January 1980

Alteration of Microbial Populations in Surface Mine Revegetation and Their Effects on Nitrogen Cycling

Margaret Mary McCarthy

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A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Engineering

UTAH STATE UNIVERSITY
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Approved:

Major Professor	Committee Member
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Committee Member	Dean of Graduate Studies

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ACKNOWLEDGMENTS

This research was supported, in part, with funds from the USDA, Forest Service SEAM (Surface Environment and Mining) program through the Intermountain Forest and Range Experiment Station, Ogden, Utah; the Decker Coal Company; and the Utah Water Research Laboratory which also provided research equipment and facilities.

I would like to thank Bland Z. Richardson of the Intermountain Forest and Range Experiment Station for the personal interest he took in this research and also Dwight Layton of the Decker Coal Company for all of the information and assistance he provided.

I wish to express my appreciation to the members of my committee: Dr. Donald B. Porcella for all of his support, encouragement and insight; Dr. Dennis B. George for the time he spent in our many discussions; and Dr. R. J. Wagonet, Dr. J. J. Jurinak and Dr. L. R. Andersen whose respective expertise in soils were invaluable.

I am deeply grateful to Dr. V. Dean Adams, not only for his scholarly advice and help but also for his friendship. I would also like to thank Darwin Sorensen for sharing his experience, knowledge and dedication with me.

I especially would like to thank my very good friends and co-workers at the Water Lab for all of their help, particularly Bert, Kevin, Mary Ann, Mary Pitts, and Scott for the enjoyment they added to my work. I would also like to thank the fine secretarial staff for all of their assistance in the completion of this dissertation.

Last, but far from least, I want to thank Charley for his patience, kindness, love and support which made each day a little bit brighter.

Margaret M. McCarthy

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LIST OF SYMBOLS

SP	sample collected in spring
S	sample collected in summer
F	sample collected in fall
W	sample collected in winter
UD	undisturbed site
SP75	site revegetated in spring 1975
SP76	site revegetated in spring 1976
SP77	site revegetated in spring 1977
total N	total nitrogen
NO_2-N	nitrite-nitrogen
NO_3-N	nitrate-nitrogen
NH_4-N	ammonium-nitrogen
ext. P	$NaHCO_3$ extractable phosphorus
total P	total phosphorus
TOC	total organic carbon
CEC	cation exchange capacity
EC_e	electrical conductivity
SAR	sodium adsorption ratio
ATP	adenosin triphosphate
r^2	coefficient of determination
MC	revegetation practice of mining company
1 cm	sample taken from surface to 1 cm
15 cm	sample taken from 1 cm to 15 cm

60 cm	sample taken from 15 cm to 60 cm
90 cm	sample taken from 60 cm to 90 cm
P1	sample taken at 3 weeks
P2	sample taken at 6 weeks
P3	sample taken at 9 weeks
P4	sample taken at 13 weeks

ABSTRACT

Alterations of Microbial Populations in Surface Mine
Revegetation and Their Effects
on Nitrogen Cycling
by
Margaret M. McCarthy, Doctor of Philosophy
Utah State University, 1980

Major Professor: Dr. Donald B. Porcella
Department: Civil and Environmental Engineering

Surface mining in the arid west results in soil disruption by altering structure, water-holding capacity and nutrient availability. Intensive revegetation efforts may end in marginal rehabilitation due to adverse chemical and physical properties of the disturbed soil. Monitoring microbial activity and soil chemistry of revegetated and undisturbed areas of a surface mine in Southeastern Montana enabled delineation of major factors participating in soil fertility were determined.

In the undisturbed site, the upper few centimeters of the soil profile contained most of the nutrients and the microbial populations. In the revegetated sites, due to mixing of various soil horizons, the concentration of nutrients in the surface soil was absent and microbial activity was consequently low.

Laboratory studies indicated the segregation and replacement of A horizon soils restored native soil nitrogen to the upper few centimeters

and reduced the need for heavy applications of inorganic nitrogen fertilizer. Also, the addition of manure to the soils increased gross yields and microbial activity. This may be due, in part, to increased availability of phosphorus in manure and to improved physical conditions of the soil with the manure applications.

(149 pages)

INTRODUCTION

The mining of surface coal in the West to satisfy present and future energy needs in the United States poses many problems of environmental significance. Those problems presently receiving the greatest attention involve land destruction and the potential for water pollution. To minimize and control these problems, the federal government and many states have enacted laws which mandate strict adherence to rehabilitation regulations. These include segregation of overburden from topsoil, limitation on the length of time land can stand barren, and posting of bonds to ensure rehabilitation.

In spite of these laws, adverse chemical and physical properties of the soil and lack of water occasionally result in marginal rehabilitation. Soil disruption alters structure, water-holding capacity and nutrient availability. Nutrient cycling, based on a chemical, biological and physical equilibria developed over decades within the soil cannot be restored without special attention being given to those microorganisms responsible for the physical-chemical changes necessary to ensure rehabilitation of the land.

The major factor responsible for the stabilization of soil aggregates is organic matter. However, organic matter itself, without biological transformation, has little if any effect on soil structure.

The microorganisms influencing soil development are heterogeneous, often difficult to culture, and nearly impossible to observe as a single group (Mishustin 1975). The number and type of bacteria inhabiting a soil depends, in part, on the organic matter content of the soil. Since

this organic fraction contains the organic carbon and nitrogen needed for microbial development, it is the dominant food reservoir (Alexander 1961). When organic matter in soil is decomposed, the nutrients released are either 1) taken up by the microorganisms for growth and maintenance or 2) reintroduced into the soil environment for plant uptake.

Nitrogen is the most frequently limiting nutrient in soils. One of the most important processes in the soil is the mineralization of organic nitrogen to ammonium-nitrogen during decomposition. Bremner (1967) estimated that generally only 1 to 3 percent of the soil organic nitrogen is mineralized during a growing season. It has also been estimated that the amount of exchangeable and soluble inorganic nitrogen rarely exceeds 2 percent of the total soil nitrogen (Harmen and Kolenbrander 1965). Atmospheric nitrogen can be fixed by certain bacteria and soil cyanophyta, becoming bound in the form of protein in biomass. This organic nitrogen is mineralized to ammonium-nitrogen by microorganisms and can be taken up by plants or microorganisms for growth or oxidized by nitrifiers to nitrite and nitrate. Processes such as nitrification are vital to sustain soil fertility.

Nitrogen can be lost from soil by leaching out of the root zone, ammonia volatilization, removal of plant crops and denitrification. In many climax communities where productivity is balanced with decomposition, losses of nitrogen may be replaced by biological nitrogen-fixation. The effects of soil disruption on the nitrogen cycle is to increase nitrogen losses. Surface mining can result in a severe decrease in soil fertility. Marginal revegetation due to low soil fertility can lead to increased costs of land restoration.

Direct on-site costs for reclamation of western lands has been estimated to vary from $618/ha ($250/acre) to as much as $6,795/ha ($2,750/acre) (Cook 1976). The costs depend on:

1) Saving and distribution of topsoil
2) Shaping of overburden
3) Seed-bed preparation
4) Planting, fertilizing, mulching and irrigation, and
5) Drainage control

These costs do not include the cost of not using the land and of protection until seedlings are well established nor does it take into account the possibility of failure or partial failure in obtaining a satisfactory covering of vegetation. Unless the fertility of a soil is to be intensively managed and amended by artificial means, microbial activities are vital to sustain soil fertility and the resultant plant production.

Approach to Study

In view of the importance of microbial activity in productive soils, microbial activity was studied in reclaimed soils disturbed by surface coal mining at the Decker Coal Mine in southeastern Montana. Commercial operations at the Decker Mine started in 1971 and revegetation activities and research have been actively conducted there since 1972. By monitoring both microbial activity and soil chemistry of these revegetated areas an attempt was made to determine major factors participating in the microbial establishment process.

This information was then used to design a laboratory experiment to study the effects on microbial development of adding various levels of

inorganic nutrients, organic amendments and other soil treatments. Also the major factors controlling nitrogen cycling in these soils were determined.

In the laboratory experiments the bioassay technique developed by Sorensen et al. (1975) and Anderson (1976) was used to determine the effects of various fertilizer and soil amendments on microbial development. Lysimeters were used to analyze those treatments determined "most promising" by the bioassay, under field-like conditions.

Correcting imbalances in the carbon-nitrogen ratios within the soil were emphasized in the bioassay. This was accomplished by the addition of nitrogen fertilizers in the form of $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2SO_4$. Also, an attempt was also made to lower the soil pH sufficiently to make other nutrients more available for uptake. The most promising treatments were then analyzed further in the lysimeter study in conjunction with evaluating the effects of various revegetation techniques on nitrogen cycling.

Objectives

The overall objective of this research was to determine which revegetation processes are needed to optimize microbial activity and thereby ensure nutrient cycling, specifically nitrogen cycling. This involved:

1) An evaluation of microbial activities in undisturbed and previously revegetated areas to determine major factors contributing to the development of microbial processes
2) A laboratory study to evaluate the capacity of selected soil amendments to enhance microbial development
3) Studying nitrogen transformations using field-like laboratory experiments

LITERATURE REVIEW

To achieve "energy independence" the United States must develop new energy sources and advance its technology in the efficient extraction and use of resources available within its borders. For the past decade coal as been considered an undesirable energy source due to potential pollution problems from both its extraction and use. Recently, coal has come to the forefront as a major available substitute for imported oil. A ton of coal has approximately the same heating value as four and a half barrels of oil (Atwood 1975). By 1985, coal could be substituted for 6.5 billion barrels of oil (Schuman et al. 1976). A substantial part of this increased coal supply is expected to come from the western United States.

Most of the coal of the western states is contained in the Great Plains Province (Figure 1). These basins are the principal target for coal development by surface mining. The remaining identified coal resources in the Northern Great Plains total approximately 1,383 billion metric tons (1,524 billion tons) of which 58.3 billion metric tons (64.2 billion tons) are considered to be strippable (Northern Great Plains Resource Program 1975). Ninety-three percent of the strippable reserves in the western states contain less than 1 percent sulfur and these reserves are 40 times more abundant than the low sulfur, strippable reserves in the eastern part of the nation (U.S. Department of the Interior 1971). However, western coal is lower in heat value and higher in ash and water content than eastern coal.

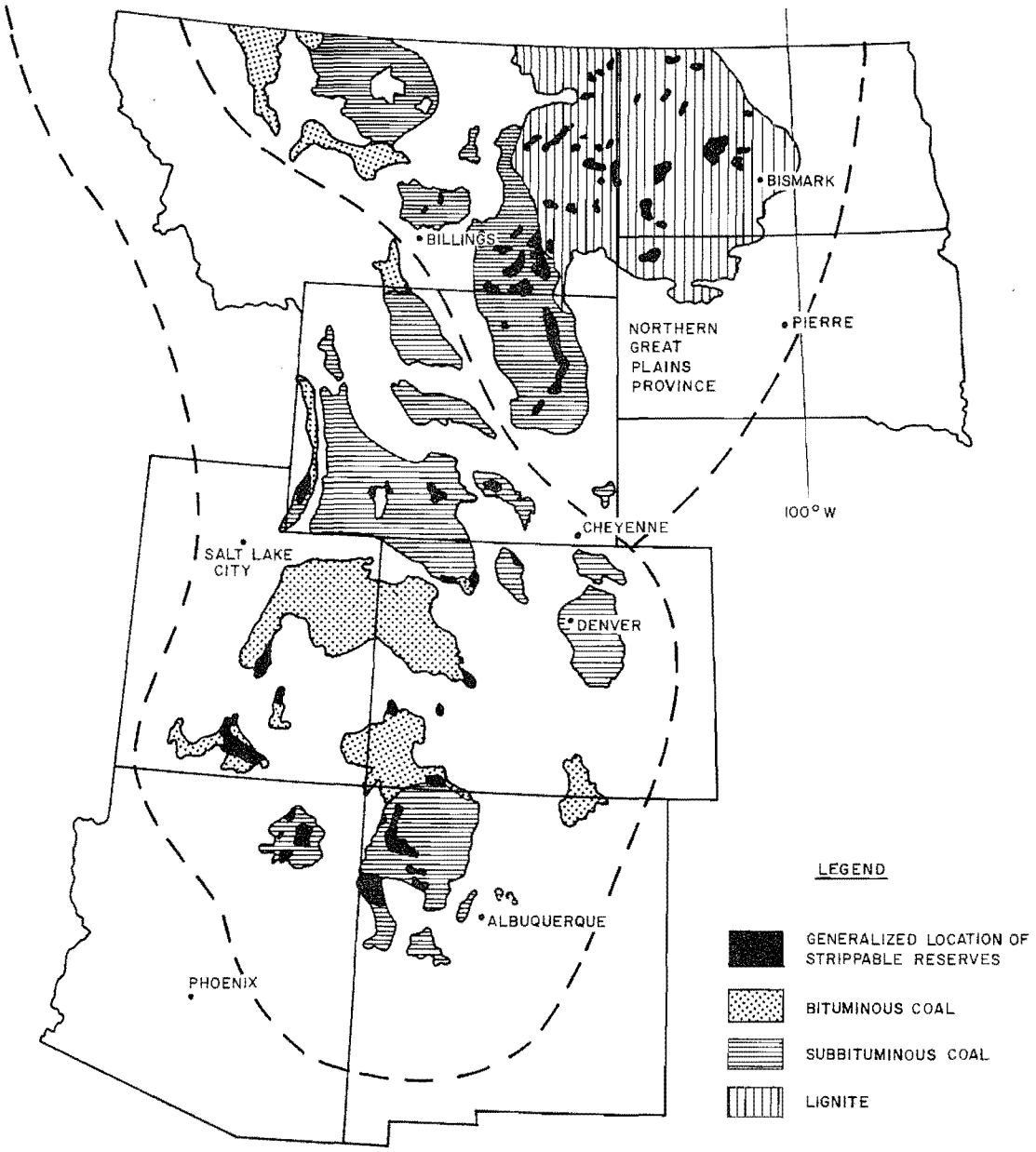


Figure 1. Location of western coal reserves (National Academy of Sciences 1974).

