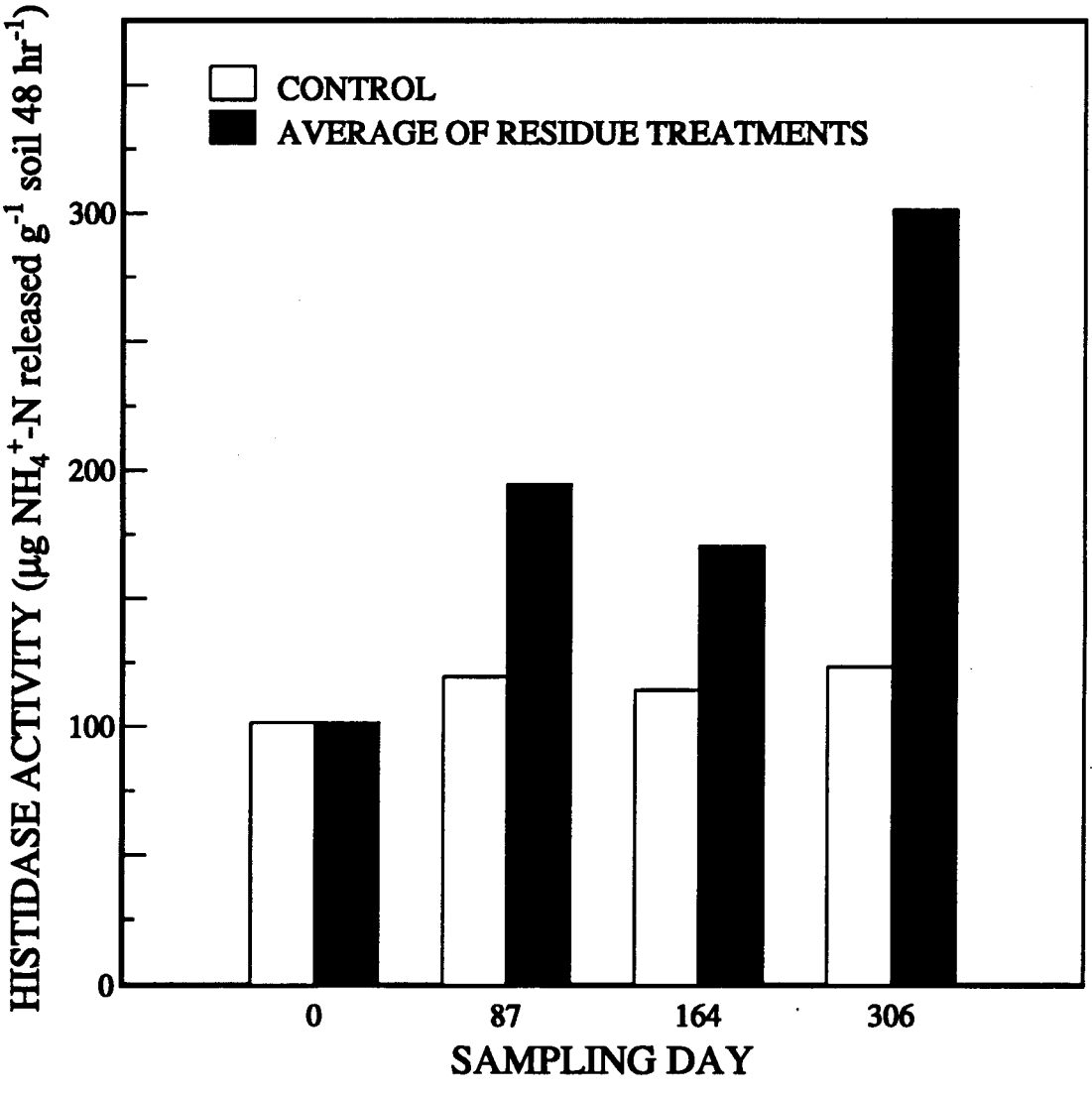

Figure 4.4. Mean effect of organic residue treatments on histidase activity, averaged over N fertilizer treatment (n = 48 for control; n = 144 for mean of residue treatments).

Protease activity in soil from the poultry manure treatment was similar to that of soil in the control treatment (Table 4.4 and Figure 4.3). Inorganic N content of the poultry manure, pea vine, and beef manure account for 11.5, 1.9, and 3.9%, respectively of the total N added in each treatment. Therefore, since more of the N in the poultry manure was inorganic, less organic N substrate was available to stimulate proteolytic enzymes in this treatment. Also, the poultry manure treatment added only one-third as much C as in either pea vine or beef manure treatment.

Pea vine and beef manure added approximately the same amount of C to the soil (21 and 24 g 2 kg^{-1} soil, respectively) and all residues were added on an equal N basis. However, pea vine treatment had a greater effect on protease and β-glucosidase activity than beef manure (Tables 4.4 and 4.5 and Figures 4.2 and 4.3). Adding a fresh energy source to soil enhances microbial growth and, therefore, has the potential to increase soil enzyme activity. Tate (1984) found that adding a readily metabolizable substrate (sugarcane leaves) to a large pool of organic material already present in a Histosol increased protease activity. Pea vine residue was not composted as were the poultry and beef manure, and it contained less lignin than the beef manure. Pea vine, therefore, was more readily metabolizable than the beef manure which may explain the higher protease and β-glucosidase activity measured in soil from the pea vine residue treatment.

Although poultry manure treatment had little effect on protease activity (Table 4.4 and Figure 4.3), it significantly increased β-glucosidase activity (Table 4.5 and Figure 4.2). There was some interaction in the pea vine field history - poultry manure residue treatment combination at crop 1 (Figure 4.2), however, with the exception of this interaction beef manure and poultry manure residue treatments had a similar effect on β-glucosidase activity when this interaction is ignored.

CONCLUSIONS

Increased soil enzyme activity resulting from recent organic N additions depended on the composition of the substance added, not on the amount of N or C applied. In the short-term, inorganic N fertilizer had little effect on soil enzyme activity. Long-term additions of inorganic N, however, resulted in low enzyme activity when compared with management practices that returned more C to the soil. The data suggest that low enzyme activity in soil that received repeated additions of inorganic N did not result from inhibition by the inorganic N itself, but rather was related to the amount and availability of C added. In the short-term, soil enzyme activity can be increased by adding a readily metabolizable organic substrate while continuing to add inorganic N.

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CHAPTER 5

SUMMARY AND PERSPECTIVES

Crop DMY and N uptake, microbial biomass, and soil enzyme activity as affected by organic residues, N fertilizer, and long-term management history during a simulated transition from inorganic to organic sources of N were discussed in Chapters 2, 3, and 4, respectively. The purpose of this chapter is to summarize and discuss these results as they relate to one another.

Soil Enzymes and Microbial Biomass

Soil microorganisms are believed to be the primary source of soil enzymes (Skujins, 1978), therefore, as expected, enzyme activity response to soil additions was similar to that of the microbial biomass (Figure 5.1). Soil from the pea vine residue treatment had the highest microbial biomass and enzyme activity generally followed by soil from the beef manure, poultry manure and control residue treatments. β-Glucosidase activity, however, was higher in soil from the poultry manure treatment than in beef manured treated soil (Figure 5.1). Since the poultry manure had no appreciable β-glucosidase activity itself, the poultry manure amendment either induced microbial production of β-glucosidase or promoted selective growth of microorganisms that constitutively produce more β-glucosidase.

Plant and Soil Biological Parameters

In general soil enzyme activity and microbial biomass are not good predictors of plant growth (Dick et al., 1988; Skujins, 1978). Since enzymes chosen for this study are involved in N and C cycling, their activity might reflect soil N availability. In the absence of inorganic N, differences in DMY and N uptake resulting from long-term soil management were similar to biological parameters measured in the control residue treatment (Figure 5.2). However, when an organic residue was added in the greenhouse soil biological and plant parameter response was not the same (Table 5.1). The poultry manure residue treatment provided the most plant available N, but did not increase soil biological parameters very much. Beef manure added in the greenhouse increased soil biological parameters without increasing DMY or N uptake. In general, organic N additions

increased the soil biological parameters measured. Nitrogen availability during a transition will depend on the composition of the organic substrate added as indicated by the observed differences in N uptake. When inorganic N was added in the greenhouse neither enzyme activities or microbial biomass were closely related to maize yield or N uptake.

Most soil biological parameters were lower at the 164 day sampling than on the 306 day sampling (Figure 5.1) even though approximately the same amount of time had elapsed between organic residue addition and soil sampling (164 and 142 days, respectively). Higher soil biological parameters at the 306 day sampling may indicate a cumulative effect. However, a more plausible explanation centers around plant response. The 164 day sampling immediately followed the harvest of crop 2, and the largest differences in DMY and N uptake among N treatments occurred during crop 2 (DMY is presented as an example in Figure 5.3). Even though all soil biological parameters weren't significantly affected by N treatment, competition between plants and microbes at this time would be expected. Competition for N may explain the lower soil biological parameter measurements obtained at the 164 day sampling. The microbes were probably N limited at the 164 day sampling.

Only β-glucosidase activity measured after the crop 2 harvest (164 day sampling) was affected by N treatment (Figure 4.1). Activity increased with the amount of N applied as did dry matter yield (Figure 5.3). Root exudates contain C substrates for β-glucosidase. Since the microbial biomass was not affected by N treatment at this sampling date, β-glucosidase may have been induced by the presence of increased C substrates resulting from N application.

When crop 4 was grown, inorganic N was only applied to the N_{1600} treatment. In the pea vine, beef manure, and control treatments, N uptake was the same for the N_0, N_{400}, and N_{800} rate within each residue treatment and more N was taken up by plants in the N_{1600} treatment. N uptake was unaffected by N treatment in the poultry manure residue treatment at crop 4. The MB_N measured after harvesting crop 4 was significantly higher in the N_{1600} treatment than in the other inorganic N treatments which were not different from one another. Apparently,

under N limiting conditions, microbial biomass and plants compete for inorganic N. When nutrients are in excess of plant requirements microbes become a nutrient sink.

The relative differences in microbial biomass and soil enzyme activity among soils from the RUP remained relatively constant throughout the experiment (Figure 5.2). The greater increase in MB_N in response to N treatment in the soil from the nitrogen RUP relative to soils from the other RUP is an exception to this. In general, regardless of past history, soil from the RUP responded similarly to recent additions of organic residues and N fertilizer.

Perspectives

In the short-term, pea vine was the best organic N source studied for increasing soil biology while maintaining plant productivity. In the short-term, choosing an organic N source as a replacement for inorganic N in a cropping system will depend on the timing and extent of N mineralization from the organic substrate. In the long-term, a substrate that builds soil organic matter, thus improving soil fertility, biological, and physical parameters (such as the manure added to the RUP) may be the best choice, if available. However, since N_2 fixation by rhizobia associated with legumes is an addition rather than a translocation of N, inclusion of legumes in cropping systems will be important in sustaining soil productivity in the future.