The Mining Operation

Surface mining is a very broad term and refers to any process of removing the earth rock and other strata in order to uncover the underlying mineral or fuel deposit. Strip mining is a type of surface mining in which the overburden is removed in narrow bands, one cut at a time (EPA 1974b).

At the Decker Mine a method known as area strip mining is practiced (Figure 2). After removal and stockpiling of topsoil, the overburden above the coal is drilled and shot with explosives, a trench or box-cut is made through the overburden to expose the deposit of ore to be removed. The overburden from the first cut is placed on unmined land away from the cut. The ore is then removed. Once the first cut is completed, a second cut is made parallel to the first, and the overburden from the succeeding cuts is deposited in the cut just previously excavated. This process is continued to the limits of the property or deposit. After the coal has been mined, the spoil material is smoothed and contoured with bulldozers and the topsoil is replaced. The prepared ground is then seeded and fertilized.

Revegetation in the Arid West

Although much has been learned about revegetation of surface mine areas in the east (EPA 1974a, Doyle 1976), revegetation in the semi-arid west is a relatively new endeavor with little base data from which to build. Surface mine areas in the western states are quite different from those in the east. Acidity has not been a major problem and is not expected to be, while climatic factors are. Mean annual precipitation

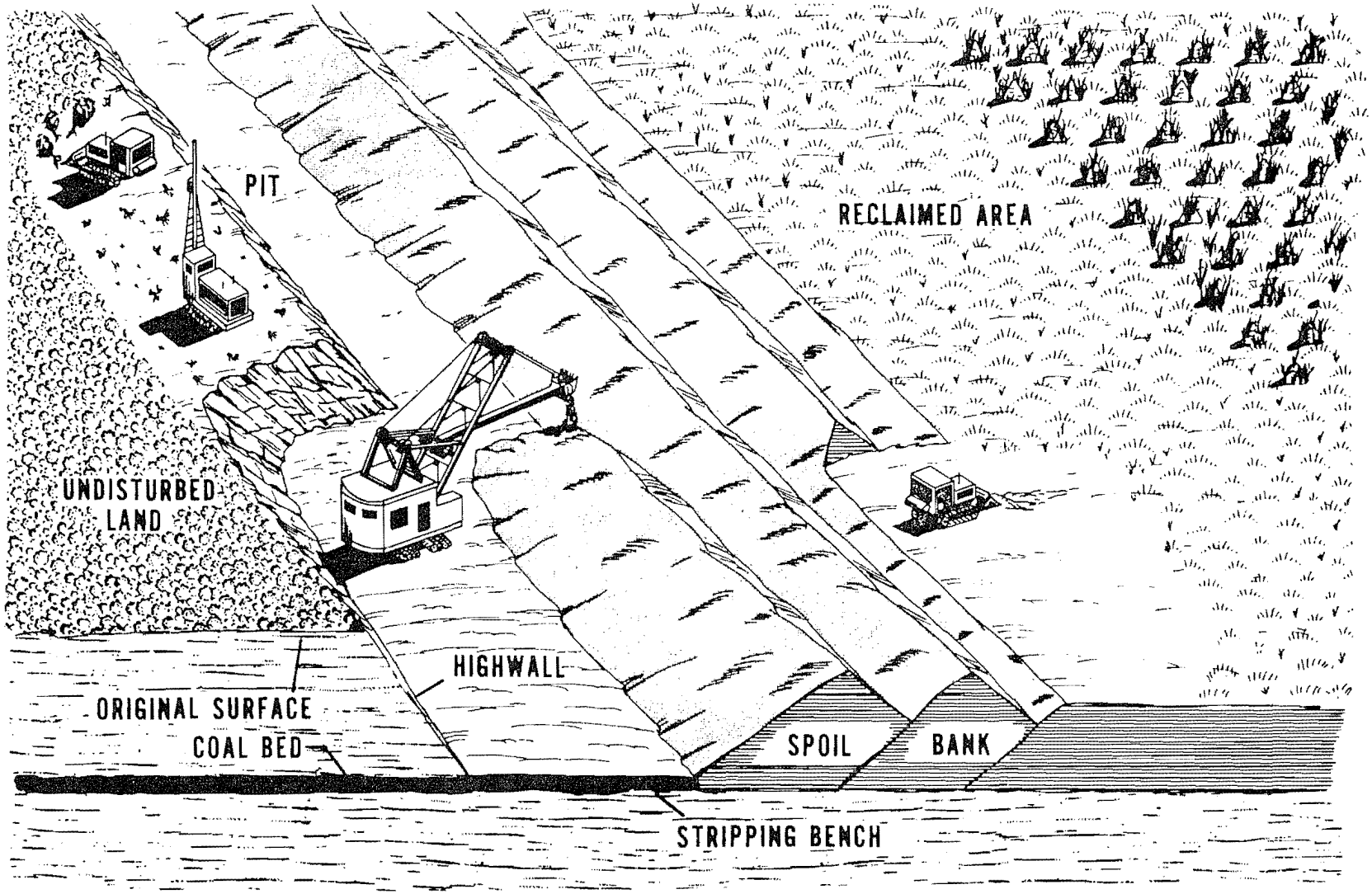


Figure 2. Area strip mining with concurrent reclamation (EPA 1974b).

in the west ranges from only 10 to 50 centimeters with seasonal temperature variations from -50 to 49°C. Soil is generally poorly developed with adequate topsoil lacking (EPA 1974b). Salinity and sodicity problems can exist. These conditions, which are manifested as available soil moisture, are the primary determining factors in successful rehabilitation of surface mines in the west (National Academy of Science 1974). The potential for rehabilitation is extremely site specific with no one method that will be applicable to all sites (Packer 1974).

Sufficient information dealing with all aspects of revegetation of western coal land is slow in coming. The potential for rehabilitation of western coal lands has been reviewed in detail by the National Academy of Sciences (1974), Packer (1974), Cook (1976), Schuman et al. (1976), and USDA Forest Service (1979a,b). All reports conclude that more information is needed. Plant and fertilizer selection has received some attention on a site by site basis (Bjugstad 1978, Aldon 1978, Howard et al. 1977, Farmer et al. 1974, Sindelar et al. 1973) but no definitive answers are available.

Farmer and Richardson (1976) have studied the hydrologic and soil properties of overburden to determine the relation between infiltration rates and time since disturbance of overburden material. They have found that sediments eroded from the overburden piles are more finely textured, have more total salts, contain more sodium and have considerably higher pH than the parent overburden material. They recommend that grading to final configuration be done as soon as feasible to minimize surface erosion potential.

The importance of microbial processes involved in the revegetation of surface mine areas in the west has been recognized (Cundell 1977). Research efforts along these lines, however, have been limited and much work is still required.

Microorganisms in Disturbed Soil

Much of the land subject to disturbance by surface mining of coal in the western states has salt-affected soils (EPA 1974b). The structural problems associated with sodium affected soils can seriously decrease gas diffusion, water activity and water permeability and thereby greatly limit the spectrum of microorganisms that can inhabit the soil. Also, the high pH of alkaline soils is limiting to many soil biochemical processes. These factors may limit the rate of microorganism community development in a disturbed soil system (Cundell 1977). Hersman (1977), working with six study plots on a mine in Colstrip, Montana, was able to differentiate native range from spoil samples based on adenosine triphosphate (ATP) concentrations. The average ATP concentration was higher at all depths in the native range plots than they were in the spoil plots.

Mulching practices, in which organic matter is applied in large amounts to the soil surface or accumulates as large amounts of dead biomass from previous years production, may result in immobilization of available nitrogens and/or phosphorus as microorganisms consume nutrients in order to decompose the carbonaceous material (Parnas 1975, 1976). This kind of nutrient imbalance may also inhibit some types of microbial processes (e.g. cellulose decomposition) as well as having adverse effects on plants (McGill et al. 1975).

Most microbial activity takes place in the top several centimeters of the soil horizon. The surface horizon of the soil is also the most variable in water content. In the semi-arid environments (especially in saline soils) microbial activity may be limited due to water stress (Wilson and Griffin 1975).

The establishment of important nutrient cycling processes in deeper disturbed soil horizons is poorly understood. The nutrient cycling processes in the root zone are very important to soil fertility and assuring their establishment may be vital to revegetation success. Microorganisms may find their way below the surface of the soil by being carried along with percolating water, and/or they may follow the development of plant roots. The plant root environment (rhizosphere) may supply simple carbonaceous substrate to microorganisms by sloughing dead tissue, exuded photosynthate, vitamins and other growth factors needed by microorganisms (Barber and Lynch 1977). This richer rhizosphere environment may stimulate heterotrophic nitrogen fixation, increase microbial respiration, and lead to solubilization of phosphorus and other nutrients (Chan et al. 1963). Any factor having an adverse effect on the development of rhizosphere processes in mine soils will depress productivity. It is, therefore, important to elucidate the process of rhizosphere development in disturbed soils.

Because nitrogen often limits productivity in agricultural and natural ecosystems the understanding of its conversions in soil is important. The intimate relationship between microorganisms to soil nitrogen is the key to understanding the soil nitrogen cycle.

Nitrogen in Soil: Sources, Sinks and Transformations, Losses

Although the nitrogen content of the soil ranges from less than 0.1 percent in desert and semi-desert to a high of only 2 percent in some organic soils (Stevenson 1965), 95 percent of the annual nitrogen flow occurs within the soil and between soil and vegetation (Rosswall 1976).

Sources of soil nitrogen include 1) precipitation, 2) adsorption of NH_3, N_2O and NO from air, 3) soil organic matter, 4) organic fertilizers, 5) biological nitrogen fixation and 6) inorganic fertilizers. It is estimated that precipitation contributes an average of 8.7 kg/ha/yr (7.8 lbs/acre/yr) the contribution due to adsorption from air is negligible (Allison 1965).

Soil organic matter can tie up 90 percent of the total nitrogen in surface soil (Bremner 1965b). This nitrogen source is available only after mineralization to an inorganic form. The principal source of organic fertilizer is cow manure. Allison (1973) reports that a ton of barnyard cow manure usually contains 5 kg nitrogen phosphorus as 2.5 kg P_2O_5 and 5 kg K. However, the release of these nutrients from the manure is slow.

Symbiotic nitrogen fixation, which is difficult to estimate, accounts for some 14 to 53 million metric tons N/yr (Quispel 1974). World fertilizer production via the Haber process is approximately 44 million metric tons of nitrogen annually (Hardy and Havelka 1975). However, a substantial portion, about 50 percent, of the applied fertilizer nitrogen is not recovered by crops (Child 1976) and the production of nitrogen fertilizer is inextricably linked with the energy situation. Eighty percent of all the hydrogen required for making ammonia is produced from natural gas or

lighter petroleum fractions and this production requires 44 billion joules (42 million BTU) to produce a metric ton of ammonia from natural gas (Skinner 1976). In light of the heavy energy dependence of fertilizer production and the fact that biological nitrogen fixation accounts for greater than 90 percent of the biological nitrogen cycle (Postgate 1974) interest in nitrogen fixation is not surprising.

However, only a limited number of microorganisms have the ability to utilize elemental nitrogen; all other organisms require combined nitrogen. Combined nitrogen in soil is largely bound to organic matter and mineral material. Bremner (1965b) estimates that well over 90 percent of the total nitrogen in most surface soils is organically combined. This large pool of organic nitrogen in soil is a potential reserve of nitrogen for nutrition of plants but, biological mineralization of this soil organic nitrogen is slow. It has been estimated that generally only 1-3 percent of the soil organic nitrogen is mineralized during a growing season (Bremner 1967) and only a few kgs N/ha exists in available mineral form. Scarsbrook (1965) defines available nitrogen as a chemical form that can be readily absorbed by plant roots. This includes nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), and ammonium-nitrogen (NH_4-N). Rarely do soils contain more than 1 percent of the total nitrogen in an available form at any one time.

Rosswall (1976) estimates the percentage of nitrogen in microbial biomass to be 4 percent. The amount of nitrogen immobilized in living microbial cells at a given time is usually estimated at less than 1 percent of the total soil nitrogen (Bremner 1967). Another process which immobilizes inorganic nitrogen is the fixation of ammonium by clay minerals in such a way that it becomes unavailable. The amounts of fixed ammonium

have been reported attaining levels above 40 percent of total soil nitrogen by Young (1962) and constituting from 1 to 25 percent of the total soil nitrogen by others (Keeney and Bremner 1966, Bremner 1967).

The process by which organic nitrogen is mineralized to inorganic nitrogen results in the release of ammonium. Although some ammonium in soils is utilized directly by plants, most of it is first oxidized to nitrite and subsequently to nitrate by soil microorganisms. The extent of this nitrification process is determined by the type of clay mineral present, the soil temperature, pH and moisture content, and the percentage of oxygen in the soil atmosphere. The importance of the nitrifying microorganisms rests upon their capacity to produce nitrate which is the major nitrogen source assimilated by higher plants (Alexander 1965). However, the detrimental consequences of ammonium oxidation are apparent. Nitrification of ammonium results in the conversion of a slowly leached, cationic form of nitrogen to a readily leached anionic form. The downward migration of these soluble anions with rainfall depends not only on the amount of rain, but also on the water-holding capacity of the soil. Nitrate ions move downward more readily in sandy than in clay or peat soils (Harmsen and Kolenbrander 1965).

Nitrogen can be lost as an indirect consequence of nitrification by the process of denitrification which produces NO, NO_2, N_2O and N_2. Denitrification is the major process that releases N to the atmosphere (Paul and Victoria 1978). Skujins and Klubek (1978) found that nearly all of the N_2 fixed by a desert algal-licken crust was lost through denitrification over a period of three weeks.

Another method by which nitrogen is lost to the atmosphere is ammonia volatilization. Soils with high $CaCO_3$ concentration, alkaline pH and low

cation exchange capacity frequently have high losses of nitrogen due to ammonia volatilization (Fenn 1975, Gasser 1969). High temperatures and decreased moisture levels also result in higher volatilization (Fenn and Kessel 1974).

The biological nitrogen cycle is responsible for a turnover of 10^8 to 10^9 metric tons of N/yr (Postgate 1974). The internal plant-soil-microorganisms cycle is larger by an order of magnitude than the fluxes to and from the global system (Rosswall 1976). The management of soil fertility increasingly depends on man's understanding and ability to manipulate the biological nitrogen cycle.

N_2 Fixation

Biological nitrogen fixation can be attributed to 1) symbiotic associations composed of a microorganism and a higher plant or 2) nonsymbiotic free living bacteria or blue-green algae. The symbiotic associations between Rhizobium and legumes are responsible for approximately 40 percent of the nitrogen fixed by biological means, and virtually all the nitrogen fixed by cultivated plants. However, there are over 10,000 species of Leguminosae of which fewer than 50 species are cultivated. There may well be other plants in the family that could be exploited for agriculture (Brill 1977). Presently, nitrogen fixation by natural symbiotic processes supplies almost as much fixed N as does the output of synthetic nitrogenous fertilizers (Childs 1976).

The importance of blue-green algae in supplying fixed nitrogen to the soil is probably limited to the initial stages of soil formation (Stevenson 1965). Rychert and Skujins (1974) estimated the annual nitrogen

fixed by desert algal crust in the Great Basin to be 10 to 100 kg N/ha/yr. However, evidence suggests that most of the nitrogen fixed by algal crusts is lost by denitrification (Skujins and West 1974). Childs (1976) cites estimates of nitrogen fixing activity of free-living bacteria as high as 117 kg N/ha/yr under somewhat artificial conditions and values of 2 kg N/ha/yr as more common for in situ experiments.

Numerous articles have been written on the biochemical mechanism by which microorganisms assimilate elemental nitrogen. The reader is referred to Skinner (1976) and Postgate (1974) for extensive reviews of advances and the future potential in biological nitrogen fixation.

Methods for the measurement of N_2 fixation include most commonly: 1) N-analysis using N_2^{15} and 2) the reduction of an alternate nitrogenase substrate, acetylene, to ethylene. The acetylene reduction test for nitrogen fixation is greater than 1000x more sensitive than tests with N_2^{15}. Hardy et al. (1973) provide a comprehensive summary of the use of the acetylene reduction (C_2H_2-C_2H_4) assay for the measurement of N_2 fixation.

Mineralization and Immobilization

The decomposition of organic matter depends on the activities of numerous different populations in the soil. Although the soil fauna is often very important in the incorporation of organic residue into the soil (Ettershank et al. 1978), it is the microflora that plays the dominant role. Campbell (1978) has reviewed the role of the microflora in the decomposition process.

Organic residues added to soil are first broken down to their basic organic components by the extracellular enzymes produced by heterotrophs.

An extensive compilation of enzymes in soil can be found in Burns (1978). The conversion of these simple organic compounds to ammonium with the release of CO_2 is referred to as mineralization. The resynthesis of inorganic constituents into organic compounds by microorganisms is the process of immobilization. Mineralization and immobilization proceed simultaneously and constantly in opposition in the soil.

The ammonium released due to mineralization is associated with a waste product overflow in microbial metabolism, the accumulated ammonium representing the quantity of substrate nitrogen in excess of the microbial demand (Alexander 1961). Immobilization is a consequence of the incorporation of ammonium and nitrate into proteins, nucleic acids, and other organic complexes contained within microbial cells.

Campbell (1978) cited data which establish the equivalence point at which N immobilization equals N mineralization. At a C/N ratio of approximately 22 and a N percentage of 2, mineralization equals immobilization. Smaller ratios (high N percentage) are associated with net mineralization and larger ratios with net immobilization.

Nitrogen turnover due to mineralization and immobilization is usually measured by tracer studies. The tracing of nitrogen transfers through soil microorganisms and their metabolites into more resistant forms in the soils allows for the elucidation of the relationship between recently synthesized microbial metabolites and soil constituents.

Nitrification

Nitrification is usually associated with the energy-yielding reaction in the metabolism of chemo-autotrophic bacteria which leads to both growth

and maintenance of nitrifiers. Numbers of nitrifiers in most soils are rarely greater than a few thousand per gram (Walker 1975). This may be due to their slow growth rate (Morrill and Dawson 1962) and their requirement for considerable amounts of ammonium or nitrite as energy sources (Soriano and Walker 1973).

It appears that a rather diverse population of ammonium oxidizers coexist in several niches within the soil which may share the property of substrate availability but vary individually in substrate concentration, pH, water potential and other parameters (Belser and Schmidt 1978). Those organisms responsible for the majority of ammonium oxidation include Nitrosomonas, Nitrosospira and Nitrosolobus. Nitrobacter is the principal, if not only, nitrite oxidizing genus detected in the terrestrial environment (Belser and Schmidt 1978, Fliermans et al. 1974).

The nitrification characteristics of soils might reasonably be expected to be altered as soil conditions change to present more favorable or unfavorable environments for the nitrifying populations. Inhibition of nitrification in soils can be induced by the accumulation of trace elements (Liang and Tabatabai 1978, Wilson 1977), increased salinity (Laura 1977, Gandhi and Paliwal 1976, Westerman and Tucker 1974) and high pH (Morrill and Dawson 1967). Molina and Rovira (1964) found that root exudate did not inhibit nitrification. Heterotrophic microorganisms in the rich rhizosphere environment quickly immobilized nitrification products making the actual process difficult to measure.

There are numerous methodologies available for studying the nitrification process in soils, two of which are widely used. Lee and Quastel (1946a,b,c) perfected the perfusion technique which allows for sampling without disruption of the soil. Macura and Malek (1958) introduced the

continuous flow method for studying microbiological processes in soil samples. This method also allows for nondisruptive sampling but permits lower concentrations of substrate to be used.

Denitrification

The amount of nitrogen lost from soils as a result of denitrification is still a major unknown quantity in studies of the nitrogen cycle. Recent interest in the extent of nitrogen loss due to denitrification has been intensified by the rising costs of nitrogen fertilizer.

Denitrification is essentially a respiratory mechanism in which nitrate, in the absence of oxygen, functions as the terminal electron acceptor. In "nitrate respiration" the reduction products, N_2O, N_2 and NO gases, are excreted by the microorganism resulting in a loss of nitrogen from the soil. Burford and Bremner (1975) found that denitrification in soils under anaerobic conditions was controlled largely by the supply of readily decomposable organic matter. They reported that analysis of soils for mineralizable carbon or water-soluble organic carbon provided a good index of their capacity for denitrification of nitrate. Gilliam et al. (1978) found that any soil condition which impeded water flow was positively correlated to denitrification.

The amount of nitrogen lost due to denitrification is generally obtained by difference from the other components of the nitrogen cycle. However, the reliability of values obtained by difference are at best no better than the reliability of the other measurements, and all errors are accumulated in the difference value (Rolston et al. 1976).

Until recently, no direct routine method was available for field determination of denitrification. The lack of methods for direct

measurement arose from the fact that N_2 is a major denitrification product. Rolston et al. (1976) made direct field measurements by determining the amounts of N^{15}-labeled N_2O and N_2 evolved from sites treated with N^{15}-labeled nitrate. However, the use of N^{15}-labeled fertilizer is prohibited by cost for routine analysis.

The finding that small concentrations of acetylene (C_2H_2) inhibit the reduction of N_2O to N_2 during denitrification has been verified for soils (Yoshinari and Knowles 1976, Smith et al. 1978). Ryden and co-workers (1978, 1979a,b) developed a technique for direct measurement of denitrification from soils based on C_2H_2 inhibition of N_2O reductase. This technique eliminates the problems associated with working in N_2-free atmospheres and eliminates the cost of isotope work. The potential now exists for studying the extent of denitrification, the distribution of denitrification products and the environmental factors affecting the latter (Ryden et al. 1979a).

Soil Organic Matter

Soil organic matter comes from several sources including plant and animal residue at various stages of decomposition, microbial cells, and substances synthesized and excreted by the soil population including plant roots. The global mass of organic carbon in soil is 30×10^{14} kg. This value more than equals the other surface carbon reservoirs combined (atmospheric $CO_2 = 7.0 \times 10^{14}$ kg, biomass $C = 4.8 \times 10^{14}$ kg, fresh water $C = 2.5 \times 10^{14}$ kg, and marine (above thermocline) $C = 5$ to 8×10^{14} kg) (Bohn 1976). Although deeper carbon reservoirs are much larger, they are physically separated from active interchange with surface carbon reservoirs (Bohn 1976).

Humic substances, the major organic constituent of soils and sediments, account for approximately 60 to 70 percent of the total soil carbon (Griffith and Schnitzer 1975). Because humus contains the organic carbon and nitrogen needed for microbial development, it is the dominant food reservoir.

Parnas (1975) has shown that the decomposition rate of any substrate is a function of the microbial growth rate on that substrate. The microbial growth rate depends upon the chemical composition of the substrate and the physical and chemical condition of the surrounding environment.

During decomposition under aerobic conditions from 20 to 40 percent of the substrate carbon is assimilated and the remainder is released as CO_2 or accumulated as waste products (Alexander 1977). Concomitant to the assimilation of carbon is the uptake of nitrogen, phosphorus, potassium and sulfur. If a microbial cell contains 5 to 15 parts of carbon to 1 part of nitrogen then a reasonable C:N ratio of the soil would be about 10:1. This ratio being a reflection of the dynamic equilibrium that results from the dominating presence of the microbial community.

Incorporation into soil of organic matter having a high C:N ratio will result in nitrogen immobilization making this nutrient unavailable for plant uptake. Nitrogen will remain unavailable until the C:N ratio is decreased to about 10:1. At this point the organic nitrogen that becomes mineralized is no longer necessary for microbial growth, and it remains in the mineral form available for plant uptake.

Parsons and Tinsley (1975) present profile distributions of C and N for some soils all over the world (Figure 3). In most of these soils the organic carbon and nitrogen accumulates near the surface with concentrations decreasing with depth. Only the basin peat, characterized

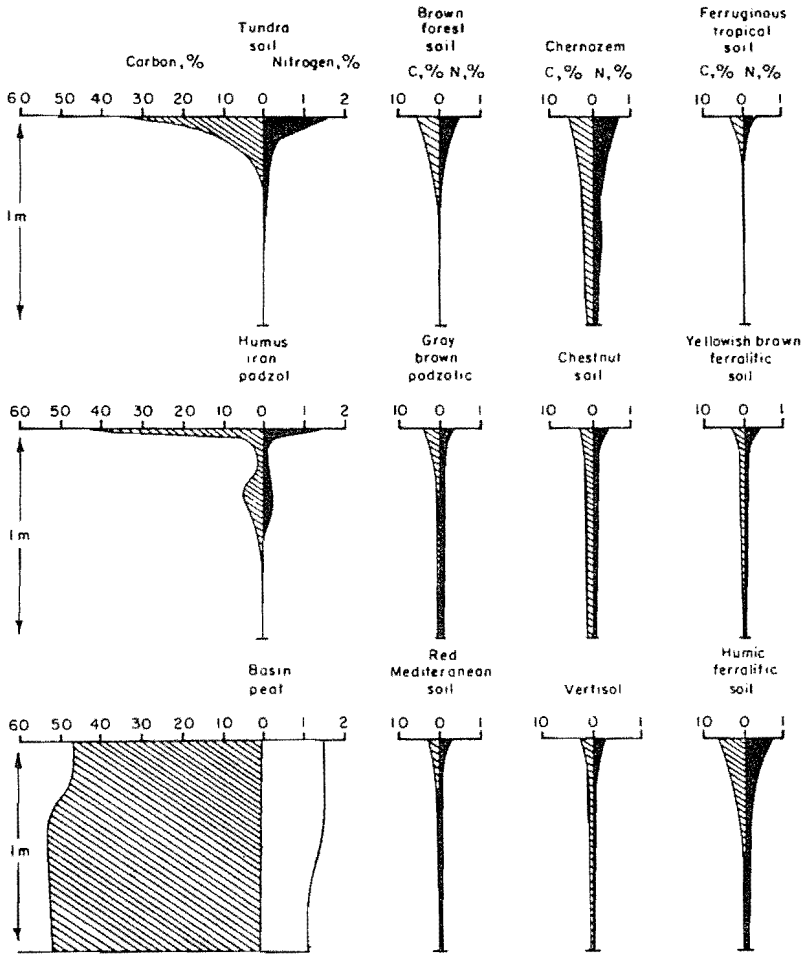


Figure 3. Distribution of carbon and nitrogen in representative soil profiles. (From Parsons and Tinsley 1975.)

by poor aeration therefore low decomposition rates, accumulate carbon and nitrogen to greater depths.

In arid regions, surface soils have C:N ratios usually between 6 to 14:1, with lower soil horizons having ratios of 2:1 or less (Porcella et al. 1973, Balph et al. 1973). Skujins and West (1974) report that the Curlew Valley soils of the Great Basin Desert have C:N ratios of from 11 to 8:1.

Soil pH and Nutrient Availability

The pH of the soil solution is, in general, dependent primarily on the type of parent material, amount of rainfall and other soil forming factors, such as microbial activity (Russell 1961). In arid and semi-arid regions where rainfall is insufficient to provide leaching, the products of weathering are localized and enhanced by high evaporative demands. The presence of lime minerals, which include primarily $CaCO_3$ and $CaMg(CO_3)_2$, is a distinguishing characteristic of soil profiles in semi-arid and arid regions (Jurinak no date). The buffer intensity of these minerals controls the magnitude of shifts in the pH of the soil solution. The general relationship between soil pH and nutrient availability is shown in Figure 4. The pH for greatest availability for most nutrients in a mineral soil is approximately 6.5.

Soil Phosphorus

Phosphorus is present in soil in a variety of inorganic and organic forms, most of which are relatively insoluble. Insoluble phosphates, not directly available to plants or microbes comprise around 95 to 99 percent of the total soil phosphates in alkaline soils (Hayman 1975). Native phosphorus in these soils is found mainly as calcium phosphates (Olsen 1953).