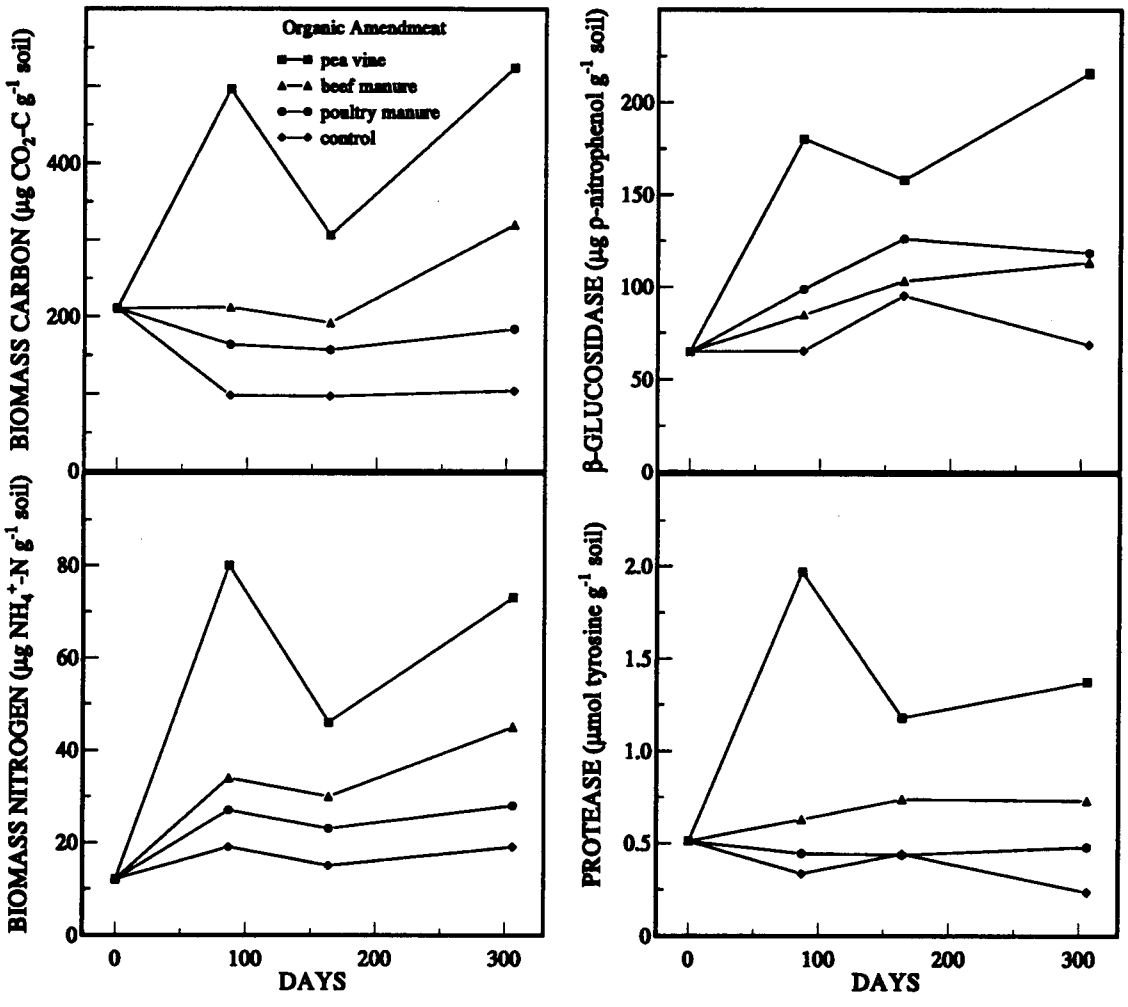

Figure 5.1. Effect of recent organic residue amendments on soil MB_C, MB_N, β-glucosidase, and protease activity, averaged over field history and N treatment (n = 48).

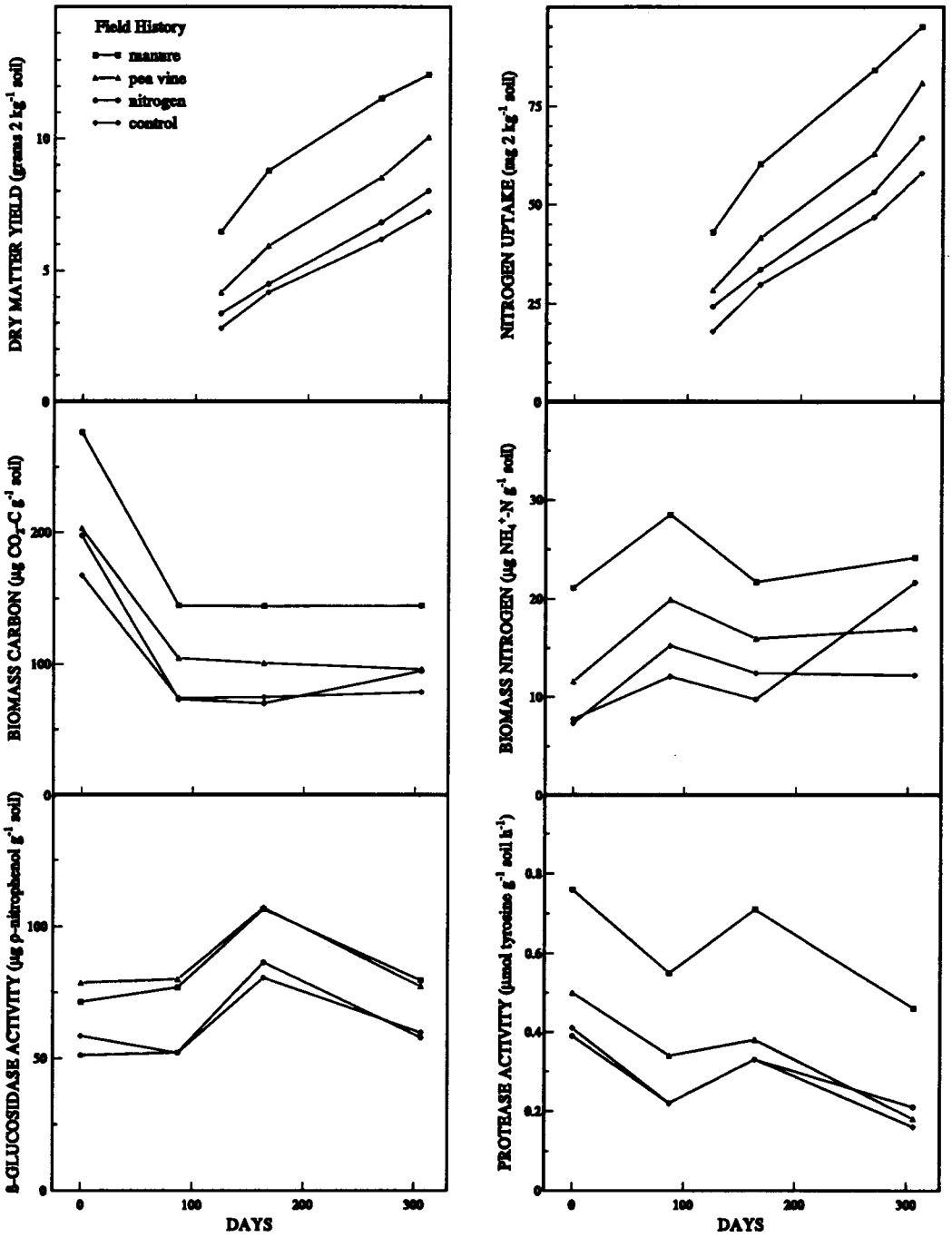

Figure 5.2. Effect of field history on plant and biological parameters in the control residue treatment, N_0 for the plant parameters (n = 3) and averaged over N rate for the biological parameters (n = 12).

Table 5.1. Effect of recent organic amendments on plant and soil biological parameters at the 306 day sampling in the N_0 treatment for the plant parameters (n = 12) and averaged over N treatment for the biological parameters (n = 48), both averaged over field history.

Organic Amendment	Protease	Glucosidase	Biomass C	Biomass N	N Uptake	Yield
	μmol TYR† g^{-1}	μg PNP‡ g^{-1}	μg CO_2-C g^{-1}	μg NH_4^+-N g^{-1}	mg N 2 kg^{-1}	g DMY 2 kg^{-1}
Pea vine	1.37 (496)§	216 (222)	524 (404)	73 (288)	470 (525)	32.6 (247)
Beef manure	0.73 (217)	118 (76)	320 (208)	45 (141)	65 (-13)	7.5 (-20)
Poultry manure	0.48 (109)	113 (67)	184 (77)	28 (48)	675 (797)	30.5 (224)
Control	0.23	67	104	19	75	9.4

† TYR represents tyrosine.
‡ PNP represents ρ-nitrophenol.
§ number in parentheses is percent increase over control.

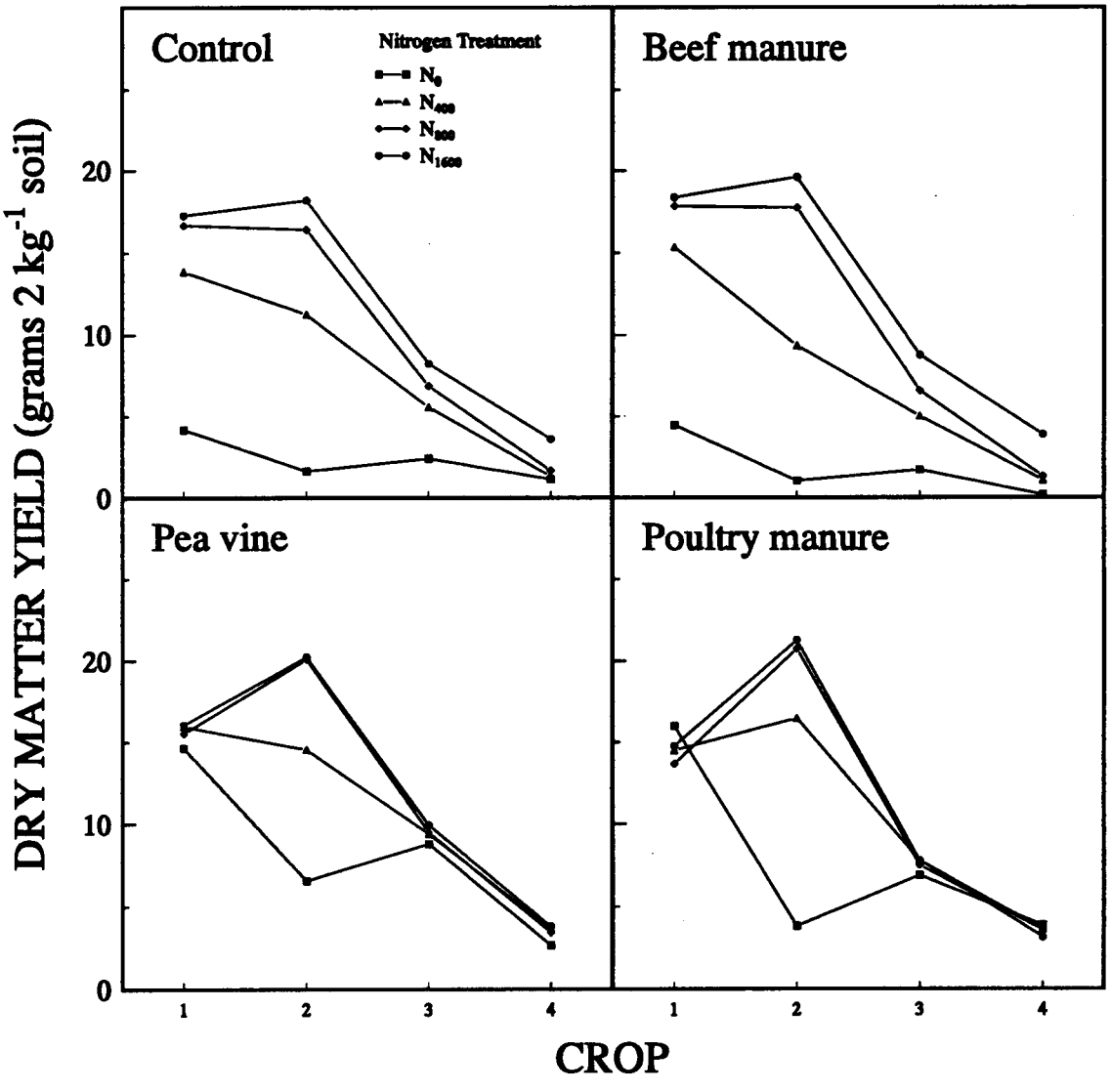

Figure 5.3. Effect of N treatment on DMY in the organic residue treatments, averaged over field history (n = 12).