The predominant dissolved orthophosphate species over the pH range 5 to 9 are $H_2PO_4^-$ and $HPO_4^=$ (Stumm and Morgan 1970). These ions are known to form complexes, chelates and insoluble salts with a number of metal ions. The extent of complexing and chelation between various phosphates and metal ions will depend upon the relative concentrations of the

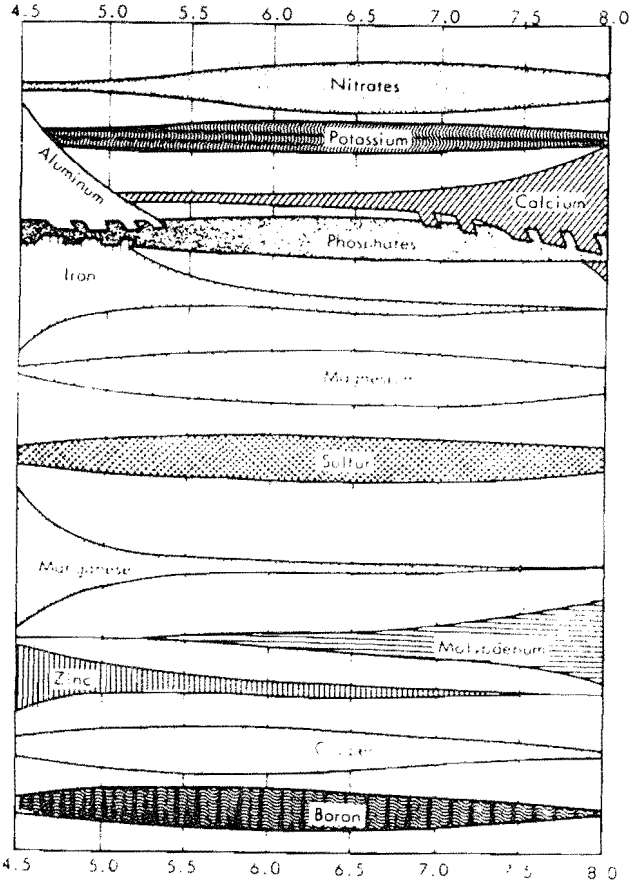


Figure 4. The relationship between soil pH and relative plant nutrient availability (the wider the bar, the greater is the availability). Where elements are shown interlocking, those two elements at that pH combine to form insoluble compounds, reducing phosphate availability (Kentucky Ag. Exp. Sta. 1970).

phosphates and the metal ions, the pH and the presence of other ligands (i.e. sulfate, carbonate, fluoride, organic species) (Stumm and Morgan, 1970).

At low phosphate levels, surface sorption is the dominant factor in determining the phosphate concentration of the soil solution. Phosphates are adsorbed on $CaCO_3$, kaolinite, montmorillonite or hydrous oxides of iron and aluminum (Figure 5). A strong tendency toward chemical bonding between phosphate groups and metal ions in a solid lattice appears to be

the underlying principle for these various "sorption" phenomena (Stumm and Morgan 1970).

Stumm and Leckie (1970) studied the sorption kinetics of the phosphate-calcite reaction and concluded that the adsorption reaction involved three steps:

1) Chemisorption of phosphate accompanied by heterogeneous formation of nuclei of amorphous calcium phosphate,

2) A slow transformation of these nuclei into crystalline apatite, and

3) Crystal growth of apatite

Therefore, deficiencies of phosphorus in plants is frequently caused, not by lack of phosphorus in the soil but, by its unavailability to

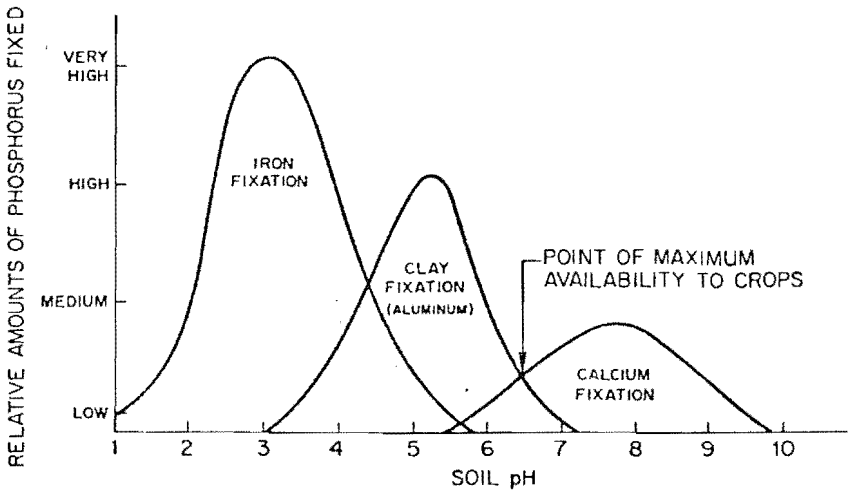


Figure 5. Soil phosphorus is fixed, or made less available, by the formation of less soluble phosphates of iron, aluminum (for clays) and calcium. Maximum phosphorus availability is at a pH of 6.5 for mineral soils (Scarseth 1962).

plants. At the microsite, microorganisms are able to mobiles these insoluble phosphorus compounds by the production of acids. These acids convert insoluble calcium hydroxyapatite to more soluble di- and monobasic phosphates with the net result of an enhanced availability of the element. The oxidation of elemental sulfur is a simple and effective means of providing the acid environment and hence utilizable phosphates.

Oxidation of Sulfur in Soils

In the early 1900's Lipman et al. (1916a,b) and McLean (1918) utilized the oxidation of sulfur in soils as a means of increasing the availability of mineral phosphates. Although sulfur oxidation in soils continues to be the subject of numerous investigations, only a few of the investigations have considered the use of sulfur for increasing phosphorus availability (Kittams 1963, Terman et al. 1964).

Sulfur as a fertilizer has received much attention lately due to recent sulfur deficiencies in plants resulting from increased use of essentially sulfur free fertilizers and the substitution of natural gas and refined hydrocarbons for high sulfur coals. The minimum level of sulfur for normal plant growth ranges from 0.3 to 1 mg per 100 g soil (Jordan and Ensminger 1958). Most soils contain between 0.1 to 0.5 mg S per g soil but it lies in the unavailable organic fraction of the soil (Burns 1967).

The oxidation of sulfur in soil depends on several variables. Li and Caldwell (1966) studied the effects of particle size, application rate and incubation time on the amount of elemental sulfur oxidized. They found that the smaller the particle size of S, the more rapid the oxidation. Oxidation of less than 100 mesh S reached a maximum of about 50 percent

after 60 days. They also found that rates of S application ranging from 10 to 100 mg per g soil was not an important factor in affecting oxidation rates.

Moser and Olsen (1953) found that heavier soils (more clay) oxidized sulfur more rapidly than lighter soils. Burns (1967) reported that mixing the sulfur with the soil maximized the soil-sulfur contact, reduced possible effects of the buildup of sulfur oxidation products and often improved sulfur-moisture relationships, all of which enhanced sulfur oxidation. Soil pH does not appear to be critical for sulfur oxidation and the process is fairly rapid in soils ranging in pH from 4.0 to 9.6 (Zajic 1969). In most soils, at near optimum moisture and temperature, the inorganic oxidation of sulfur is insignificant in comparison with the microbial conversions (Alexander 1961).

Both autotrophs and heterotrophs are capable of oxidizing inorganic sulfur compounds. The bacteria using such molecules for energy are the most important in soils and are chiefly members of the genus Thiobacillus. These bacteria are strict aerobes and obtain all energy from oxidation of sulfur compounds with CO_2 serving as their only carbon source (Zajic 1969). The following equation typifies the transformations catalyzed by these bacteria.

$$S + 1.5\ O_2 + H_2O \rightarrow H_2SO_4$$

The effect of sulfur oxidation on soil pH however, is quite variable. Differential oxidation in different soils with variable buffering capacities make it practically impossible to make exact predictions of pH change.

Manure

Cattle produce nearly 1 billion metric tons of manure each year (U.S. Dept. Agri. 1968). The problems associated with its collection, transport and disposal will not be discussed here. Of importance is the beneficial use of manure for aiding plant growth.

Manure is partially decomposed organic matter which functions similarly to natural soil organic matter and hastens humus accumulation within the soil (Bartholomew 1965). Cattle manure approximates 25 percent dry matter and usually consists of from 2 to 8 percent nitrogen, 0.2 to 1 percent phosphorus, 1 to 3 percent potassium, 1 to 1.5 percent magnesium, 1 to 3 percent sodium (Donahue et al. 1977), and approximately 89 percent carbon, oxygen and hydrogen (Walsh and Hensler 1971). In comparison with chemical fertilizers, manure is low grade, supplying small quantities of plant nutrients per unit of dry weight. However, besides increasing the soil organic matter content and nutrient levels, incorporation of manure into the soil improves the soil tilth and water intake, increases the size of the water-stable soil aggregates (Guttay et al. 1956), increases the water holding capacity (Salter et al. 1967), and significantly reduces the soil bulk density as well as the modulus of rupture (Tiarks et al. 1974). Manure also contains many micronutrients (i.e. 0.03 percent iron, 0.07 percent boron, 0.01 percent zinc, 0.01 percent manganese, 0.003 percent copper) which are not found in chemical fertilizers (Walsh and Hensler 1971). The nitrification process in soils may be improved by manure additions due primarily to an increase of the ammonium substrates and improved environmental conditions in the soil (Alexander 1965).

Recommended application rates solely for plant growth enhancement range from 33.6 to 56 metric ton/ha (15 to 25 tons per acre) on irrigated lands in the western states (Meek et al. 1974). Weeks et al. (1972) found that there is no economic advantage for manure applications much in excess of 45 metric tons/ha (20 tons per acre), however, only 20 percent of this amount can be used on non-irrigated dryland grains in the west (Donahue et al. 1977).

Microbial Bioassay for Soils

Nutrient imbalances, salinity, pH, and lack of a stable microflora often make the revegetation of arid and semi-arid surface mines a difficult and lengthy process. The quantity of nutrients or amendments to be added is difficult to assess because of the complex biological, physical and chemical reactions dynamically occurring in the soil water system. The availability of a nutrient to a plant or microorganism is difficult to measure and techniques used are subject to much controversy. In some cases, a nutrient is assimilated by an organism as fast as it is made available and thus a chemical assay of the nutrient availability will give poor correlation with observed growth (Alexander 1971).

Since the microbial component plays an important part in the fertility status of a soil, microbiological methods for determining nutrient deficiencies and toxicities have long been regarded as being rapid and simple in comparison with those methods requiring higher plants. Algal bioassays have become a standard tool in aquatic biology (EPA 1971, 1973) yielding valuable information about toxicities and nutrient limitations to productivity in aquatic systems. The basic principles of the algal bioassay have been expanded, with appropriate modification, to soil

systems by Sorensen et al. (1975) in the form of a soil algal bioassay. This bioassay is used as a screening tool to determine the deficiencies and toxicities of any number of elements depending on the original design of the bioassay.

MATERIALS AND METHODS

The largest region in the Northern Great Plains coal province is the Fort Union region which encompasses the western half of North Dakota and parts of South Dakota and Montana. The Powder River region, southern extension of the Fort Union region, continues from southern Montana into northeastern Wyoming. This region contains nearly 218 billion metric tons of sub-bituminous coal (Glass 1972). The coal seam averaging about 15 meters (50 feet) thick, is buried under approximately 18 meters (60 feet) of overburden.

The Decker Mine operates in a part of the Tongue River member of the Powder River Region. The soils in the area of the mine are classified as clayey and loamy Ustic Aridisols, often containing a large amount of sodium (Farmer and Richardson 1976). The average precipitation in this region is approximately 36 centimeters (14 inches) with 50 percent of it occurring between April and July (Bjugstad 1978). The major grasses of the area include Festuce (fescus), Agropyron (wheatgrass), Stipa (needle-grass) and Bouteloua (bluestem) (Wright and Wright 1948). Overgrazing in parts of the region, however, has resulted in an increase in Artemesia (sagebrush).

This research was divided into two phases. Phase one consisted of a field study to monitor the effects of revegetation on selected variables relating to soil chemistry and microbial activities at the Decker Mine. The purpose of this study was to identify those chemical variables which have the greatest influence on microbial growth and development. Phase two, a laboratory study, was an endeavor to analyze the effects of those

variables determined in phase one to have the most influence on the microbial community.

Field Study

Soil samples were collected quarterly for one year, from three areas at the Decker Mine which had been revegetated in the spring of 1975, 1976, and 1977 respectively and from one undisturbed area (Sp 75, Sp 76, Sp 77, and UD). Samples were taken using a simple random sampling design (Petersen and Calvin 1965) at three locations on each site to a depth of 90 cm. The surface centimeter, 1-15 cm, 15-60 cm and 60-90 cm horizons from each of the triplicates were composited and then subsampled for analysis. The chemical, biological and physical analyses performed on each composite sample are shown in Table 1.

Table 1. Analyses performed on samples.

Chemical	Biological	Physical
pH (in water)	Respiration	CEC[c]
Total Nitrogen	Dehydrogenase	Sieve Analysis[b]
Extractable Nitrate-N	N_2-fixation	Hydrometer Analysis[b]
Extractable Nitrite-N		% Field Moisture
Exchangeable Ammonium-N		Moisture Potential[a]
Total Phosphorus		
Bicarbonate Extractable Phosphorus		
Total Organic Carbon		
Soluble Salts (Ca^{++}, Mg^{++}, Na^{+}, K^{+}, $SO_4^{=}$, Cl^{-})[c]		
EC_e[c]		

[a]Analysis performed on May samples only.
[b]Analyses performed on August samples only.
[c]Analyses performed on November samples only.

Analytical Techniques

Analytical techniques employed were as follows:

1) Soil pH was measured on a 1:1 (w/w) suspension of soil in DDW (Peech 1965). A one to one ratio was used to eliminate potential errors due to possible poor contact between the soil and the glass electrode at lower soil water content and also to eliminate erroneously high pH values due to dilution at higher water content.

2) Total nitrogen (N) was determined with a Coleman model 29 nitrogen analyzer (micro-Dumas nitrogen technique, Coleman Instruments 1968). In this method of determining N, the sample is heated with copper oxide at a high temperature (usually above 600 C) in a stream of purified CO_2, and the gases liberated are led over hot Cu to reduce nitrogen oxides to N_2, and then over copper oxide to convert CO to CO_2. The N_2-CO_2 mixture thus obtained is collected in a nitrometer containing concentrated alkali, which absorbs the CO_2, and the volume of N_2 gas is measured. The Dumas method can give inflated results with highly organic soils due to incomplete combustion with formation of methane or other hydrocarbons instead of CO_2 (Bremner 1965c).

3) Exchangeable ammonium-N, nitrate-N and nitrite-N were extracted using 2 M KCl. The extracts were analyzed directly for nitrate-N and nitrite-N and, following steam distillation (Bremner 1965a), for ammonium-N using a Technicon Auto-Analyzer II (APHA 1975). This method permits quantitative extraction of inorganic forms of nitrogen which account for the available forms of nitrogen in the soil.

4) Total phosphorus was determined using persulfate digestion of the total sample followed with analysis on the Technicon Auto-Analyzer II (APHA 1975). The total phosphorus content of the soil includes all of the orthophosphate, condensed phosphate (pyro-, meta-, and poly-phosphates), and organically bound phosphate.

5) Phosphorus, soluble in sodium bicarbonate (Olsen and Dean 1965), was analyzed by the ascorbic acid technique (Strickland and Parsons 1972). Moser et al. (1959) found a high correlation between $NaHCO_3$ extractable phosphorus and uptake of phosphorus by plants.

6) Total organic carbon was determined with an Oceanography model 0524B total carbon system (Oceanography International Corp. no date). This method involves a high temperature, wet oxidation of organic carbon to carbon dioxide, the concentration of which is determined using a nondispersive infrared analyzer.

7) Soluble salts in saturation extracts were determined by titration (Mg^{++} and Ca^{++}), atomic absorption (Na^{+} and K^{+}), and using the Technicon Auto-Analyzer II ($SO_4^{=}$ and Cl^{-}) (APHA 1975). The adverse effect of soluble salts in the soil is the reduction of the osmotic potential and hence the water potential, thereby reducing water availability.

8) Respiration rates were measured by analyzing CO_2 evolution in a closed container using flame ionization gas chromatography as described by Colket et al. (1974). The measurement of CO_2 released due to the breakdown of carbonaceous substrates by microorganisms has been extensively used as an index of biological activity in soil. These respiration rates have been correlated with other measures of microbial activity, organic matter content and nitrogen transformations.

9) Dehydrogenase activity was assayed using a modification of the method described by Casida et al. (1964). Dehydrogenase activity is an index of soil biological activities and correlates highly with other indices of biological activity (Skujins 1973). The measurement of dehydrogenase enzyme activity, particularly succinate dehydrogenase, is accomplished by the reduction of 2,3,5-triphenyltetrazolium chloride to 2,3,5-triphenyltetrazolium formazan, a compound extracted from the soil and read spectrophotometrically. Tetrazolium salts are low redox potential indicators which upon reduction form water insoluble formazans.

10) Nitrogen fixation rates were estimated using the acetylene reduction technique described by Hardy et al. (1973). The assay procedure involves utilization of the nitrogenase-catalyzed reduction of acetylene (C_2H_2) to ethylene (C_2H_4) coupled with flame ionization gas chromatographic analysis and is based on the inhibition of N_2-fixation by C_2H_2 and the reduction of C_2H_2 to C_2H_4.

11) Sieve analysis and hydrometer analysis were done as described in Dunn et al. (1977). A soil classification system based on particle size and particle size distribution reflects the soil textural properties which have an important bearing on the physical properties of the soil.

12) Percent field moisture was determined gravimetrically by oven drying a field sample transported to the lab in a sealed container.

13) Soil moisture potential was measured using electronic dew point (psychrometric techniques) in a laboratory sample chamber. Moisture potential is an energy term which describes the work necessary to

extract water from a system relative to pure free water (Brown and Van Haveren 1972). Soil moisture potential can be accurately estimated from measurements of the equilibrium relative humidity of the soil atmosphere by use of the thermocouple psychrometer (Wiebe et al. 1971).

14) Electrical conductivity (EC_e) and cation exchange capacity (CEC) were determined at the Soils, Plant and Water Analysis Lab, Utah State University, Logan. The electrical conductivity of a soil extract gives some indication of the total concentration of ionized constituents in the soil solution. The cation exchange capacity relates the sum total of the ability of the soil to attract and hold cations due to the excess negative charge.

15) ATP concentrations were measured using a composite of the methods outlined by Karl and LaRock (1975) and Paul and Johnson (1977). As shown in the work of Hersman (1977) and others, ATP measurements provide a good estimation of the total microbial activity in the soil. The extracted ATP is assayed by means of the firefly bioluminescent reaction using a commercial ATP photometer (SAI Tech. Co. 1975).

The bioluminescent reaction yields a photon of light for each ATP molecule consumed:

$$\text{luciferin (red.)} + \text{ATP} + O_2 \xrightarrow[Mg+2]{\text{luciferase}} \text{luciferin (oxd.)} + \text{AMP} + \text{P} - \text{P} + H_2O + h_v$$

Laboratory Study

Both topsoil (defined by guidelines set forth by the State of Montana) and overburden (that material which lies between the topsoil and the

coal) were obtained from the Decker Mine in sufficient quantity for both phases of the laboratory study. In the bioassay, that fraction of the topsoil which was less than 1.40 mm in diameter was used. The material used in the lysimeters was that fraction of both the topsoil and the overburden which was less than 2 cm in diameter.

Bioassay

The experimental design of the bioassay (Table 2) was based on results compiled from the field samples. The amendments and treatment levels chosen were added to topsoil samples only. Nitrogen fertilizer in the form of $Ca(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2SO_4$ was added in amounts equal to or greater than the concentration presently used by the mining company. Phosphorus was added at various levels alone, and in combination with nitrogen additions.

Low levels of sulfur were added to some samples to see if oxidation of sulfur to sulfuric acid would lower the pH sufficiently to 1) make nutrients such as phosphorus and trace elements such as zinc and iron available to plants and 2) produce gypsum from calcium carbonate, which plays an important role in the conversion of sodium saturated clay to calcium clay and therefore increase soil permeability to water.

Physical conditions, namely light, temperature and moisture content, were set and maintained to ensure optimal growth conditions for soil cyanophytes (Table 3).

The bioassay technique followed closely the procedure outlined by Anderson (1976). Fifty grams of air-dried topsoil were placed into a petri dish and the moisture potential maintained at -1 bar throughout the experiment, using deionized distilled water. Algal inoculation was done as

Table 2. Experimental design of bioassay.

Treatment Number	Treatment[a]	Level	Form	Method of Addition
1	Control	-	-	-
2	MC[b]	0.04 mgN/g 0.08 mgP/g 1.1 mg/g wood fiber	$Ca(NO_3)_2$ $CaHPO_4$	Solution Suspension Dry
3	MC + Sulfur (S)	Same as 2 + 0.01mgS/g		Surface application
4	Nitrogen (N)	0.04 mgN/g	$Ca(NO_3)_2$	Solution
5	Nitrogen (N)	0.08 mgN/g	$Ca(NO_3)_2$	Solution
6	Nitrogen (N)	0.04 mgN/g	$(NH_4)_2SO_4$	Solution
7	Nitrogen (N)	0.08 mgN/g	$(NH_4)_2SO_4$	Solution
8	Phosphorus (P)	0.08 mgP/g	$CaHPO_4$	Suspension
9	N + P	(Same as treatments 4 + 8)		
10	N + P	(Same as treatments 5 + 8)		
11	N + P	(Same as treatments 6 + 8)		
12	N + P	(Same as treatments 7 + 8)		
13	N + P + S	(Same as treatments 4 + 8 + 0.01 mg S^o/g)		
14	N + P + S	(Same as treatments 6 + 8 + 0.01 mg S^o/g)		

[a] 3 replicates/treatment.

[b] Treatment presently used by the mining company.

Table 3. Maintenance levels for control of physical conditions.

Parameter	Level
Light intensity[a]	2100 lux
Temperature[a]	24 $\pm$ 2°C
Moisture potential	-1 bar

[a] APHA (1975).

described by Anderson (1976) with addition of amendments just prior to inoculation.

Twenty-four hours following inoculation, dehydrogenase activity was measured on one replicate of each treatment to have an initial estimate of the microbial population. Three weeks following inoculation the treatments were sampled for chlorophyll-a and dehydrogenase activity. The treatments were then air dried for 24 hours, rewet to a water potential of -1 bar and incubated in the dark at 26°C for 1 week. Following this time period the bioassay was terminated and samples were taken for dehydrogenase activity, ATP, extractable phosphorus, nitrate-N, nitrite-N and exchangeable ammonium-N.

Lysimeters

The lysimeter study, based on the results of the bioassay, permitted detailed analysis of the nutrient status and microbial response of the soil. Six teflon lined steel drums (60 cm (d) x 90 cm (h)) with 1 cm drainage pipes in the bottom were used as lysimeters (Figure 6). Each lysimeter was filled as follows. Overburden was placed in the bottom to a depth of 15 cm. B horizon soil was placed over the overburden to a depth of 45 cm and A horizon topsoil was placed to a depth of 15 cm over the B horizon. With the lysimeters constructed in this manner, sampling could be done to correspond with sampling depths used in the field studies.

Lysimeters were maintained in a controlled environment with a light intensity of 2100 lux and with temperatures ranging from 21 to 27°C. Plant species chosen to be used in the lysimeter study were those currently being used at the mine (Table 4). Potential germination of this seed mixture was measured in a petri dish containing DDW moistened filter

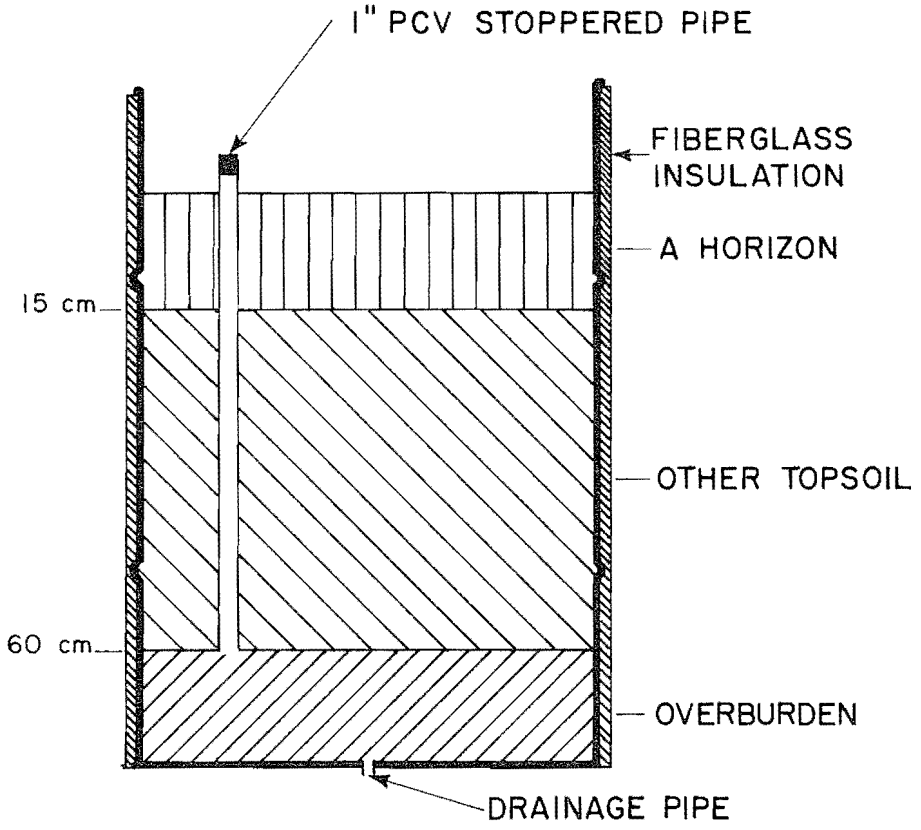


Figure 6. Cross-sectional view of lysimeter. (Note: PVC pipe is inserted into sampling hole to prevent disruption of soil around hole.)

Table 4. Decker Coal Company seed mixture.

The following seed mixture was applied at 28.5 lbs per acre (32 kg/ha):		
Species	lbs/acre P.L.S.	kg/ha
Agropyron smithii (western wheatgrass)	6.0	6.7
Agropyron dasystachyum (thickspike wheatgrass)	3.0	3.4
Agropyron trachycaulum (slender wheatgrass)	3.0	3.4
Agropyron sp. (Whitmar wheatgrass)	3.0	3.4
Stipa viridula (green needlegrass)	4.0	4.5
Atriplex canescens (four-wing saltbush)	0.5	0.6
Agropyron trichophorum (pubescent wheatgrass)	1.0	1.1
Bromus inermis (smooth bronegrass)	1.0	1.1
Omobrychis viciaefolia (sanfoin)	3.0	3.4
Boutelova curtipendula (sideoats grama)	2.0	2.2
Orysopsis hymenoides (Indian ricegrass)	2.0	2.2
	Total 28.5 lbs/acre	32 kg/ha

papers. The germination ratio averaged 0.50 which compares well with expected germination ratios for native seeds (J. Wagonet, personal communication). Rainfall records from the mining company averaged over the three previous growing seasons were used to establish a watering schedule (Figure 7). In this way, snowmelt and storm events could be simulated to correspond with field conditions. Each lysimeter was wrapped in fiberglass insulation to minimize temperature changes at the sides of the lysimeter.

The amendments and level of treatment chosen for use in the lysimeter study were those evaluated to be the "most promising" from the bioassay study (Table 5). In addition, one lysimeter was set up as a control with no treatment, and a second lysimeter was treated according to revegetation techniques employed at the mine.