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APPENDICES

Appendix A. Plant dry matter yield and N concentration - crop 1.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
PV0051	13.89	2.15	0.086	0.071
PV0052	12.54	3.18	0.110	0.075
PV0053	10.67	2.12	0.121	0.074
PV0081	14.15	3.41	0.090	0.064
PV0082	11.52	2.18	0.162	0.094
PV0083	13.02	3.03	0.143	0.072
PV0091	14.74	3.15	0.095	0.056
PV0092	11.65	2.81	0.121	0.074
PV0093	10.65	1.84	0.145	0.076
PV0101	9.02	1.45	0.138	0.102
PV0102	9.89	2.21	0.099	0.065
PV0103	13.50	2.80	0.098	0.075
PV1051	18.46	3.88	0.133	0.082
PV1052	12.77	2.53	0.207	0.109
PV1053	13.60	2.46	0.170	0.121
PV1081	15.36	3.10	0.166	0.079
PV1082	12.51	2.10	0.199	0.116
PV1083	8.13	2.71	0.201	0.094
PV1091	15.64	2.56	0.168	0.118
PV1092	12.72	2.91	0.149	0.095
PV1093	15.05	3.31	0.177	0.102
PV1101	11.99	1.85	0.220	0.118
PV1102	10.63	1.91	0.219	0.110
PV1103	13.12	2.19	0.202	0.089
PV2051	15.52	1.96	0.218	0.150
PV2052	13.36	2.38	0.262	0.151
PV2053	12.09	1.72	0.257	0.155
PV2081	16.11	2.90	0.253	0.124
PV2082	13.31	2.52	0.272	0.159
PV2083	14.02	2.42	0.231	0.144
PV2091	15.48	3.17	0.197	0.128
PV2092	9.58	2.06	0.254	0.147
PV2093	14.42	1.83	0.235	0.169
PV2101	10.11	1.42	0.282	0.172
PV2102	14.25	2.46	0.234	0.119
PV2103	11.74	1.61	0.279	0.147
PV2A051	14.51	2.04	0.184	0.141
PV2A052	14.64	2.19	0.224	0.144
PV2A053	15.40	2.19	0.231	0.145
PV2A081	14.67	2.84	0.215	0.126
PV2A082	10.69	1.82	0.275	0.191
PV2A083	16.04	2.36	0.258	0.147
PV2A091	15.09	2.68	0.211	0.114
PV2A092	11.90	1.90	0.269	0.149
PV2A093	15.87	2.15	0.243	0.142
PV2A101	18.04	3.33	0.185	0.100
PV2A102	12.61	2.49	0.259	0.113
PV2A103	5.35	1.97	0.275	0.147

Appendix A, continued. Plant dry matter yield and N concentration - crop 1.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
BM0051	4.15	1.22	0.068	0.055
BM0052	3.02	0.96	0.078	0.053
BM0053	4.27	1.26	0.081	0.048
BM0081	5.82	1.50	0.061	0.048
BM0082	3.75	0.99	0.088	0.055
BM0083	3.14	0.90	0.084	0.054
BM0091	4.12	1.17	0.083	0.053
BM0092	3.44	1.08	0.085	0.060
BM0093	2.84	0.90	0.079	0.059
BM0101	2.59	0.65	0.067	0.061
BM0102	2.10	0.74	0.084	0.052
BM0103	2.40	0.60	0.088	0.058
BM1051	15.92	2.99	0.083	0.058
BM1052	9.69	2.17	0.120	0.079
BM1053	12.79	2.47	0.118	0.060
BM1081	13.72	3.05	0.103	0.064
BM1082	12.63	2.76	0.113	0.058
BM1083	12.10	3.08	0.108	0.064
BM1091	14.69	2.68	0.089	0.063
BM1092	12.30	3.06	0.107	0.072
BM1093	10.70	1.81	0.122	0.069
BM1101	14.26	3.16	0.081	0.068
BM1102	2.07	0.22	0.235	0.213
BM1103	11.01	1.83	0.108	0.081
BM2051	15.88	2.72	0.179	0.076
BM2052	12.14	3.02	0.118	0.075
BM2053	15.23	2.73	0.162	0.079
BM2081	16.09	3.55	0.173	0.087
BM2082	14.51	3.20	0.167	0.098
BM2083	11.11	2.02	0.209	0.098
BM2091	18.35	3.53	0.141	0.083
BM2092	14.06	2.73	0.185	0.097
BM2093	15.29	3.20	0.159	0.100
BM2101	18.56	3.32	0.100	0.092
BM2102	12.97	2.71	0.170	0.096
BM2103	14.61	2.39	0.167	0.105
BM2A051	17.76	3.70	0.143	0.072
BM2A052	16.57	3.09	0.141	0.091
BM2A053	13.98	2.15	0.190	0.096
BM2A081	18.41	2.77	0.157	0.086
BM2A082	15.69	3.24	0.171	0.082
BM2A083	14.72	2.33	0.191	0.089
BM2A091	17.29	4.02	0.151	0.081
BM2A092	13.24	2.07	0.186	0.103
BM2A093	14.29	3.07	0.167	0.079
BM2A101	17.40	3.11	0.152	0.078
BM2A102	13.50	1.99	0.139	0.080
BM2A103	13.55	2.57	0.174	0.084

Appendix A, continued. Plant dry matter yield and N concentration - crop 1.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
PM0051	13.99	1.33	0.239	0.099
PM0052	11.21	1.56	0.247	0.160
PM0053	12.49	1.34	0.236	0.131
PM0081	16.05	2.72	0.184	0.075
PM0082	14.25	2.75	0.209	0.122
PM0083	13.92	1.99	0.207	0.119
PM0091	12.81	1.92	0.290	0.108
PM0092	14.94	2.65	0.246	0.103
PM0093	15.79	2.92	0.271	0.087
PM0101	16.54	1.93	0.171	0.079
PM0102	12.48	2.77	0.219	0.103
PM0103	11.90	1.59	0.231	0.101
PM1051	14.67	1.92	0.234	0.124
PM1052	12.78	2.21	0.255	0.166
PM1053	14.63	1.84	0.249	0.144
PM1081	13.39	1.91	0.259	0.167
PM1082	13.66	2.10	0.262	0.175
PM1083	11.87	1.50	0.265	0.189
PM1091	17.77	2.49	0.261	0.131
PM1092	10.77	1.51	0.242	0.176
PM1093	5.78	1.60	0.273	0.191
PM1101	14.13	1.92	0.249	0.155
PM1102	8.74	1.18	0.256	0.169
PM1103	13.52	1.93	0.272	0.126
PM2051	16.93	2.81	0.245	0.157
PM2052	12.94	2.63	0.283	0.190
PM2053	13.71	2.35	0.262	0.184
PM2081	9.33	2.98	0.243	0.177
PM2082	7.02	2.02	0.232	0.181
PM2083	14.04	2.12	0.266	0.176
PM2091	15.72	1.84	0.269	0.179
PM2092	14.15	2.30	0.277	0.197
PM2093	7.99	1.26	0.256	0.251
PM2101	7.44	1.24	0.287	0.224
PM2102	9.02	1.41	0.258	0.231
PM2103	11.24	1.54	0.261	0.234
PM2A051	14.74	2.02	0.273	0.226
PM2A052	13.38	2.22	0.266	0.195
PM2A053	10.59	1.32	0.283	0.246
PM2A081	13.30	1.92	0.237	0.166
PM2A082	10.17	2.60	0.289	0.175
PM2A083	10.70	1.49	0.279	0.000
PM2A091	16.16	2.44	0.256	0.178
PM2A092	12.42	2.11	0.212	0.195
PM2A093	12.40	2.00	0.177	0.251
PM2A101	16.77	2.37	0.268	0.155
PM2A102	12.29	2.35	0.166	0.172
PM2A103	11.16	1.37	0.189	0.240

Appendix A, continued. Plant dry matter yield and N concentration - crop 1.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
C0051	3.47	0.77	0.074	0.048
C0052	1.87	0.50	0.081	0.071
C0053	2.65	0.88	0.077	0.053
C0081	5.33	1.58	0.073	0.049
C0082	5.64	1.82	0.064	0.061
C0083	3.89	1.13	0.073	0.066
C0091	3.53	0.95	0.073	0.059
C0092	3.56	1.07	0.073	0.060
C0093	2.66	0.73	0.064	0.064
C0101	2.11	0.67	0.059	0.060
C0102	2.30	0.86	0.067	0.055
C0103	1.81	0.65	0.068	0.071
C1051	14.01	2.33	0.092	0.073
C1052	11.38	2.50	0.101	0.066
C1053	10.88	2.14	0.123	0.063
C1081	13.56	2.82	0.088	0.072
C1082	11.29	2.14	0.143	0.085
C1083	11.53	2.50	0.113	0.075
C1091	12.85	2.09	0.088	0.074
C1092	11.36	2.33	0.099	0.074
C1093	10.89	2.17	0.104	0.079
C1101	12.18	2.55	0.094	0.066
C1102	9.74	2.08	0.120	0.069
C1103	9.78	1.40	0.146	0.086
C2051	17.32	2.89	0.147	0.098
C2052	9.49	1.99	0.173	0.106
C2053	11.24	1.78	0.193	0.120
C2081	18.03	3.80	0.153	0.089
C2082	16.15	2.73	0.167	0.111
C2083	13.59	2.97	0.185	0.102
C2091	16.22	2.29	0.151	0.093
C2092	13.78	3.08	0.160	0.100
C2093	14.90	3.05	0.172	0.106
C2101	14.65	2.34	0.171	0.126
C2102	13.40	2.46	0.178	0.122
C2103	10.72	1.16	0.162	0.080
C2A051	17.49	3.22	0.125	0.088
C2A052	14.53	2.57	0.160	0.107
C2A053	14.19	1.99	0.178	0.095
C2A081	20.34	4.00	0.133	0.084
C2A082	12.64	1.87	0.214	0.109
C2A083	13.82	1.81	0.187	0.101
C2A091	4.64	0.96	0.232	0.165
C2A092	13.55	2.39	0.168	0.094
C2A093	13.86	1.91	0.182	0.098
C2A101	16.88	2.27	0.127	0.107
C2A102	14.59	2.51	0.158	0.112
C2A103	12.09	1.44	0.207	0.063

Appendix A, continued. Plant dry matter yield and N concentration - crop 2.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
PV0051	5.54	1.60	0.086	0.057
PV0052	4.52	0.88	0.110	0.071
PV0053	5.22	1.22	0.121	0.073
PV0081	6.74	1.85	0.090	0.053
PV0082	5.05	1.16	0.162	0.065
PV0083	5.71	1.35	0.143	0.057
PV0091	5.69	1.57	0.095	0.054
PV0092	4.75	1.30	0.121	0.057
PV0093	3.60	0.83	0.145	0.060
PV0101	6.31	1.61	0.138	0.056
PV0102	5.47	1.42	0.099	0.063
PV0103	4.14	1.04	0.098	0.052
PV1051	13.41	3.48	0.133	0.055
PV1052	12.55	2.90	0.207	0.068
PV1053	12.53	2.73	0.170	0.074
PV1081	13.77	3.24	0.166	0.063
PV1082	12.89	3.23	0.199	0.065
PV1083	6.77	1.21	0.201	0.077
PV1091	12.26	2.70	0.168	0.062
PV1092	11.16	2.50	0.149	0.070
PV1093	11.44	2.69	0.177	0.071
PV1101	13.09	3.01	0.220	0.058
PV1102	12.04	2.48	0.219	0.062
PV1103	10.84	1.62	0.202	0.073
PV2051	19.65	5.12	0.218	0.076
PV2052	14.89	3.29	0.262	0.094
PV2053	15.31	2.94	0.257	0.093
PV2081	18.83	4.71	0.253	0.070
PV2082	13.56	2.78	0.272	0.105
PV2083	15.88	2.98	0.231	0.090
PV2091	18.02	3.92	0.197	0.072
PV2092	13.89	3.29	0.254	0.085
PV2093	16.43	2.87	0.235	0.083
PV2101	17.78	3.52	0.282	0.091
PV2102	16.60	3.92	0.234	0.081
PV2103	18.14	2.76	0.279	0.080
PV2A051	20.58	5.23	0.184	0.086
PV2A052	15.84	3.39	0.224	0.103
PV2A053	16.00	2.62	0.231	0.111
PV2A081	18.74	4.00	0.215	0.081
PV2A082	15.46	3.32	0.275	0.122
PV2A083	17.15	3.12	0.258	0.104
PV2A091	19.85	4.56	0.211	0.078
PV2A092	12.43	2.02	0.269	0.130
PV2A093	16.65	2.93	0.243	0.103
PV2A101	18.82	3.69	0.185	0.087
PV2A102	13.93	2.89	0.259	0.103
PV2A103	16.79	2.82	0.275	0.091

Appendix A, continued. Plant dry matter yield and N concentration - crop 2.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
BM0051	0.41	0.36	0.068	0.069
BM0052	0.50	0.32	0.078	0.072
BM0053	0.62	0.34	0.081	0.070
BM0081	0.81	0.55	0.061	0.063
BM0082	0.85	0.38	0.088	0.079
BM0083	1.14	0.50	0.084	0.071
BM0091	0.57	0.54	0.083	0.064
BM0092	0.53	0.45	0.085	0.071
BM0093	0.48	0.48	0.079	0.088
BM0101	0.14	0.47	0.067	0.068
BM0102	0.58	0.48	0.084	0.071
BM0103	0.79	0.43	0.088	0.064
BM1051	6.84	1.75	0.083	0.062
BM1052	8.55	2.08	0.120	0.064
BM1053	6.33	1.15	0.118	0.067
BM1081	9.61	2.72	0.103	0.058
BM1082	9.34	2.65	0.113	0.060
BM1083	8.98	1.82	0.108	0.066
BM1091	5.37	1.68	0.089	0.079
BM1092	6.79	1.33	0.107	0.061
BM1093	7.62	1.55	0.122	0.064
BM1101	7.68	1.99	0.081	0.054
BM1102	11.12	2.91	0.235	0.066
BM1103	5.70	1.19	0.108	0.076
BM2051	18.57	4.14	0.179	0.062
BM2052	13.37	3.21	0.118	0.066
BM2053	15.17	2.97	0.162	0.065
BM2081	15.52	3.16	0.173	0.059
BM2082	14.51	3.12	0.167	0.065
BM2083	13.48	3.00	0.209	0.066
BM2091	17.66	3.86	0.141	0.061
BM2092	13.41	2.47	0.185	0.079
BM2093	13.42	3.00	0.159	0.100
BM2101	15.22	3.16	0.100	0.064
BM2102	12.30	2.58	0.170	0.080
BM2103	13.33	2.27	0.167	0.069
BM2A051	17.41	3.40	0.143	0.079
BM2A052	15.95	3.18	0.141	0.080
BM2A053	14.81	2.23	0.190	0.082
BM2A081	19.76	4.56	0.157	0.069
BM2A082	13.81	3.70	0.171	0.096
BM2A083	17.74	3.24	0.191	0.090
BM2A091	17.39	3.88	0.151	0.070
BM2A092	16.31	3.18	0.186	0.086
BM2A093	15.44	2.44	0.167	0.093
BM2A101	17.60	3.57	0.152	0.083
BM2A102	16.11	3.44	0.139	0.086
BM2A103	14.14	1.86	0.174	0.093

Appendix A, continued. Plant dry matter yield and N concentration - crop 2.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
PM0051	3.24	0.88	0.239	0.051
PM0052	3.21	1.11	0.247	0.057
PM0053	2.06	0.82	0.236	0.057
PM0081	2.94	1.03	0.184	0.053
PM0082	3.60	0.90	0.209	0.052
PM0083	2.88	0.59	0.207	0.059
PM0091	3.52	1.08	0.290	0.056
PM0092	2.26	0.66	0.246	0.059
PM0093	2.10	0.60	0.271	0.051
PM0101	3.07	1.04	0.171	0.054
PM0102	3.01	0.97	0.219	0.053
PM0103	3.06	0.91	0.231	0.061
PM1051	14.62	3.34	0.234	0.063
PM1052	13.59	3.14	0.255	0.082
PM1053	12.77	2.48	0.249	0.061
PM1081	18.35	4.18	0.259	0.064
PM1082	12.31	2.73	0.262	0.063
PM1083	15.88	3.00	0.265	0.067
PM1091	10.78	2.80	0.261	0.055
PM1092	15.37	2.98	0.242	0.074
PM1093	9.31	1.23	0.273	0.099
PM1101	14.59	2.80	0.249	0.067
PM1102	15.80	2.97	0.256	0.074
PM1103	10.86	1.78	0.272	0.064
PM2051	19.24	4.45	0.245	0.090
PM2052	14.28	2.64	0.283	0.118
PM2053	18.04	3.21	0.262	0.103
PM2081	18.42	3.80	0.243	0.075
PM2082	15.59	3.28	0.232	0.106
PM2083	16.38	3.29	0.266	0.092
PM2091	20.07	3.74	0.269	0.073
PM2092	15.65	3.38	0.277	0.078
PM2093	16.22	2.48	0.256	0.150
PM2101	20.54	2.83	0.287	0.111
PM2102	17.15	2.91	0.258	0.113
PM2103	18.22	3.02	0.261	0.095
PM2A051	17.84	3.32	0.273	0.164
PM2A052	16.36	3.19	0.266	0.142
PM2A053	17.22	2.13	0.283	0.154
PM2A081	23.09	5.14	0.237	0.105
PM2A082	17.76	3.43	0.289	0.107
PM2A083	18.23	3.03	0.279	0.143
PM2A091	20.72	5.50	0.256	0.082
PM2A092	16.72	2.82	0.212	0.134
PM2A093	17.88	2.47	0.177	0.123
PM2A101	16.98	3.94	0.268	0.083
PM2A102	16.56	2.84	0.166	0.124
PM2A103	15.89	1.96	0.189	0.149

Appendix A, continued. Plant dry matter yield and N concentration - crop 2.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
C0051	1.24	0.67	0.074	0.053
C0052	0.55	0.36	0.081	0.081
C0053	0.32	0.24	0.077	0.104
C0081	1.70	0.78	0.073	0.055
C0082	1.64	0.73	0.064	0.066
C0083	1.66	0.45	0.073	0.053
C0091	1.74	0.69	0.073	0.054
C0092	1.23	0.70	0.073	0.057
C0093	0.57	0.38	0.064	0.070
C0101	1.12	0.53	0.059	0.063
C0102	1.10	0.51	0.067	0.062
C0103	0.60	0.27	0.068	0.084
C1051	10.48	2.55	0.092	0.053
C1052	9.13	1.89	0.101	0.061
C1053	4.24	0.71	0.123	0.109
C1081	12.55	2.89	0.088	0.051
C1082	9.76	2.38	0.143	0.052
C1083	9.65	1.89	0.113	0.061
C1091	19.83	2.54	0.088	0.055
C1092	9.12	2.44	0.099	0.074
C1093	2.51	0.46	0.104	0.110
C1101	19.18	2.24	0.094	0.049
C1102	3.12	0.49	0.120	0.108
C1103	4.13	0.78	0.146	0.095
C2051	15.80	3.33	0.147	0.069
C2052	13.76	2.43	0.173	0.074
C2053	14.06	2.19	0.193	0.075
C2081	14.32	2.69	0.153	0.071
C2082	12.43	2.35	0.167	0.078
C2083	13.69	1.99	0.185	0.075
C2091	13.64	2.56	0.151	0.067
C2092	14.51	2.78	0.160	0.073
C2093	13.26	2.26	0.172	0.077
C2101	13.92	2.56	0.171	0.076
C2102	12.96	3.11	0.178	0.072
C2103	14.31	2.17	0.162	0.073
C2A051	16.59	2.67	0.125	0.084
C2A052	14.38	2.48	0.160	0.088
C2A053	17.53	2.97	0.178	0.083
C2A081	18.25	3.93	0.133	0.072
C2A082	13.54	3.44	0.214	0.103
C2A083	17.84	2.81	0.187	0.077
C2A091	1.33	0.72	0.232	0.390
C2A092	13.80	2.81	0.168	0.098
C2A093	14.61	2.25	0.182	0.102
C2A101	17.15	2.83	0.127	0.082
C2A102	12.43	1.87	0.158	0.108
C2A103	14.19	1.98	0.207	0.099

Appendix A, continued. Plant dry matter yield and N concentration - crop 3.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
PV0051	7.42	1.48	0.163	0.057
PV0052	8.94	1.69	0.198	0.071
PV0053	1.80	0.29	0.366	0.073
PV0081	11.05	2.11	0.165	0.053
PV0082	9.08	2.14	0.194	0.065
PV0083	6.26	1.64	0.283	0.057
PV0091	9.49	2.22	0.188	0.054
PV0092	5.05	1.07	0.317	0.057
PV0093	6.56	1.64	0.246	0.060
PV0101	8.34	1.82	0.218	0.056
PV0102	7.81	1.64	0.297	0.063
PV0103	5.07	0.97	0.291	0.052
PV1051	8.69	1.66	0.246	0.055
PV1052	8.26	1.41	0.231	0.068
PV1053	5.82	1.39	0.253	0.074
PV1081	8.62	1.58	0.337	0.063
PV1082	7.62	1.36	0.351	0.065
PV1083	7.20	1.07	0.260	0.077
PV1091	10.24	1.89	0.229	0.062
PV1092	8.28	1.61	0.312	0.070
PV1093	6.16	1.18	0.245	0.071
PV1101	8.80	1.72	0.262	0.058
PV1102	8.80	1.76	0.292	0.062
PV1103	6.39	1.43	0.241	0.073
PV2051	8.73	1.65	0.273	0.076
PV2052	8.95	1.32	0.309	0.094
PV2053	5.29	1.14	0.316	0.093
PV2081	6.92	0.84	0.351	0.070
PV2082	9.95	1.85	0.299	0.105
PV2083	6.14	1.00	0.368	0.090
PV2091	9.84	2.09	0.277	0.072
PV2092	10.17	2.14	0.257	0.085
PV2093	5.88	1.11	0.319	0.083
PV2101	8.93	1.46	0.323	0.091
PV2102	8.89	1.51	0.240	0.081
PV2103	7.17	1.07	0.271	0.080
PV2A051	9.85	1.66	0.322	0.086
PV2A052	8.40	1.51	0.288	0.103
PV2A053	5.82	0.95	0.314	0.111
PV2A081	8.56	1.25	0.357	0.081
PV2A082	10.23	2.10	0.354	0.122
PV2A083	6.90	1.20	0.317	0.104
PV2A091	9.62	1.63	0.328	0.078
PV2A092	9.22	1.99	0.362	0.130
PV2A093	6.43	0.94	0.355	0.103
PV2A101	9.24	1.41	0.324	0.087
PV2A102	9.89	1.75	0.337	0.103
PV2A103	7.53	1.19	0.343	0.091