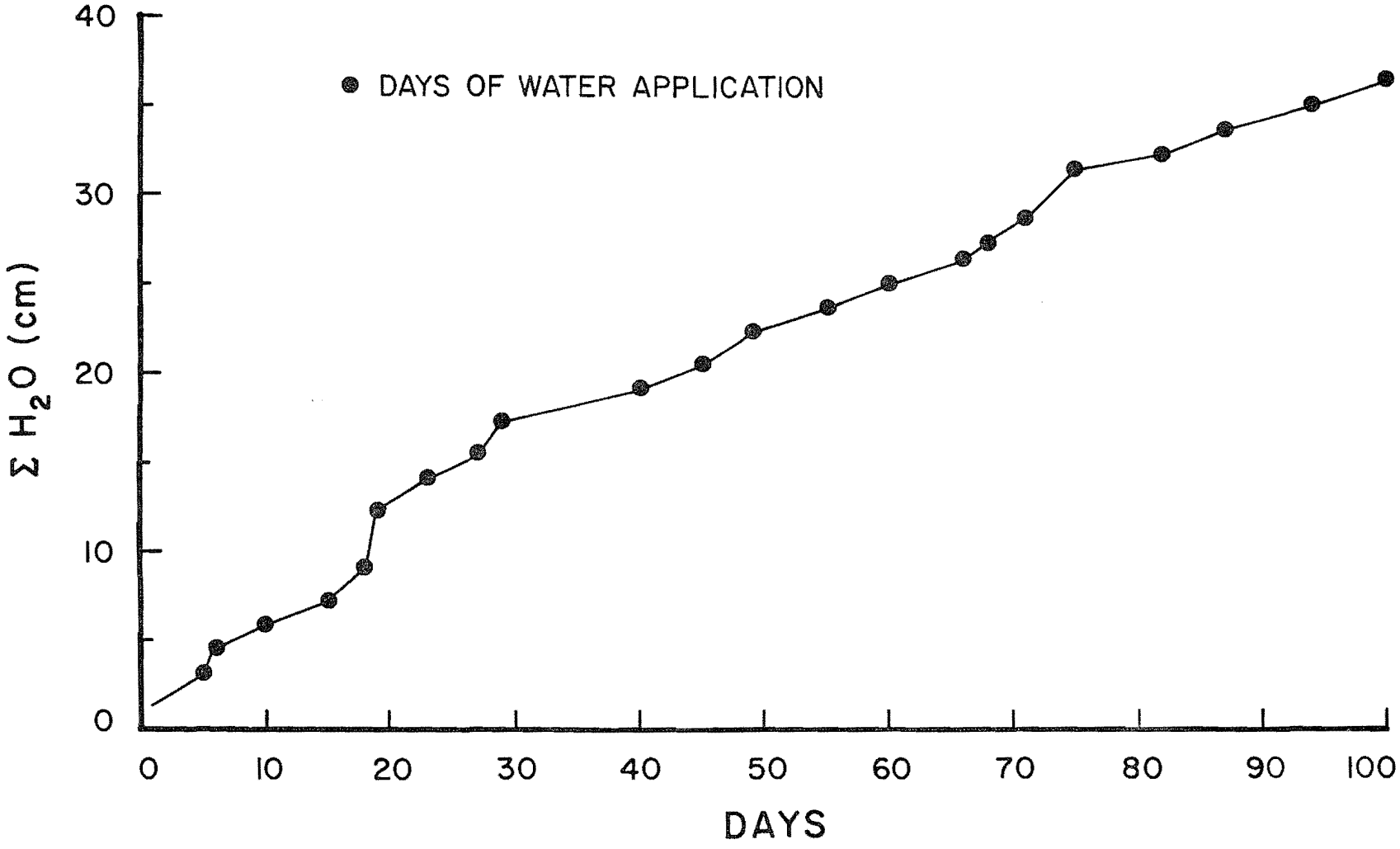


Figure 7. Watering schedule during lysimeter experiments.

Table 5. Lysimeter treatments.

treatment \ lysimeter	1	2	3	4	5
no treatment	*				
manure $\frac{9\ mt}{ha}$			*		
0.04 $\frac{mgN}{g}$ $Ca(NO_3)_2$; ($\frac{81\ kg\ N}{ha}$)		*		*	
0.04 $\frac{mgN}{g}$ $(NH_4)_2\ SO_4$					*
0.08 $\frac{mgP}{g}$ $CaHPO_4$; ($\frac{162\ kg\ P}{ha}$)		*		*	*
0.01 $\frac{mgS}{g}$ S^o; ($\frac{33\ kg}{ha}$)				*	*
wood fiber $\frac{2.24\ mt}{ha}$		*	*	*	*
seed mix $\frac{32\ kg}{ha}$	*	*	*	*	*

The lysimeters were maintained for 13 weeks with samples taken in triplicate at four different times. Sampling of the lysimeters was done with a 2.5 cm diameter soil auger to the following three depth increments: 0-1 cm, 1-15 cm and 15-60 cm. Immediately after sampling, the holes left in the soil were filled with stoppered, 1" PVC pipe to prevent disruption of the soil bordering the hole.

Parameters analyzed in the lysimeter study were, in general, the same parameters as those reported for the field study (Table 1). ATP analysis was performed in lieu of measuring respiration. Ammonium acetate-acetic acid extractable sulfate was measured using the technique described by Bardsley and Lancaster (1965).

Statistics

Statistical analyses included a factorial design analysis of variance on a Burroughs 6700 computer with a STATPAC (Hurst 1972) program (STATPAC/FCTCVR). The program calculates the mean square values of the data for the different combinations of variables and then the various F values for the combinations are estimated using the mean square of any combination as the numerator and the mean square of the overall combination as the denominator. The F values were compared for the probability of erroneously rejecting the null hypothesis at the 1 and 5 percent levels for the different degrees of freedom for the different combinations (Zar 1974).

Following analysis of variance a Duncan's multiple range test was carried out to detect differences between any possible pair of means. All significant differences were determined at $P \geq 0.95$.

RESULTS

Field Study

Samples brought back from the field were analyzed for microbial activity and specific chemical parameters as previously discussed. The tabulated data for these analyses are listed in Appendix A. The results presented in this section are 1) the effects of sampling season, site and depth on the biological and chemical parameters tested, 2) an overview of the status of these parameters in the undisturbed and revegetated areas, and 3) an attempt to relate chemical parameters with biological activity to provide specific functional relationships.

The experimental design of the field study allowed for comparisons of biological and chemical parameters among sampling sites and among sampling seasons and sampling depths. Therefore, for any particular variable, the effects of three factors (sampling season, site and depth), acting simultaneously were assessed.

Effects of sampling season, site and depth

A summary of the results from a three factor analysis of variance is tabulated in Table 6. Analysis of the data by season shows a significant difference at the 99 percent level of confidence among sampling seasons for the following parameters: nitrate-N, ammonium-N, total phosphorus, total organic carbon and respiration activity. There was no significant difference among sampling seasons for total nitrogen, nitrite-N, extractable phosphorus or dehydrogenase activity.

Table 6. Summary of analysis of variance from field data.

	Season	Site	Depth	Season x Site	Season x Depth	Site x Depth
df	3	3	3	9	9	9
Total N	0.831	12.908**	5.457**	2.125*	1.610	6.006**
NO_2-N	2.445	1.061	4.583**	0.672	3.124**	1.075
NO_3-N	4.618**	5.460**	4.945**	6.304**	1.921	2.734**
NH_4-N	27.637**	1.481	0.561	1.392	0.918	0.540
Ext. P	2.372	3.985**	33.654**	0.844	2.458*	1.395
Total P	6.116**	4.772**	17.837**	3.877**	0.739	1.815
TOC	5.662**	1.308	9.640**	1.114	1.447	4.022**
Dehydrogenase	0.647	8.200**	21.659**	0.333	0.591	7.849**
df	2	3	3	6	6	9
Respiration	18.121**	3.352*	0.880	1.372	2.820*	0.855

Critical values

df: numerator	5%*	1%**
denominator 191		
3	2.65	3.88
9	1.93	2.50
denominator 143		
2	3.06	4.76
3	2.67	3.92
6	2.16	2.93

*,** Significant at the 0.05 and 0.01 levels, respectively.

Analysis of the data by sites shows a significant difference at the 99 percent level of confidence among sampling sites for total nitrogen, nitrate-N, extractable phosphorus, total phosphorus and dehydrogenase activity and a significant difference at the 95 percent level of significance for respiration activity. There was no significant difference among sampling sites for nitrite-N, ammonium-N or total organic carbon.

Analysis of the data by depth showed a significant difference at the 99 percent level of confidence among sampling depths for total nitrogen, nitrite-N, nitrate-N, extractable phosphorus, total phosphorus, total organic carbon, and dehydrogenase activity. There was no significant difference among sampling depths for ammonium-N or respiration activity.

The data were further analyzed to check for interactions between factors. The null hypothesis (H_o) that variable differences among seasons are independent of differences between sites was rejected at the 99 percent level of significance for the following parameters: nitrate-N, and total phosphorus, and at the 95 percent level of significance for total nitrogen. The H_o: variable differences among seasons are independent of differences between depths was rejected at the 99 percent significance level for nitrite-N and at the 95 percent level of significance for extractable phosphorus and respiration activity. In like manner, the H_o: variable differences among sites are independent of differences between depths was rejected at the 99 percent level of significance for total nitrogen, nitrate-N, total organic carbon and dehydrogenase activity.

However, rejection of equality among means using analysis of variance does not imply that all means are different from one another. To examine the differences between all possible pairs of means a Duncan's multiple range test was employed. Also, due to interactions between factors, as previously discussed, the factors were separated to eliminate these interactions from the statistical analysis.

Biological and chemical parameters

Total nitrogen. A Duncan's multiple range analysis of the total nitrogen data by sampling depth ranks the sampling sites from lowest

concentration of total nitrogen at the top of the listing to the greatest concentration of total nitrogen at the bottom of the listing (Table 7a). Any group of sampling sites which are not significantly different from each other are connected by a line of stars to the right of the ranking list.

At the sampling depth of 1 cm, only the Sp 75 site approached the total nitrogen concentration of the undisturbed site. At the 15 cm depth, all revegetated sites have significantly lower total nitrogen concentrations than the undisturbed site. At the two lower sampling depths the Sp 75 site has the greatest concentration of total nitrogen.

Only the undisturbed site exhibited a concentration gradient of total nitrogen from surface to lower depths (Table 7b). All other sites exhibited uniform concentrations of total nitrogen with depth.

Nitrite-N. To determine differences among depths for nitrite-N concentrations, a Duncan's multiple range test was performed on each sampling season individually (Table 8). Only the 1 cm sample of the fall sampling period differed significantly from the other sampling depths of the same period. There was no significant difference in nitrite-N concentration among sampling depths for the other sampling periods.

Nitrate-N. Due to interactions between season and site, and site and depth, differences in nitrate concentrations for the three factors were analyzed separately (Table 9a). For all three revegetated sites, the 90 cm sample contained the highest concentration of nitrate-N. Only the undisturbed site exhibited a decrease in nitrate-N concentration from the surface to lower depths.

Examination was made for differences in nitrate-N concentrations among sampling seasons for each site (Table 9b). Sampling season had no

Table 7. Summary of Duncan's multiple range analysis[a] of total nitrogen (mg/g), depth x site, for all seasons (b) and site x depth for all seasons (a).[b]

(a)	1 cm		15 cm		60 cm		90 cm	
		AVERAGE		AVERAGE		AVERAGE		AVERAGE
	REVEG SP 76	0.66833	REVEG SP 76	0.58667	REVEG SP 77	0.58750	REVEG SP 76	0.37000
	REVEG SP 77	0.78333	REVEG SP 75	0.60750	REVEG SP 76	0.61333	REVEG SP 77	0.44917
	REVEG SP 75	0.99167	REVEG SP 77	0.66333	UNDISTURBED	0.74417	UNDISTURBED	0.45500
	UNDISTURBED	2.0133	UNDISTURBED	1.2683	REVEG SP 75	1.3142	REVEG SP 75	1.3842
3	REVEG SP 76	*	3 REVEG SP 76	*	4 REVEG SP 77	*	3 REVEG SP 76	*
		*		*		*		*
4	REVEG SP 77	*	2 REVEG SP 75	*	3 REVEG SP 76	*	4 REVEG SP 77	*
		*		*		*		*
2	REVEG SP 75	* *	4 REVEG SP 77	*	1 UNDISTURBED	* *	1 UNDISTURBED	*
		* *		*		* *		*
1	UNDISTURBED	*	1 UNDISTURBED	*	2 REVEG SP 75	*	2 REVEG SP 75	*
		*		*		*		*

(b)	UD		SP 75		SP 76		SP 77	
		AVERAGE		AVERAGE		AVERAGE		AVERAGE
	DEPTH 90CM	0.45500	DEPTH 15CM	0.60750	DEPTH 90CM	0.37000	DEPTH 90CM	0.44917
	DEPTH 60CM	0.74417	DEPTH 01CM	0.99167	DEPTH 15CM	0.58667	DEPTH 60CM	0.58750
	DEPTH 15CM	1.2683	DEPTH 60CM	1.3142	DEPTH 60CM	0.61333	DEPTH 15CM	0.66333
	DEPTH 01CM	2.0133	DEPTH 90CM	1.3842	DEPTH 01CM	0.66833	DEPTH 01CM	0.78333
4	DEPTH 90CM	*	2 DEPTH 15CM	*	4 DEPTH 90CM		4 DEPTH 90CM	*
		*		*				*
3	DEPTH 60CM	*	1 DEPTH 01CM	*	2 DEPTH 15CM	*	3 DEPTH 60CM	*
		*		*		*		*
2	DEPTH 15CM	* *	3 DEPTH 60CM	*	3 DEPTH 60CM	*	2 DEPTH 15CM	*
		* *		*		*		*
1	DEPTH 01CM	*	4 DEPTH 90CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	*
		*		*		*		

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

effect on nitrate-N concentrations for any site except the Sp 77 site. The Sp 77 site had a significantly higher nitrate-N concentration at the summer sampling season when compared to the other seasons.