Appendix A, continued. Plant dry matter yield and N concentration - crop 3.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
BM0051	1.40	0.83	0.153	0.069
BM0052	0.46	0.68	0.102	0.072
BM0053	0.69	0.45	0.138	0.070
BM0081	2.71	1.11	0.141	0.063
BM0082	1.02	0.66	0.105	0.079
BM0083	1.46	0.73	0.143	0.071
BM0091	1.43	0.95	0.139	0.064
BM0092	0.31	0.65	0.124	0.071
BM0093	0.33	0.51	0.108	0.088
BM0101	1.15	0.60	0.133	0.068
BM0102	0.75	0.82	0.109	0.071
BM0103	0.41	0.57	0.116	0.064
BM1051	4.19	1.60	0.141	0.062
BM1052	1.40	0.54	0.147	0.064
BM1053	2.76	0.78	0.178	0.067
BM1081	6.28	1.81	0.114	0.058
BM1082	2.51	0.93	0.194	0.060
BM1083	4.13	1.00	0.191	0.066
BM1091	4.88	1.54	0.141	0.079
BM1092	2.70	1.06	0.183	0.061
BM1093	4.98	1.28	0.143	0.064
BM1101	4.62	0.85	0.163	0.054
BM1102	2.57	1.21	0.152	0.066
BM1103	4.11	1.13	0.131	0.076
BM2051	5.07	1.21	0.212	0.062
BM2052	4.62	1.30	0.163	0.066
BM2053	5.72	1.25	0.202	0.065
BM2081	6.83	1.69	0.149	0.059
BM2082	5.00	1.22	0.182	0.065
BM2083	3.38	0.74	0.266	0.066
BM2091	6.10	1.27	0.217	0.061
BM2092	6.72	1.96	0.174	0.079
BM2093	3.95	0.92	0.235	0.100
BM2101	4.46	0.98	0.238	0.064
BM2102	6.41	1.96	0.187	0.080
BM2103	5.02	1.16	0.213	0.069
BM2A051	7.06	1.34	0.275	0.079
BM2A052	7.09	1.47	0.340	0.080
BM2A053	7.42	1.07	0.310	0.082
BM2A081	8.41	1.55	0.326	0.069
BM2A082	6.62	1.29	0.326	0.096
BM2A083	6.44	1.46	0.288	0.090
BM2A091	8.09	1.49	0.303	0.070
BM2A092	7.63	1.66	0.310	0.086
BM2A093	7.70	1.29	0.282	0.093
BM2A101	7.48	1.23	0.299	0.083
BM2A102	6.26	1.77	0.311	0.086
BM2A103	7.60	1.69	0.293	0.093

Appendix A, continued. Plant dry matter yield and N concentration - crop 3.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
PM0051	6.73	1.08	0.289	0.051
PM0052	3.53	0.69	0.292	0.057
PM0053	6.23	1.05	0.290	0.057
PM0081	6.36	1.08	0.310	0.053
PM0082	5.44	0.85	0.327	0.052
PM0083	0.80	0.94	0.319	0.059
PM0091	5.21	0.66	0.325	0.056
PM0092	9.05	1.58	0.304	0.059
PM0093	6.18	0.90	0.324	0.051
PM0101	6.92	1.23	0.262	0.054
PM0102	8.01	1.58	0.319	0.053
PM0103	5.19	1.08	0.279	0.061
PM1051	5.96	1.20	0.344	0.063
PM1052	8.23	1.80	0.310	0.082
PM1053	6.36	0.93	0.317	0.061
PM1081	5.64	0.82	0.373	0.064
PM1082	5.14	1.10	0.324	0.063
PM1083	7.52	1.44	0.290	0.067
PM1091	6.47	0.90	0.311	0.055
PM1092	6.74	0.99	0.301	0.074
PM1093	8.03	1.09	0.290	0.099
PM1101	5.29	0.81	0.317	0.067
PM1102	7.77	1.92	0.247	0.074
PM1103	7.07	1.39	0.247	0.064
PM2051	6.10	0.79	0.302	0.090
PM2052	7.41	1.47	0.309	0.118
PM2053	6.39	1.05	0.288	0.103
PM2081	5.78	0.78	0.344	0.075
PM2082	5.56	1.17	0.354	0.106
PM2083	6.59	1.11	0.288	0.092
PM2091	7.01	1.25	0.314	0.073
PM2092	6.03	1.11	0.305	0.078
PM2093	5.19	0.71	0.294	0.150
PM2101	6.33	0.97	0.312	0.111
PM2102	7.68	1.50	0.328	0.113
PM2103	6.58	1.05	0.317	0.095
PM2A051	5.94	0.85	0.339	0.164
PM2A052	7.58	1.33	0.311	0.142
PM2A053	6.45	1.06	0.327	0.154
PM2A081	7.24	1.01	0.337	0.105
PM2A082	7.70	1.59	0.335	0.107
PM2A083	7.15	1.34	0.346	0.143
PM2A091	6.01	0.80	0.334	0.082
PM2A092	5.12	0.85	0.317	0.134
PM2A093	6.83	0.95	0.338	0.123
PM2A101	6.47	1.18	0.349	0.083
PM2A102	6.60	1.28	0.325	0.124
PM2A103	6.84	1.04	0.327	0.149

Appendix A, continued. Plant dry matter yield and N concentration - crop 3.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
C0051	1.59	0.77	0.109	0.053
C0052	1.61	0.86	0.079	0.081
C0053	1.42	0.71	0.097	0.104
C0081	2.27	1.03	0.111	0.055
C0082	1.65	0.82	0.091	0.066
C0083	1.65	0.87	0.086	0.053
C0091	2.03	0.87	0.093	0.054
C0092	1.93	0.79	0.094	0.057
C0093	1.44	0.70	0.087	0.070
C0101	1.65	0.79	0.104	0.063
C0102	0.95	0.48	0.082	0.062
C0103	1.50	0.66	0.090	0.084
C1051	4.35	1.12	0.154	0.053
C1052	4.08	1.20	0.126	0.061
C1053	4.71	1.17	0.127	0.109
C1081	4.64	1.24	0.168	0.051
C1082	5.29	1.41	0.111	0.052
C1083	3.86	1.01	0.144	0.061
C1091	3.65	0.93	0.185	0.055
C1092	4.95	1.39	0.132	0.074
C1093	3.90	1.07	0.141	0.110
C1101	4.23	0.92	0.174	0.049
C1102	4.18	0.99	0.118	0.108
C1103	5.05	1.52	0.110	0.095
C2051	4.89	1.13	0.230	0.069
C2052	5.62	1.59	0.171	0.074
C2053	5.52	1.24	0.198	0.075
C2081	4.46	0.89	0.242	0.071
C2082	6.41	1.91	0.155	0.078
C2083	4.57	0.99	0.209	0.075
C2091	4.86	1.30	0.239	0.067
C2092	6.23	1.54	0.177	0.073
C2093	5.52	1.12	0.192	0.077
C2101	5.06	1.03	0.259	0.076
C2102	6.13	1.51	0.171	0.072
C2103	7.07	1.81	0.150	0.073
C2A051	7.08	1.37	0.364	0.084
C2A052	8.61	1.49	0.310	0.088
C2A053	5.03	1.06	0.356	0.083
C2A081	6.80	1.50	0.295	0.072
C2A082	8.72	1.85	0.286	0.103
C2A083	7.01	1.43	0.323	0.077
C2A091	6.52	1.53	0.330	0.390
C2A092	7.68	1.35	0.330	0.098
C2A093	5.32	0.93	0.367	0.102
C2A101	6.03	1.11	0.363	0.082
C2A102	6.41	1.32	0.335	0.108
C2A103	7.16	1.47	0.331	0.099

Appendix A, continued. Plant dry matter yield and N concentration - crop 4.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
PV0051	3.37	0.72	0.212	0.115
PV0052	2.96	0.64	0.209	0.119
PV0053	2.76	0.48	0.392	0.213
PV0081	2.69	0.51	0.211	0.116
PV0082	2.42	0.53	0.229	0.128
PV0083	2.29	0.54	0.252	0.132
PV0091	-	0.46	0.253	0.141
PV0092	2.08	0.39	0.260	0.121
PV0093	3.03	0.62	0.206	0.108
PV0101	2.43	0.62	0.198	0.101
PV0102	3.03	0.61	0.240	0.119
PV0103	2.62	0.63	0.220	0.119
PV1051	2.82	0.58	0.271	0.125
PV1052	3.07	0.73	0.197	0.110
PV1053	2.66	0.62	0.206	0.103
PV1081	3.07	0.55	0.263	0.134
PV1082	3.27	0.63	0.238	0.118
PV1083	2.96	0.50	0.199	0.103
PV1091	3.28	0.67	0.227	0.106
PV1092	3.12	0.69	0.197	0.092
PV1093	2.52	0.49	0.258	0.118
PV1101	3.01	0.47	0.290	0.138
PV1102	2.99	0.63	0.227	0.115
PV1103	3.38	0.56	0.261	0.102
PV2051	3.52	0.49	0.348	0.168
PV2052	3.11	0.58	0.227	0.115
PV2053	3.22	0.50	0.363	0.179
PV2081	3.37	0.49	0.381	0.202
PV2082	2.49	0.36	0.210	0.124
PV2083	3.21	0.57	0.353	0.198
PV2091	2.58	0.56	0.226	0.109
PV2092	2.65	0.51	0.198	0.093
PV2093	2.34	0.39	0.352	0.174
PV2101	2.94	0.55	0.266	0.132
PV2102	3.19	0.63	0.265	0.129
PV2103	2.32	0.33	0.389	0.209
PV2A051	2.12	0.36	0.455	0.250
PV2A052	3.92	0.55	0.445	0.240
PV2A053	2.88	0.47	0.421	0.268
PV2A081	3.72	0.55	0.439	0.262
PV2A082	3.17	0.50	0.428	0.282
PV2A083	3.31	0.54	0.420	0.290
PV2A091	3.80	0.65	0.427	0.252
PV2A092	3.81	0.65	0.425	0.260
PV2A093	2.87	0.47	0.413	0.243
PV2A101	3.05	0.49	0.426	0.234
PV2A102	3.05	0.47	0.437	0.288
PV2A103	3.42	0.58	0.433	0.263

Appendix A, continued. Plant dry matter yield and N concentration - crop 4.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
BM0051	0.53	0.40	0.145	0.098
BM0052	0.23	0.38	0.153	0.078
BM0053	0.41	0.32	0.173	0.083
BM0081	-	0.46	0.181	0.248
BM0082	0.07	0.35	0.155	0.103
BM0083	0.03	0.29	0.175	0.106
BM0091	0.65	0.50	0.166	0.096
BM0092	0.05	0.22	0.202	0.100
BM0093	0.19	0.34	0.151	0.076
BM0101	0.37	0.39	0.165	0.087
BM0102	0.19	0.30	0.174	0.092
BM0103	0.76	0.43	0.147	0.087
BM1051	0.59	0.24	0.152	0.090
BM1052	0.37	0.39	0.153	0.077
BM1053	1.37	0.53	0.150	0.094
BM1081	0.65	0.29	0.160	0.100
BM1082	0.93	0.57	0.146	0.083
BM1083	0.30	0.58	0.148	0.087
BM1091	0.21	0.38	0.175	0.111
BM1092	0.99	0.53	0.146	0.092
BM1093	0.45	0.36	0.148	0.092
BM1101	0.30	0.40	0.151	0.091
BM1102	0.26	0.66	0.147	0.088
BM1103	1.07	0.38	0.152	0.082
BM2051	1.61	0.41	0.141	0.090
BM2052	1.08	0.47	0.148	0.095
BM2053	0.59	0.46	0.132	0.101
BM2081	1.20	0.55	0.149	0.091
BM2082	2.75	0.47	0.129	0.098
BM2083	2.23	0.64	0.182	0.106
BM2091	-	0.24	0.185	0.115
BM2092	0.76	0.42	0.131	0.088
BM2093	1.36	0.56	0.131	0.097
BM2101	1.84	0.57	0.152	0.095
BM2102	1.75	0.53	0.148	0.088
BM2103	2.16	0.74	0.128	0.093
BM2A051	2.76	0.62	0.406	0.084
BM2A052	4.04	0.70	0.391	0.192
BM2A053	3.62	0.59	0.413	0.214
BM2A081	2.59	0.50	0.403	0.263
BM2A082	3.36	0.52	0.395	0.212
BM2A083	2.98	0.50	0.350	0.250
BM2A091	3.01	0.46	0.418	0.258
BM2A092	3.78	0.65	0.401	0.223
BM2A093	3.52	0.52	0.398	0.235
BM2A101	3.72	0.58	0.422	0.241
BM2A102	3.06	0.49	0.420	0.219
BM2A103	3.45	0.62	0.409	0.252

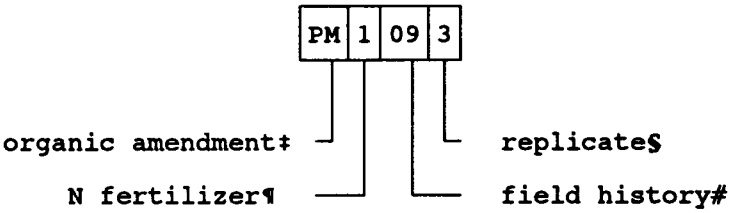
Appendix A, continued. Plant dry matter yield and N concentration - crop 4.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
PM0051	3.53	0.62	0.356	0.173
PM0052	3.55	0.49	0.388	0.196
PM0053	2.71	0.39	0.367	0.217
PM0081	3.90	0.72	0.341	0.185
PM0082	3.35	0.54	0.376	0.196
PM0083	2.96	0.40	0.397	0.209
PM0091	-	0.38	0.389	0.228
PM0092	3.16	0.73	0.229	0.136
PM0093	2.53	0.29	0.381	0.208
PM0101	3.00	0.48	0.325	0.172
PM0102	2.97	0.64	0.214	0.285
PM0103	3.47	0.47	0.371	0.209
PM1051	3.62	0.56	0.386	0.232
PM1052	2.81	0.51	0.388	0.225
PM1053	2.95	0.51	0.379	0.174
PM1081	2.08	0.43	0.397	0.250
PM1082	2.85	0.38	0.390	0.247
PM1083	3.47	0.51	0.373	0.221
PM1091	3.66	0.54	0.402	0.191
PM1092	2.81	0.56	0.389	0.225
PM1093	3.57	0.41	0.353	0.179
PM1101	3.09	0.46	0.370	0.236
PM1102	3.05	0.75	0.351	0.207
PM1103	3.35	0.41	0.326	0.156
PM2051	1.79	0.45	0.403	0.241
PM2052	3.97	0.60	0.383	0.213
PM2053	3.09	0.38	0.405	0.226
PM2081	-	0.40	0.424	0.285
PM2082	3.40	0.57	0.398	0.239
PM2083	2.68	0.37	0.390	0.256
PM2091	2.83	0.51	0.395	0.246
PM2092	1.98	0.41	0.382	0.240
PM2093	3.26	0.44	0.401	0.225
PM2101	3.43	0.57	0.387	0.207
PM2102	3.75	0.51	0.379	0.193
PM2103	2.76	0.39	0.388	0.200
PM2A051	1.82	0.38	0.435	0.275
PM2A052	3.46	0.53	0.394	0.253
PM2A053	2.82	0.46	0.451	0.272
PM2A081	2.73	0.46	0.413	0.279
PM2A082	2.81	0.51	0.402	0.293
PM2A083	2.85	0.49	0.407	0.268
PM2A091	2.76	0.52	0.414	0.293
PM2A092	2.20	0.48	0.395	0.293
PM2A093	2.40	0.44	0.410	0.273
PM2A101	2.79	0.52	0.393	0.266
PM2A102	2.96	0.71	0.376	0.118
PM2A103	2.21	0.35	0.381	0.294

Appendix A, continued. Plant dry matter yield and N concentration - crop 4.

Sample†	Shoot DMY	Root DMY	Shoot TKN	Root TKN
	grams		mg N g^{-1}	
C0051	0.46	0.32	0.122	0.083
C0052	1.65	0.42	0.126	0.089
C0053	0.37	0.38	0.133	0.081
C0081	0.56	0.39	0.140	0.091
C0082	0.52	0.33	0.143	0.094
C0083	0.59	0.35	0.127	0.082
C0091	0.57	0.34	0.150	0.088
C0092	0.25	0.32	0.139	0.082
C0093	2.81	0.36	0.119	0.074
C0101	0.52	0.38	0.130	0.083
C0102	0.73	0.32	0.124	0.099
C0103	0.72	0.41	0.118	0.071
C1051	0.49	0.25	0.189	0.118
C1052	0.68	0.45	0.114	0.074
C1053	0.95	0.36	0.142	0.078
C1081	0.61	0.70	0.146	0.086
C1082	0.89	0.47	0.143	0.085
C1083	0.90	0.42	0.133	0.077
C1091	0.75	0.36	0.155	0.093
C1092	1.97	0.48	0.124	0.070
C1093	0.90	0.51	0.136	0.080
C1101	0.57	0.35	0.137	0.085
C1102	1.35	0.38	0.140	0.072
C1103	0.95	0.35	0.138	0.078
C2051	1.47	0.51	0.153	0.096
C2052	0.79	0.47	0.134	0.088
C2053	0.90	0.50	0.139	0.084
C2081	1.58	0.58	0.176	0.100
C2082	1.42	0.54	0.130	0.088
C2083	1.24	0.53	0.153	0.091
C2091	1.61	0.57	0.153	0.099
C2092	0.94	0.33	0.129	0.082
C2093	1.43	0.43	0.141	0.079
C2101	1.22	0.51	0.178	0.099
C2102	0.83	0.40	0.141	0.081
C2103	1.01	0.38	0.140	0.075
C2A051	2.82	0.50	0.384	0.217
C2A052	3.49	0.48	0.411	0.256
C2A053	1.30	0.24	0.395	0.288
C2A081	4.83	0.57	0.424	0.259
C2A082	3.65	0.54	0.420	0.247
C2A083	3.91	0.59	0.405	0.225
C2A091	1.98	0.31	0.420	0.303
C2A092	3.62	0.57	0.439	0.205
C2A093	2.37	0.42	0.400	0.253
C2A101	1.79	0.42	0.460	0.265
C2A102	3.38	0.57	0.431	0.231
C2A103	3.47	0.44	0.420	0.247

† Sample identification example:



‡ organic amendment: PV = pea vine
BM = beef manure
PM = poultry manure
C = control

$ replicate: 1 and 2 = lower slope position
3 = upper slope position

¶ N fertilizer: 0 = N_0
1 = N_{400}
2 = N_{800}
2A= N_{1600}

# field history: 05 = 90 kg N
08 = manure
09 = pea vine
10 = 0 kg N

Appendix B. Biological Parameters - 87 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
PV0051	2.7	215	157	190	502	61
PV0052	1.5	314	173	152	453	56
PV0053	1.5	580	256	138	454	76
PV1051	2.6	316	171	205	537	73
PV1052	2.1	379	194	130	399	68
PV1053	1.5	278	189	174	405	79
PV2051	2.6	319	118	114	372	77
PV2052	1.8	371	185	134	541	66
PV2053	2.1	631	284	135	440	78
PV2A051	2.0	284	154	128	526	70
PV2A052	1.5	416	178	111	382	-
PV2A053	1.9	-	248	119	454	76
PV0081	2.4	433	138	139	436	86
PV0082	1.6	512	171	170	552	93
PV0083	1.6	-	185	187	474	92
PV1081	2.5	330	115	120	537	91
PV1082	1.7	536	173	163	492	86
PV1083	1.7	488	193	210	522	96
PV2081	2.3	423	120	148	-	101
PV2082	2.7	-	221	172	593	91
PV2083	1.2	-	200	150	431	100
PV2A081	2.6	471	157	198	582	97
PV2A082	2.3	381	198	156	515	73
PV2A083	2.3	182	188	147	570	86
PV0091	2.0	399	148	110	419	77
PV0092	2.3	417	192	141	557	80
PV0093	1.1	467	179	120	476	70
PV1091	2.8	382	164	130	-	82
PV1092	2.6	385	200	125	479	82
PV1093	1.6	-	185	160	561	-
PV2091	2.6	458	175	127	571	85
PV2092	2.0	508	224	120	547	68
PV2093	1.3	173	175	102	422	77
PV2A091	2.5	346	132	130	564	87
PV2A092	1.8	448	210	117	572	75
PV2A093	1.6	279	178	139	483	81
PV0101	2.1	469	149	152	459	70
PV0102	2.2	419	178	222	536	85
PV0103	1.4	550	173	178	515	84
PV1101	1.6	458	185	161	616	74
PV1102	2.2	429	195	157	458	74
PV1103	1.3	421	189	173	461	90
PV2101	2.1	399	151	108	450	75
PV2102	1.7	483	186	220	554	74
PV2103	1.8	335	192	143	464	83
PV2A101	2.4	382	175	155	585	73
PV2A102	1.6	580	178	128	437	68
PV2A103	1.4	-	178	157	473	76

Appendix B, continued. Biological Parameters - 87 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
BM0051	0.6	169	60	-	148	29
BM0052	1.0	437	63	71	177	23
BM0053	0.5	469	93	95	146	33
BM1051	0.9	222	71	90	219	36
BM1052	0.7	460	69	97	181	23
BM1053	0.5	338	88	117	205	30
BM2051	0.7	311	65	83	171	28
BM2052	0.6	400	69	69	152	21
BM2053	0.9	323	117	87	162	32
BM2A051	0.5	319	92	81	269	27
BM2A052	0.5	281	73	87	178	28
BM2A053	0.6	387	86	126	157	32
BM0081	0.8	374	96	60	249	44
BM0082	0.7	622	80	102	275	41
BM0083	0.4	370	125	81	183	37
BM1081	0.8	354	104	82	288	42
BM1082	0.7	460	76	75	287	49
BM1083	0.6	495	113	97	279	46
BM2081	0.9	259	86	68	272	48
BM2082	0.8	391	81	99	337	42
BM2083	0.5	402	94	80	281	-
BM2A081	0.6	266	91	80	258	45
BM2A082	0.8	369	90	102	296	37
BM2A083	0.6	340	101	104	235	40
BM0091	0.7	321	99	63	182	39
BM0092	0.4	356	95	91	191	33
BM0093	0.4	319	102	95	271	35
BM1091	0.8	326	97	113	274	38
BM1092	0.4	473	81	62	279	34
BM1093	0.5	416	103	99	232	36
BM2091	0.7	289	101	59	186	-
BM2092	0.8	387	91	134	-	40
BM2093	0.6	434	101	84	187	35
BM2A091	0.7	211	88	79	221	32
BM2A092	0.8	465	102	62	153	38
BM2A093	0.4	302	115	70	199	35
BM0101	0.6	157	55	54	123	25
BM0102	0.8	175	58	111	-	29
BM0103	0.3	233	78	103	169	31
BM1101	0.8	209	68	83	236	29
BM1102	0.6	327	55	111	176	27
BM1103	0.4	179	91	250	202	29
BM2101	0.6	265	70	42	144	27
BM2102	0.7	356	56	77	208	37
BM2103	0.4	-	75	125	154	31
BM2A101	0.6	253	61	73	184	29
BM2A102	0.7	211	61	52	148	34
BM2A103	0.5	-	78	110	238	32