Comparing the nitrate-N concentration by site for each season at each depth showed a trend in the relationship between the undisturbed site and the revegetated sites (Table 10). In the upper sampling depths, the undisturbed site generally had a nitrate-N concentration as high or higher than the revegetated sites. At the lower sampling depths the nitrate-N

Table 8. Summary of Duncan's multiple range analysis[a] of nitrite-N (µg/g), depth x season for all sites.[b]

	SP		S		Fall		W	
	AVERAGE		AVERAGE		AVERAGE		AVERAGE	
	DEPTH 01CM	.47917E-01	DEPTH 15CM	.42083E-01	DEPTH 15CM	.38750E-01	DEPTH 15CM	.45000E-01
	DEPTH 15CM	.52083E-01	DEPTH 60CM	.49167E-01	DEPTH 60CM	.39167E-01	DEPTH 90CM	.54167E-01
	DEPTH 60CM	.53750E-01	DEPTH 01CM	.70833E-01	DEPTH 90CM	.47083E-01	DEPTH 01CM	.56250E-01
	DEPTH 90CM	.68333E-01	DEPTH 90CM	0.14292	DEPTH 01CM	.87500E-01	DEPTH 60CM	.60833E-01
1	DEPTH 01CM	*	2 DEPTH 15CM	*	2 DEPTH 15CM	*	2 DEPTH 15CM	*
		*		*		*		*
2	DEPTH 15CM	*	3 DEPTH 60CM	*	3 DEPTH 60CM	*	4 DEPTH 90CM	*
		*		*		*		*
3	DEPTH 60CM	*	1 DEPTH 01CM	*	4 DEPTH 90CM	*	1 DEPTH 01CM	*
		*		*		*		*
4	DEPTH 90CM	*	4 DEPTH 90CM	*	1 DEPTH 01CM	*	3 DEPTH 60CM	*
		*		*		*		*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

Table 9. Summary of Duncan's multiple range analysis[a] of nitrate-N (µg/g), depth x site for all seasons (a) and season by site for all depths (b).[b]

(a)	UD		SP 75		SP 76		SP 77	
	AVERAGE		AVERAGE		AVERAGE		AVERAGE	
	DEPTH 60CM	0.60667	DEPTH 15CM	0.78250	DEPTH 60CM	0.64750	DEPTH 15CM	1.1558
	DEPTH 90CM	0.65917	DEPTH 60CM	1.7783	DEPTH 15CM	0.72833	DEPTH 01CM	1.1567
	DEPTH 15CM	1.1125	DEPTH 01CM	2.5708	DEPTH 01CM	1.3592	DEPTH 60CM	3.2383
	DEPTH 01CM	1.6700	DEPTH 90CM	2.6742	DEPTH 90CM	2.3425	DEPTH 90CM	3.5417
3	DEPTH 60CM	*	2 DEPTH 15CM	*	3 DEPTH 60CM	*	2 DEPTH 15CM	*
		*		*		*		*
4	DEPTH 90CM	*	3 DEPTH 60CM	*	2 DEPTH 15CM	*	1 DEPTH 01CM	*
		*		*		*		*
2	DEPTH 15CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	* *	3 DEPTH 60CM	*
		*		*		* *		*
1	DEPTH 01CM	*	4 DEPTH 90CM	*	4 DEPTH 90CM	*	4 DEPTH 90CM	*
		*		*		*		*

(b)	UD		SP 75		SP 76		SP 77	
	AVERAGE		AVERAGE		AVERAGE		AVERAGE	
	SPRING	0.87250	SUMMER	1.4050	FALL	0.88417	WINTER	0.98583
	FALL	0.87250	FALL	1.6733	WINTER	1.2208	SPRING	1.1767
	SUMMER	1.0175	WINTER	1.7575	SUMMER	1.3350	FALL	1.2092
	WINTER	1.2858	SPRING	2.9700	SPRING	1.6375	SUMMER	5.7208
1	SPRING	*	2 SUMMER	*	3 FALL	*	4 WINTER	*
		*		*		*		*
3	FALL	*	3 FALL	*	4 WINTER	*	1 SPRING	*
		*		*		*		*
2	SUMMER	*	4 WINTER	*	2 SUMMER	*	3 FALL	*
		*		*		*		*
4	WINTER	*	1 SPRING	*	1 SPRING	*	2 SUMMER	*
		*		*		*		*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

Table 10. Summary of Duncan's multiple range analysis[a] of nitrate-N (µg/g), site x depth for each season.[b]

Spring

1 cm	AVERAGE	15 cm	AVERAGE	60 cm	AVERAGE	90 cm	AVERAGE
REVEG SP 77	1.1400	REVEG SP 75	0.49667	UNDISTURBED	0.53667	UNDISTURBED	0.54333
UNDISTURBED	1.4267	REVEG SP 76	0.66333	REVEG SP 76	0.98333	REVEG SP 77	1.6567
REVEG SP 76	2.2967	REVEG SP 77	0.72667	REVEG SP 77	1.1833	REVEG SP 76	2.6067
REVEG SP 75	4.7400	UNDISTURBED	0.98333	REVEG SP 75	3.8933	REVEG SP 75	2.7500
4 REVEG SP 77	*	2 REVEG SP 75	*	1 UNDISTURBED	*	1 UNDISTURBED	*
	*		*		*		*
1 UNDISTURBED	*	3 REVEG SP 76	* *	3 REVEG SP 76	* *	4 REVEG SP 77	*
	*		* *		* *		*
3 REVEG SP 76	*	4 REVEG SP 77	*	4 REVEG SP 77	*	3 REVEG SP 76	*
	*		*		*		*
2 REVEG SP 75	*	1 UNDISTURBED	*	2 REVEG SP 75	*	2 REVEG SP 75	*
	*		*		*		*

Summer

1 cm	AVERAGE	15 cm	AVERAGE	60 cm	AVERAGE	90 cm	AVERAGE
REVEG SP 75	1.2300	REVEG SP 76	0.76333	UNDISTURBED	0.50667	UNDISTURBED	1.0267
REVEG SP 77	1.2300	REVEG SP 75	0.89667	REVEG SP 76	0.58000	REVEG SP 75	2.3167
REVEG SP 76	1.3000	UNDISTURBED	1.1333	REVEG SP 75	1.1767	REVEG SP 76	2.6967
UNDISTURBED	1.4033	REVEG SP 77	2.2467	REVEG SP 77	9.6167	REVEG SP 77	9.7900
2 REVEG SP 75	*	3 REVEG SP 76	*	1 UNDISTURBED	*	1 UNDISTURBED	*
	*		*		*		*
4 REVEG SP 77	*	2 REVEG SP 75	*	3 REVEG SP 76	*	2 REVEG SP 75	*
	*		*		*		*
3 REVEG SP 76	* *	1 UNDISTURBED	*	2 REVEG SP 75	*	3 REVEG SP 76	*
	* *		*		*		*
1 UNDISTURBED	*	4 REVEG SP 77	*	4 REVEG SP 77	*	4 REVEG SP 77	*
	*		*		*		*

Fall

1 cm	AVERAGE	15 cm	AVERAGE	60 cm	AVERAGE	90 cm	AVERAGE
REVEG SP 75	0.93000	REVEG SP 75	0.54000	UNDISTURBED	0.55000	UNDISTURBED	0.38667
REVEG SP 76	1.1067	REVEG SP 76	0.71000	REVEG SP 76	0.75333	REVEG SP 76	0.96667
REVEG SP 77	1.3267	UNDISTURBED	0.83000	REVEG SP 77	0.96000	REVEG SP 77	1.6433
UNDISTURBED	1.7233	REVEG SP 77	0.90667	REVEG SP 75	1.0200	REVEG SP 75	4.2033
2 REVEG SP 75	*	2 REVEG SP 75	*	1 UNDISTURBED	*	1 UNDISTURBED	*
	*		*		*		*
3 REVEG SP 76	* *	3 REVEG SP 76	*	3 REVEG SP 76	* *	3 REVEG SP 76	*
	* *		*		* *		*
4 REVEG SP 77	* *	1 UNDISTURBED	* *	4 REVEG SP 77	*	4 REVEG SP 77	*
	* *		* *		*		*
1 UNDISTURBED	*	4 REVEG SP 77	*	2 REVEG SP 75	*	2 REVEG SP 75	*
	*		*		*		*

Winter

1 cm	AVERAGE	15 cm	AVERAGE	60 cm	AVERAGE	90 cm	AVERAGE
REVEG SP 76	0.73333	REVEG SP 77	0.74333	REVEG SP 76	0.27333	UNDISTURBED	0.68000
REVEG SP 77	0.93000	REVEG SP 76	0.77667	UNDISTURBED	0.83333	REVEG SP 77	1.0767
UNDISTURBED	2.1267	REVEG SP 75	1.1967	REVEG SP 75	1.0233	REVEG SP 75	1.4267
REVEG SP 75	3.3833	UNDISTURBED	1.5033	REVEG SP 77	1.1933	REVEG SP 76	3.1000
3 REVEG SP 76	*	4 REVEG SP 77	*	3 REVEG SP 76	*	1 UNDISTURBED	*
	*		*		*		*
4 REVEG SP 77	*	3 REVEG SP 76	*	1 UNDISTURBED	*	4 REVEG SP 77	*
	*		*		*		*
1 UNDISTURBED	*	2 REVEG SP 75	*	2 REVEG SP 75	* *	2 REVEG SP 75	*
	*		*		* *		*
2 REVEG SP 75	*	1 UNDISTURBED	*	4 REVEG SP 77	*	3 REVEG SP 76	*
	*		*		*		*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

concentration was consistently lower in the undisturbed site than in the revegetated sites.

Ammonium-N. Determination of which of the seasons affected the ammonium-N concentration the greatest was achieved using the Duncan's multiple range test (Table 11). The ammonium-N concentration in the summer samples was significantly higher than the ammonium-N concentration in the winter samples. There was no significant difference in the ammonium-N concentration between the other seasons.

Extractable phosphorus. Results from the analysis of variance test indicated that there was a statistically significant difference at the 99 percent confidence level in extractable phosphorus between sites. The Duncan's multiple range test however, was unable to detect differences between sites (Table 12a). This reflects the fact that the analysis of variance is a more powerful test than is the multiple range test and Type II errors are more likely to occur in multiple range testing than in performing an analysis of variance (Zar 1974).

Differences in extractable phosphorus among depths were analyzed by season using the Duncan's multiple range test (Table 12b). The extractable phosphorus concentration in the 1 cm sampling depth was significantly different from the concentration in all other depths in every sampling season.

Total phosphorus. Determination of differences among depths for total phosphorus concentration was achieved using the Duncan's multiple range test (Table 13a). Since no interactions affected differences in total phosphorus concentration with depth, all seasons and sites were lumped together for the multiple range test. Only the 1 cm depth and the

Table 11. Summary of Duncan's multiple range analysis[a] of ammonium-N (µg/g) by seasons, for all sites and depths.[b]

		AVERAGE
	WINTER	1.9646
	FALL	7.7319
	SPRING	12.599
	SUMMER	21.163
4	WINTER	*
		*
3	FALL	* *
		* *
1	SPRING	* *
		* *
2	SUMMER	*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For treatment descriptions see Table 19.

Table 12. Summary of Duncan's multiple range analysis[a] of extractable-P (µg/g) by sites, for all seasons and depths (a), and depth x season, for all sites (b).[b]

(a)

	AVERAGE
REVEG SP 76	3.9794
REVEG SP 75	6.0221
REVEG SP 77	6.1775
UNDISTURBED	8.2919
3 REVEG SP 76	*
	*
2 REVEG SP 75	*
	*
4 REVEG SP 77	*
	*
1 UNDISTURBED	*

(b)

SP		S		Fall		W	
	AVERAGE		AVERAGE		AVERAGE		AVERAGE
DEPTH 90CM	2.6083	DEPTH 90CM	2.4733	DEPTH 90CM	1.8842	DEPTH 60CM	3.6500
DEPTH 60CM	2.7583	DEPTH 15CM	4.0692	DEPTH 60CM	2.3842	DEPTH 90CM	3.8083
DEPTH 15CM	4.5417	DEPTH 60CM	4.2225	DEPTH 15CM	4.8958	DEPTH 15CM	5.8917
DEPTH 01CM	21.308	DEPTH 01CM	9.3358	DEPTH 01CM	10.860	DEPTH 01CM	13.192
4 DEPTH 90CM	*	4 DEPTH 90CM	*	4 DEPTH 90CM	*	3 DEPTH 60CM	*
	*		*		*		*
3 DEPTH 60CM	*	2 DEPTH 15CM	*	3 DEPTH 60CM	*	4 DEPTH 90CM	*
	*		*		*		*
2 DEPTH 15CM	*	3 DEPTH 60CM	*	2 DEPTH 15CM	*	2 DEPTH 15CM	*
	*		*		*		*
1 DEPTH 01CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	*
	*		*		*		*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

Table 13. Summary of Duncan's multiple range analysis[a] of total phosphorus (mg/g) by depth, for all seasons and sites (a); season x site, for all depths (b); site x season, for all depths (c), and by sites for all seasons and depths (d).[b]

(a)

	AVERAGE
DEPTH 15CM	0.47956
DEPTH 60CM	0.50923
DEPTH 90CM	0.55015
DEPTH 01CM	0.56548

2 DEPTH 15CM	*
	*
3 DEPTH 60CM	* *
	* *
4 DEPTH 90CM	* *
	* *
1 DEPTH 01CM	*
	*

(b)

UD	AVERAGE	SP 75	AVERAGE	SP 76	AVERAGE	SP 77	AVERAGE
SPRING	0.48042	FALL	0.53717	WINTER	0.47658	WINTER	0.45883
SUMMER	0.49600	WINTER	0.54375	SPRING	0.48842	SPRING	0.53325
WINTER	0.50725	SUMMER	0.56800	FALL	0.50817	SUMMER	0.53942
FALL	0.58317	SPRING	0.57567	SUMMER	0.57692	FALL	0.54467

1 SPRING	*	3 FALL	*	4 WINTER	*	4 WINTER	*
	*		*		*		*
2 SUMMER	*	4 WINTER	*	1 SPRING	*	1 SPRING	*
	*		*		*		*
4 WINTER	*	2 SUMMER	*	3 FALL	*	2 SUMMER	*
	*		*		*		*
3 FALL	*	1 SPRING	*	2 SUMMER	*	3 FALL	*
	*		*		*		*

(c)

SP	AVERAGE	S	AVERAGE	Fall	AVERAGE	W	AVERAGE
UNDISTURBED	0.48042	UNDISTURBED	0.49600	REVEG SP 76	0.50817	REVEG SP 77	0.45883
REVEG SP 76	0.48842	REVEG SP 77	0.53942	REVEG SP 75	0.53717	REVEG SP 76	0.47658
REVEG SP 77	0.53325	REVEG SP 75	0.56800	REVEG SP 77	0.54467	UNDISTURBED	0.50725
REVEG SP 75	0.57567	REVEG SP 76	0.57692	UNDISTURBED	0.58317	REVEG SP 75	0.54375

1 UNDISTURBED	*	1 UNDISTURBED	*	3 REVEG SP 76	*	4 REVEG SP 77	*
	*		*		*		*
3 REVEG SP 76	*	4 REVEG SP 77	*	2 REVEG SP 75	* *	3 REVEG SP 76	* *
	*		*		* *		* *
4 REVEG SP 77	* *	2 REVEG SP 75	*	4 REVEG SP 77	* *	1 UNDISTURBED	* *
	* *		*		* *		* *
2 REVEG SP 75	*	3 REVEG SP 76	*	1 UNDISTURBED	*	2 REVEG SP 75	*
	*		*		*		*

(d)

	AVERAGE
REVEG SP 76	0.51252
UNDISTURBED	0.51671
REVEG SP 77	0.51904
REVEG SP 75	0.55615

3 REVEG SP 76	*
	*
1 UNDISTURBED	*
	*
4 REVEG SP 77	*
	*
2 REVEG SP 75	*
	*

[a] Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b] For definition of terms see page xii.