Appendix B, continued. Biological Parameters - 87 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
PM0051	0.6	309	109	41	122	19
PM0052	0.2	254	83	57	132	19
PM0053	0.3	283	80	47	163	20
PM1051	0.2	361	98	47	144	20
PM1052	0.4	303	101	63	113	23
PM1053	0.3	437	79	43	109	17
PM2051	0.4	431	84	55	124	24
PM2052	0.6	316	109	60	169	13
PM2053	0.2	238	69	30	114	18
PM2A051	0.2	322	80	61	126	20
PM2A052	0.5	305	100	44	108	17
PM2A053	0.2	255	81	46	125	11
PM0081	0.7	615	113	37	193	44
PM0082	0.9	615	96	71	207	37
PM0083	0.3	473	79	39	249	38
PM1081	0.6	613	144	41	270	38
PM1082	0.7	328	88	47	176	32
PM1083	0.4	601	119	46	206	41
PM2081	0.7	495	103	39	158	40
PM2082	0.8	489	99	60	270	33
PM2083	0.4	571	121	33	208	41
PM2A081	0.7	419	109	52	279	41
PM2A082	1.0	411	101	73	266	29
PM2A083	0.4	448	117	47	180	43
PM0091	0.6	572	162	30	174	29
PM0092	0.4	427	170	73	148	24
PM0093	0.5	583	132	54	218	24
PM1091	0.5	619	186	47	162	35
PM1092	0.2	360	120	33	138	31
PM1093	0.3	555	171	49	154	23
PM2091	0.4	553	150	38	127	25
PM2092	0.6	308	158	40	169	28
PM2093	0.4	505	141	60	259	23
PM2A091	0.5	631	175	58	114	27
PM2A092	0.7	608	190	56	135	23
PM2A093	0.3	412	138	35	149	27
PM0101	0.3	262	104	25	106	-
PM0102	-	491	84	30	-	15
PM0103	0.2	401	109	51	164	22
PM1101	0.3	348	117	47	157	24
PM1102	0.6	439	99	52	158	-
PM1103	0.3	486	103	50	148	21
PM2101	0.3	395	104	25	110	20
PM2102	0.7	273	101	23	145	18
PM2103	0.3	383	89	39	142	21
PM2A101	0.3	365	95	51	153	32
PM2A102	0.6	291	100	35	129	23
PM2A103	0.2	258	83	35	154	18

Appendix B, continued. Biological Parameters - 87 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
C0051	0.2	140	52	18	61	14
C0052	0.3	138	52	25	59	15
C0053	0.2	374	58	28	108	15
C1051	0.2	129	53	21	80	14
C1052	0.2	178	48	17	46	12
C1053	0.2	356	62	37	99	11
C2051	0.2	121	47	21	65	13
C2052	0.5	194	51	34	41	5
C2053	0.2	383	52	26	100	13
C2A051	0.2	139	48	29	104	15
C2A052	0.2	136	49	19	51	7
C2A053	0.1	366	54	33	64	12
C0081	0.5	207	88	25	135	30
C0082	0.8	280	69	44	178	28
C0083	0.6	420	83	40	117	27
C1081	0.3	163	75	34	172	28
C1082	0.6	336	71	45	124	27
C1083	0.5	317	79	30	159	34
C2081	0.4	185	76	22	148	29
C2082	0.5	182	73	33	143	25
C2083	0.6	520	81	38	166	31
C2A081	0.5	160	76	38	-	30
C2A082	0.7	158	75	32	127	25
C2A083	0.6	308	79	32	126	-
C0091	0.3	187	76	25	118	19
C0092	0.5	251	77	58	118	14
C0093	0.5	387	69	17	82	23
C1091	0.3	158	79	35	130	24
C1092	0.4	265	75	35	49	18
C1093	0.5	408	106	54	106	23
C2091	0.1	190	83	43	118	20
C2092	0.4	160	79	16	79	15
C2093	0.2	278	76	36	82	24
C2A091	0.2	217	86	20	120	23
C2A092	0.5	206	82	20	149	21
C2A093	0.4	332	70	29	102	16
C0101	0.1	143	48	21	48	14
C0102	0.2	186	49	11	64	22
C0103	0.4	388	61	23	131	14
C1101	0.2	111	45	17	84	15
C1102	0.3	147	47	23	81	16
C1103	0.2	241	59	18	75	15
C2101	0.2	145	50	18	58	14
C2102	0.2	106	46	15	54	17
C2103	0.2	327	55	14	60	14
C2A101	0.2	92	52	14	74	15
C2A102	0.2	155	49	24	78	13
C2A103	0.2	409	62	27	82	15

Appendix B, continued. Biological Parameters - 164 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
PV0051	1.6	363	133	72	288	37
PV0052	1.0	187	117	91	294	50
PV0053	0.9	397	140	69	194	41
PV1051	1.4	448	154	95	271	37
PV1052	-	-	-	-	-	-
PV1053	-	-	136	-	-	-
PV2051	1.4	420	188	87	296	35
PV2052	1.3	120	133	82	323	39
PV2053	0.9	278	189	46	167	25
PV2A051	0.8	305	148	81	322	31
PV2A052	1.0	187	119	70	284	33
PV2A053	0.6	308	152	67	182	25
PV0081	1.5	319	140	75	359	58
PV0082	1.4	205	116	97	384	60
PV0083	1.1	261	124	78	258	56
PV1081	2.0	423	152	87	376	63
PV1082	1.5	146	153	103	371	49
PV1083	0.8	288	122	77	318	58
PV2081	1.1	495	159	73	384	58
PV2082	1.1	302	152	-	379	57
PV2083	1.2	435	138	57	306	55
PV2A081	1.7	420	187	80	358	52
PV2A082	1.3	223	149	54	273	36
PV2A083	0.8	343	145	37	257	-
PV0091	1.4	485	159	78	361	60
PV0092	0.7	307	152	59	357	60
PV0093	1.1	370	180	59	279	-
PV1091	1.4	448	161	85	386	55
PV1092	1.3	315	155	84	351	50
PV1093	0.9	362	178	41	249	47
PV2091	1.5	404	215	77	385	49
PV2092	0.8	320	206	91	366	42
PV2093	0.5	336	162	46	278	43
PV2A091	1.4	442	197	79	429	47
PV2A092	1.0	337	218	62	392	49
PV2A093	0.6	378	192	33	228	36
PV0101	2.4	403	124	82	276	37
PV0102	1.8	234	128	83	332	38
PV0103	0.3	253	143	59	233	41
PV1101	1.9	491	206	80	347	47
PV1102	1.3	308	123	80	347	48
PV1103	1.1	264	155	79	137	41
PV2101	1.2	500	185	64	310	43
PV2102	1.5	286	155	101	336	49
PV2103	0.8	275	150	46	217	39
PV2A101	1.3	538	183	74	275	41
PV2A102	1.1	262	184	73	358	60
PV2A103	0.6	324	173	43	218	39

Appendix B, continued. Biological Parameters - 164 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
BM0051	0.6	316	93	98	178	32
BM0052	0.4	129	57	77	168	29
BM0053	0.3	352	86	92	147	27
BM1051	0.7	318	97	109	176	-
BM1052	0.4	150	63	110	187	34
BM1053	0.6	368	97	68	102	24
BM2051	1.1	409	121	68	179	18
BM2052	0.7	124	60	85	166	23
BM2053	0.6	416	114	65	128	16
BM2A051	0.9	288	120	105	200	23
BM2A052	0.5	114	74	102	191	27
BM2A053	0.4	399	134	98	114	18
BM0081	1.6	306	119	86	270	35
BM0082	0.8	164	74	90	262	41
BM0083	0.4	433	103	50	206	40
BM1081	1.1	449	139	88	268	37
BM1082	0.8	225	79	97	275	42
BM1083	0.7	425	111	83	190	39
BM2081	1.0	491	144	92	212	27
BM2082	0.6	201	82	83	249	37
BM2083	0.9	474	119	49	153	30
BM2A081	0.9	347	165	74	261	33
BM2A082	0.8	162	85	70	268	37
BM2A083	0.5	408	130	38	155	30
BM0091	0.8	384	144	109	184	34
BM0092	0.8	174	76	73	223	34
BM0093	0.5	702	104	41	154	34
BM1091	1.4	443	143	117	230	37
BM1092	0.8	238	74	132	266	35
BM1093	0.6	530	107	71	162	31
BM2091	0.8	482	138	60	242	35
BM2092	0.6	159	73	102	228	32
BM2093	0.6	552	132	98	196	38
BM2A091	1.1	511	162	93	232	30
BM2A092	0.8	137	96	72	218	24
BM2A093	0.9	629	146	57	157	25
BM0101	0.9	311	98	85	180	28
BM0102	0.4	170	55	86	178	28
BM0103	0.4	235	87	58	123	30
BM1101	1.0	361	119	84	210	31
BM1102	0.7	145	55	98	177	24
BM1103	0.5	173	98	56	131	27
BM2101	1.1	321	119	104	-	26
BM2102	0.7	145	56	92	169	25
BM2103	0.5	255	102	85	152	25
BM2A101	1.1	251	126	76	196	23
BM2A102	0.7	125	71	99	211	28
BM2A103	0.6	236	101	65	148	26

Appendix B, continued. Biological Parameters - 164 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
PM0051	0.2	425	129	40	152	16
PM0052	0.3	213	97	32	149	18
PM0053	0.4	435	105	39	90	15
PM1051	0.4	356	123	51	-	23
PM1052	0.3	252	101	23	105	9
PM1053	0.4	368	114	44	91	13
PM2051	0.1	332	132	41	117	9
PM2052	0.4	242	104	33	116	14
PM2053	-	-		292	489	-
PM2A051	0.1	277	123	39	104	11
PM2A052	0.5	337	118	41	146	14
PM2A053	0.2	325	115	33	39	21
PM0081	0.6	547	121	66	233	37
PM0082	0.6	364	110	66	253	30
PM0083	0.6	355	109	59	168	36
PM1081	0.8	392	173	49	223	28
PM1082	0.8	433	105	40	214	29
PM1083	0.5	411	129	40	169	30
PM2081	0.8	446	129	43	224	30
PM2082	0.5	393	102	21	196	27
PM2083	0.7	676	147	41	188	31
PM2A081	0.4	557	136	83	274	35
PM2A082	0.7	502	129	28	214	30
PM2A083	0.5	611	149	36	148	29
PM0091	0.5	514	154	38	172	27
PM0092	0.7	348	134	49	218	32
PM0093	0.5	309	158	37	170	32
PM1091	0.6	544	154	39	163	24
PM1092	0.6	384	129	33	159	17
PM1093	0.3	260	153	26	125	27
PM2091	0.3	490	159	61	156	21
PM2092	0.4	478	140	28	139	20
PM2093	0.3	256	133	22	94	30
PM2A091	0.3	434	167	61	200	22
PM2A092	0.6	417	152	25	129	22
PM2A093	0.4	210	140	15	77	15
PM0101	0.4	271	111	36	129	20
PM0102	0.5	425	89	38	154	25
PM0103	0.3	170	113	20	110	23
PM1101	0.3	305	108	39	144	18
PM1102	0.3	290	90	24	142	21
PM1103	0.2	187	120	25	112	22
PM2101	0.3	330	114	37	115	17
PM2102	0.5	335	105	35	114	21
PM2103	0.5	236	136	16	77	13
PM2A101	0.3	352	137	48	148	18
PM2A102	0.5	318	111	22	117	23
PM2A103	0.3	307	126	14	128	26

Appendix B, continued. Biological Parameters - 164 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
C0051	0.5	213	77	18	87	12
C0052	0.3	162	65	9	26	14
C0053	0.5	176	78	37	79	19
C1051	0.4	215	90	19	85	7
C1052	0.4	169	67	17	69	8
C1053	0.2	252	93	32	48	10
C2051	0.3	230	100	18	78	5
C2052	0.4	180	85	28	67	12
C2053	0.2	253	93	27	62	8
C2A051	0.3	196	107	27	84	8
C2A052	0.3	193	82	31	83	5
C2A053	0.2	173	102	28	70	10
C0081	1.0	284	97	29	191	29
C0082	0.8	195	87	30	175	32
C0083	0.6	193	95	31	111	24
C1081	1.3	378	109	33	171	23
C1082	0.7	179	82	42	166	23
C1083	0.5	289	109	15	106	18
C2081	0.9	310	123	51	184	19
C2082	0.5	237	91	33	143	20
C2083	0.5	212	118	30	110	15
C2A081	0.8	331	120	37	146	18
C2A082	0.6	279	112	21	130	19
C2A083	0.4	146	136	26	98	20
C0091	0.9	258	105	24	140	18
C0092	0.4	352	87	19	134	21
C0093	0.3	214	106	15	71	16
C1091	0.6	355	118	28	118	15
C1092	0.3	291	90	20	94	11
C1093	0.3	163	118	8	69	17
C2091	0.3	358	117	27	104	12
C2092	0.4	395	94	27	105	11
C2093	0.3	168	117	18	74	13
C2A091	0.4	293	99	33	93	-
C2A092	0.2	314	104	39	144	22
C2A093	0.2	175	129	13	66	17
C0101	0.4	204	63	11	74	14
C0102	0.2	141	59	16	91	13
C0103	0.3	173	77	13	54	17
C1101	0.6	220	82	14	78	11
C1102	0.3	176	66	9	76	11
C1103	0.3	121	88	19	49	13
C2101	0.5	194	85	20	76	7
C2102	0.3	197	74	18	83	10
C2103	0.3	170	89	13	40	9
C2A101	0.4	213	106	30	111	17
C2A102	0.2	264	67	21	112	14
C2A103	0.2	113	112	12	53	14

Appendix B, continued. Biological Parameters - 306 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
PV0051	1.2	504	232	143	514	76
PV0052	0.6	582	169	-	501	72
PV0053	1.3	1068	201	135	548	55
PV1051	1.5	430	164	133	514	67
PV1052	0.8	476	148	110	504	60
PV1053	1.6	1241	264	3	519	58
PV2051	1.6	420	183	137	433	58
PV2052	0.8	419	155	146	551	57
PV2053	1.2	956	285	158	563	69
PV2A051	1.8	577	189	90	481	92
PV2A052	0.7	501	143	82	566	99
PV2A053	1.3	1161	217	114	451	34
PV0081	2.2	268	179	146	549	89
PV0082	0.7	541	143	97	539	82
PV0083	0.8	777	218	132	520	76
PV1081	2.1	387	163	98	510	86
PV1082	1.3	444	136	124	565	76
PV1083	1.2	647	209	112	430	58
PV2081	1.9	619	212	109	610	96
PV2082	1.4	314	200	135	647	92
PV2083	1.7	1003	211	132	601	81
PV2A081	1.5	759	212	118	507	81
PV2A082	0.9	617	180	105	588	109
PV2A083	1.5	926	472	137	602	62
PV0091	1.6	541	183	119	475	68
PV0092	1.0	591	180	90	632	92
PV0093	1.4	425	228	84	428	69
PV1091	2.5	510	185	120	519	65
PV1092	1.2	477	182	136	542	62
PV1093	2.2	796	274	146	574	80
PV2091	2.0	615	248	140	596	85
PV2092	1.1	680	225	137	654	74
PV2093	2.0	921	334	128	576	83
PV2A091	1.8	621	220	91	481	112
PV2A092	1.0	739	168	74	565	59
PV2A093	1.2	1025	269	94	495	79
PV0101	1.3	797	381	111	448	61
PV0102	0.8	450	129	115	419	47
PV0103	1.2	808	251	118	452	68
PV1101	2.0	516	190	140	451	75
PV1102	1.1	358	130	115	494	67
PV1103	1.1	602	254	124	526	73
PV2101	1.9	476	396	127	496	69
PV2102	1.1	695	155	113	538	55
PV2103	1.5	562	219	171	433	31
PV2A101	1.4	865	199	77	423	48
PV2A102	0.9	694	196	95	599	88
PV2A103	1.3	628	274	99	533	86

Appendix B, continued. Biological Parameters - 306 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
BM0051	0.6	518	98	163	295	41
BM0052	0.5	394	83	131	352	41
BM0053	0.2	434	109	134	263	35
BM1051	0.3	860	110	135	329	36
BM1052	0.8	589	108	162	386	-
BM1053	0.6	423	99	148	250	32
BM2051	0.7	483	123	142	311	37
BM2052	0.5	444	85	136	353	41
BM2053	0.3	511	129	161	261	28
BM2A051	0.3	540	119	63	262	72
BM2A052	0.8	441	103	66	289	68
BM2A053	0.8	424	114	126	207	25
BM0081	0.9	987	143	134	353	47
BM0082	1.0	521	99	126	395	49
BM0083	0.6	827	111	130	314	50
BM1081	1.0	648	139	151	357	50
BM1082	1.2	591	120	142	482	55
BM1083	0.9	642	119	160	364	49
BM2081	1.0	723	129	190	367	46
BM2082	1.2	623	111	145	491	39
BM2083	1.0	977	118	166	305	39
BM2A081	1.0	471	155	121	399	59
BM2A082	1.0	603	122	106	398	89
BM2A083	0.7	717	136	152	316	62
BM0091	0.9	681	131	136	360	46
BM0092	0.8	410	109	113	373	50
BM0093	1.0	514	104	162	290	42
BM1091	1.2	942	142	148	338	48
BM1092	1.1	744	122	133	371	51
BM1093	0.4	682	111	172	290	43
BM2091	0.7	573	146	155	297	41
BM2092	1.0	474	108	127	360	46
BM2093	0.8	470	147	157	258	45
BM2A091	0.6	593	158	70	255	56
BM2A092	0.7	683	123	62	275	75
BM2A093	0.4	506	114	94	298	34
BM0101	0.6	441	86	122	239	34
BM0102	0.8	437	84	129	311	41
BM0103	0.2	461	99	157	286	42
BM1101	0.8	472	90	145	293	39
BM1102	0.9	410	85	121	337	38
BM1103	0.5	437	100	151	314	42
BM2101	0.8	675	110	129	323	33
BM2102	1.0	334	83	115	341	41
BM2103	0.5	432	92	126	281	37
BM2A101	0.5	467	111	66	264	46
BM2A102	0.7	517	88	-	267	15
BM2A103	0.4	390	105	92	220	37

Appendix B, continued. Biological Parameters - 306 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
PM0051	0.6	532	150	48	203	23
PM0052	0.5	459	92	60	207	26
PM0053	0.4	863	115	67	161	13
PM1051	0.6	532	140	48	185	20
PM1052	0.4	525	93	61	188	24
PM1053	0.4	801	121	68	153	19
PM2051	0.5	566	129	54	199	19
PM2052	0.4	441	97	60	220	28
PM2053	0.6	679	108	70	162	16
PM2A051	0.4	473	91	42	182	60
PM2A052	0.3	467	86	41	177	50
PM2A053	0.7	951	110	44	124	25
PM0081	0.8	608	156	42	275	33
PM0082	0.7	537	123	104	316	45
PM0083	0.8	533	127	56	200	29
PM1081	0.7	944	145	64	230	28
PM1082	0.6	377	109	63	258	34
PM1083	0.8	652	135	46	206	31
PM2081	0.4	685	119	53	226	19
PM2082	0.5	501	122	52	258	36
PM2083	0.7	897	131	89	178	20
PM2A081	0.3	546	121	74	220	30
PM2A082	0.4	727	107	54	227	50
PM2A083	0.5	896	105	55	160	29
PM0091	0.7	632	136	41	200	27
PM0092	0.7	565	139	84	240	36
PM0093	0.3	851	157	76	45	8
PM1091	0.5	644	156	37	198	25
PM1092	0.6	798	116	55	215	27
PM1093	0.3	895	108	81	86	11
PM2091	0.7	471	155	39	199	22
PM2092	0.5	616	114	44	207	26
PM2093	0.3	707	120	63	111	18
PM2A091	0.5	553	109	33	174	26
PM2A092	0.5	954	88	39	171	22
PM2A093	0.2	758	96	31	144	23
PM0101	0.4	641	146	29	190	23
PM0102	0.5	275	125	52	220	19
PM0103	0.2	717	92	47	145	-
PM1101	0.5	532	114	27	142	18
PM1102	0.6	442	92	67	192	24
PM1103	0.3	883	110	56	156	14
PM2101	0.4	497	148	30	192	18
PM2102	0.5	451	103	48	155	14
PM2103	0.3	887	120	52	128	19
PM2A101	0.3	540	127	58	134	70
PM2A102	0.4	425	97	36	130	56
PM2A103	0.2	835	81	38	152	49

Appendix B, continued. Biological Parameters - 306 day sampling.

Sample†	protease‡	β-glucosidase§	histidase¶	respiration#	biomass C††	biomass N‡‡
C0051	0.2	209	47	27	87	-
C0052	0.3	193	58	41	78	15
C0053	0.2	215	59	19	174	28
C1051	0.2	154	60	35	75	7
C1052	0.3	337	66	71	106	15
C1053	0.1	384	50	28	194	30
C2051	0.3	188	54	39	82	10
C2052	0.3	332	72	45	77	11
C2053	0.1	232	61	25	59	6
C2A051	0.2	162	62	32	64	38
C2A052	0.2	189	63	45	80	48
C2A053	0.1	209	42	28	61	30
C0081	0.6	152	76	34	165	23
C0082	0.7	277	77	47	191	29
C0083	0.3	255	69	31	140	23
C1081	0.6	247	74	48	173	19
C1082	0.5	262	80	64	159	27
C1083	0.3	264	73	32	132	19
C2081	0.6	229	73	44	147	20
C2082	0.5	348	86	62	158	28
C2083	0.2	293	85	39	126	18
C2A081	0.3	238	78	35	107	22
C2A082	0.5	209	100	52	155	35
C2A083	0.2	273	86	37	86	26
C0091	0.4	252	67	41	125	20
C0092	0.1	182	87	12	89	13
C0093	0.1	154	75	19	106	15
C1091	0.2	202	74	38	124	15
C1092	0.0	311	87	22	88	9
C1093	0.1	273	83	20	97	14
C2091	0.3	279	73	28	125	12
C2092	0.1	302	69	38	91	14
C2093	0.1	292	94	20	76	12
C2A091	0.1	115	59	34	97	-
C2A092	0.1	406	84	65	89	28
C2A093	0.1	232	77	16	45	36
C0101	0.2	242	45	21	107	11
C0102	0.1	250	53	28	77	14
C0103	0.1	228	54	20	86	13
C1101	0.2	172	51	27	78	12
C1102	0.2	330	67	25	70	12
C1103	0.1	299	66	25	68	10
C2101	0.2	165	53	31	100	11
C2102	0.0	295	62	25	80	9
C2103	0.2	277	73	21	78	11
C2A101	0.1	151	59	15	92	13
C2A102	0.1	226	63	25	54	16
C2A103	0.2	264	71	10	53	14

† Sample identification - see Appendix A. for key.

‡ Protease units - μmol tyrosine g^{-1} soil h^{-1}.

§ β-Glucosidase units - μg ρ-nitrophenol g^{-1} soil h^{-1}.

¶ Histidase units - μg NH_4^+-N g^{-1} soil 48 h^{-1}.

Respiration units - μg CO_2-C g^{-1} soil 10 d^{-1}.

†† Biomass C units - μg CO_2-C g^{-1} soil.

‡‡ Biomass N units - μg NH_4^+-N g^{-1} soil.