15 cm depth samples differed significantly. These depths represented the greatest and lowest total phosphorus concentrations respectively.

Analysis of the total phosphorus concentrations by sampling season for each site showed season had little effect on the total phosphorus concentration (Table 13b).

Comparisons of total phosphorus concentrations among sites for each season showed no clear pattern of any site having a consistently higher or lower total phosphorus concentration than any other site (Table 13c). When total phosphorus concentrations were compared among sites, lumping all seasons and depths, no differences were found (Table 13d).

Total organic carbon. Comparisons of total organic carbon among seasons for all sampling sites and depths was accomplished using a Duncan's multiple range test (Table 14a). However, as with the multiple comparisons of extractable phosphorus, the test was not powerful enough to detect differences among the means. Only the undisturbed site exhibited a differentiation in organic carbon concentrations between upper and lower depths (Table 14b). For all other sites, there was no difference in organic carbon concentration between depths.

Profile distributions of C and N for the four sites sampled, averaged over all seasons, are shown in Figure 8. As previously stated, only the undisturbed site exhibited a decrease in total organic carbon concentration from surface to 90 cm, for either nutrient. The three revegetated sites showed no change in concentration with depth. The C:N ratios, ranging from 5 to 15, are lowest in the undisturbed site and highest in the recently revegetated Sp 77 site.

Respiration. Respiration measurements were made on samples from the spring, summer, and fall sampling periods only. Determination of

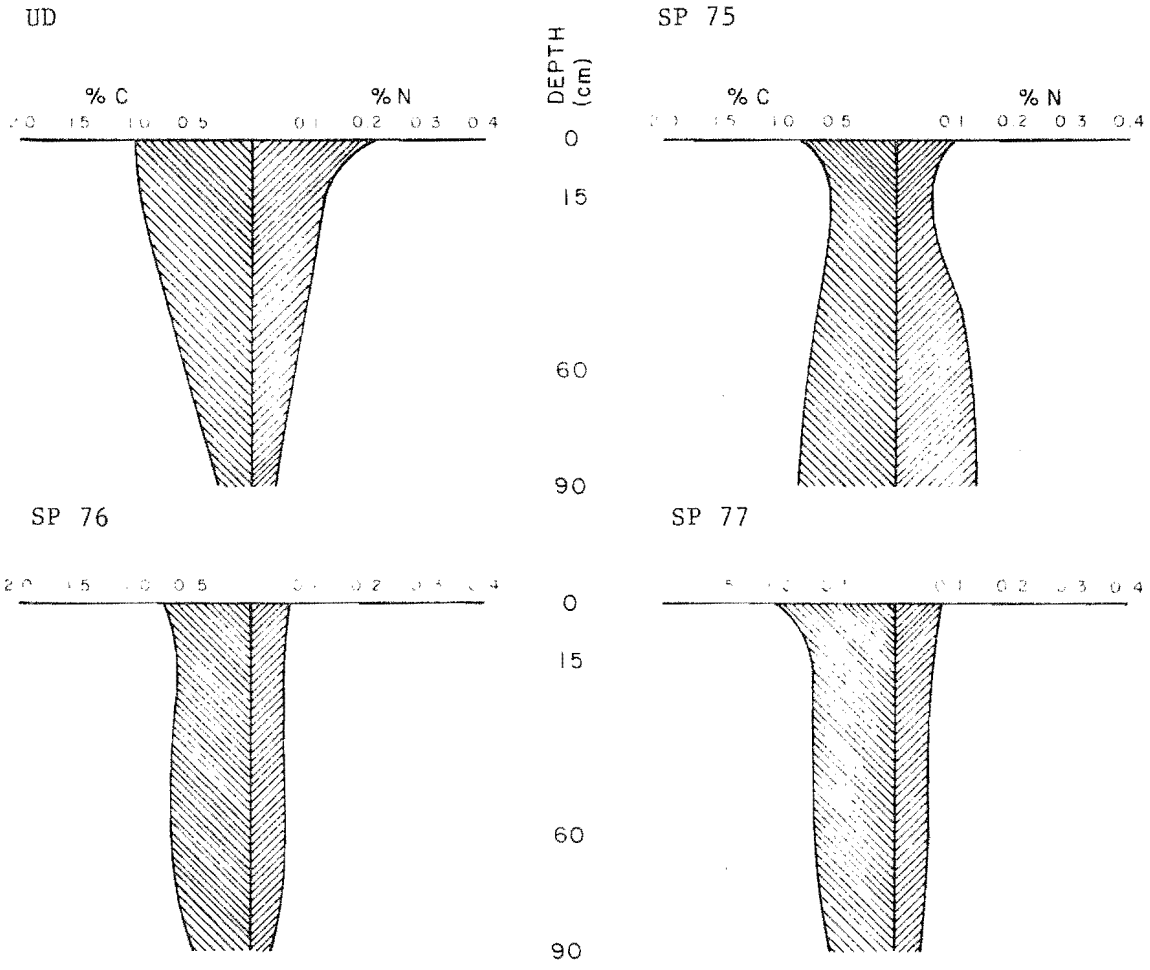


Figure 8. Profile distributions for C and N.

differences in respiration activity between sampling seasons at each sampling depth was performed using the Duncan's multiple range test (Table 15a). Only at the 60 cm depth was there any difference in respiration between sampling seasons.

Differences in respiration activity among sites were also tested for using the Duncan's multiple range test. However, no differences were detected by this test in spite of the fact that the analysis of variance indicated a difference among means at the 95 percent level of confidence (Table 15b).

Table 14. Summary of Duncan's multiple range analysis[a] of total organic carbon (mg/g) by season, for all sites and depths (a) and depth x site, for all seasons (b).[b]

(a)

	AVERAGE
FALL	5.7906
SUMMER	6.6362
SPRING	6.7288
WINTER	8.2865
3 FALL	*
	*
2 SUMMER	*
	*
1 SPRING	*
	*
4 WINTER	*
	*

(b)

UD	AVERAGE		SP 75	AVERAGE		SP 76	AVERAGE		SP 77	AVERAGE
DEPTH 90CM	2.9500		DEPTH 15CM	5.3892		DEPTH 90CM	4.9042		DEPTH 90CM	5.0350
DEPTH 60CM	5.8042		DEPTH 60CM	6.9442		DEPTH 15CM	5.6208		DEPTH 60CM	6.8317
DEPTH 01CM	9.2292		DEPTH 01CM	8.1633		DEPTH 60CM	6.8183		DEPTH 15CM	7.2142
DEPTH 15CM	9.6450		DEPTH 90CM	8.2675		DEPTH 01CM	7.2400		DEPTH 01CM	9.7117
4 DEPTH 90CM	*	2	DEPTH 15CM	*	4	DEPTH 90CM	*	4	DEPTH 90CM	*
	*			*			*			*
3 DEPTH 60CM	* *	3	DEPTH 60CM	*	2	DEPTH 15CM	*	3	DEPTH 60CM	*
	* *			*			*			*
1 DEPTH 01CM	*	1	DEPTH 01CM	*	3	DEPTH 60CM	*	2	DEPTH 15CM	*
	*			*			*			*
2 DEPTH 15CM	*	4	DEPTH 90CM	*	1	DEPTH 01CM	*	1	DEPTH 01CM	*
	*			*			*			*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

Table 15. Summary of Duncan's multiple range analysis[a] of respiration (μg C x 10^{-4}), 3 seasons x depth, for all sites (a) and by sites for 3 seasons and all depths (b).

(a)

1 cm	AVERAGE		15 cm	AVERAGE		60 cm	AVERAGE		90 cm	AVERAGE
FALL	4.0942		SUMMER	5.2417		SUMMER	3.8525		SUMMER	3.9833
SPRING	9.9708		FALL	5.4467		FALL	5.8800		FALL	5.4300
SUMMER	11.093		SPRING	11.176		SPRING	11.206		SPRING	10.517
3 FALL	*	2	SUMMER	*	2	SUMMER	*	2	SUMMER	*
	*			*			*			*
1 SPRING	*	3	FALL	*	3	FALL	* *	3	FALL	*
	*			*			* *			*
2 SUMMER	*	1	SPRING	*	1	SPRING	*	1	SPRING	*
	*			*			*			*

(b)

	AVERAGE
REVEG SP 77	5.2606
REVEG SP 76	7.5628
UNDISTURBED	7.7186
REVEG SP 75	8.7547
4 REVEG SP 77	*
	*
3 REVEG SP 76	*
	*
1 UNDISTURBED	*
	*
2 REVEG SP 75	*
	*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

Dehydrogenase activity. Comparisons for differences in dehydrogenase activity among depths was performed using the Duncan's multiple range test (Table 16a). The 1 cm depth samples exhibited significantly higher dehydrogenase activity than any other sampling depth for all sites except the Sp 76 site.

Analyzing for differences in dehydrogenase activity among sites at each depth using Duncan's multiple range test indicated that only the 1 cm sample of the undisturbed site was significantly higher in dehydrogenase activity than any other site at that depth (Table 16b). At all other sampling depths there were no differences among sites.

Figure 9 is a visual representation of differences in dehydrogenase activities among sampling sites and depths. The 1 cm sample of the undisturbed site is notably higher in dehydrogenase activity than any other site at any depth.

Nitrogen fixation. No nitrogen fixation activity was detected in any of the samples taken during any of the sampling seasons. However, an algal crust sample taken from an alternative site at the mine did contain some heterocyst forming blue-green algae species (notably Anabaena). Therefore, the potential for nitrogen fixation activity does exist in some undisturbed areas of the mine.

Percent moisture and pH. There was no difference among sites for either percent moisture or pH when tested with a Duncan's multiple range analysis (Table 17). Differences among seasons and depths were not analyzed for.

Table 16. Summary of Duncan's multiple range analysis[a] of dehydrogenase activity (µg formazan/g), depth x site, for all seasons (a) and site x depth, for all seasons (b).[b]

(a)

UD	AVERAGE	SP 75	AVERAGE	SP 76	AVERAGE	SP 77	AVERAGE
DEPTH 90CM	0.	DEPTH 60CM	0.	DEPTH 60CM	2.0983	DEPTH 90CM	1.4825
DEPTH 60CM	1.1583	DEPTH 90CM	0.	DEPTH 15CM	2.5283	DEPTH 60CM	1.4892
DEPTH 15CM	9.6842	DEPTH 15CM	4.4725	DEPTH 90CM	3.8508	DEPTH 15CM	3.4908
DEPTH 01CM	227.81	DEPTH 01CM	72.438	DEPTH 01CM	9.3942	DEPTH 01CM	28.050
4 DEPTH 90CM	*	3 DEPTH 60CM	*	3 DEPTH 60CM	*	4 DEPTH 90CM	*
	*		*		*		*
3 DEPTH 60CM	*	4 DEPTH 90CM	*	2 DEPTH 15CM	*	3 DEPTH 60CM	*
	*		*		*		*
2 DEPTH 15CM	*	2 DEPTH 15CM	*	4 DEPTH 90CM	*	2 DEPTH 15CM	*
	*		*		*		*
1 DEPTH 01CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	*
	*		*		*		*

(b)

1 cm	AVERAGE	15 cm	AVERAGE	60 cm	AVERAGE	90 cm	AVERAGE
REVEG SP 76	9.3942	REVEG SP 76	2.5283	REVEG SP 75	0.	UNDISTURBED	0.
REVEG SP 77	28.050	REVEG SP 77	3.4908	UNDISTURBED	1.1583	REVEG SP 75	0.
REVEG SP 75	72.438	REVEG SP 75	4.4725	REVEG SP 77	1.4892	REVEG SP 77	1.4825
UNDISTURBED	227.81	UNDISTURBED	9.6842	REVEG SP 76	2.0983	REVEG SP 76	3.8508
3 REVEG SP 76	*	3 REVEG SP 76	*	2 REVEG SP 75	*	1 UNDISTURBED	*
	*		*		*		*
4 REVEG SP 77	*	4 REVEG SP 77	*	1 UNDISTURBED	*	2 REVEG SP 75	*
	*		*		*		*
2 REVEG SP 75	*	2 REVEG SP 75	*	4 REVEG SP 77	*	4 REVEG SP 77	*
	*		*		*		*
1 UNDISTURBED	*	1 UNDISTURBED	*	3 REVEG SP 76	*	3 REVEG SP 76	*
	*		*		*		*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

Table 17. Summary of Duncan's multiple range analysis[a] of pH (a) and percent moisture (b) by sites for all seasons and depths.

(a)

	AVERAGE
REVEG SP 75	8.2025
UNDISTURBED	8.2138
REVEG SP 77	8.3806
REVEG SP 76	8.5175
2 REVEG SP 75	*
	*
1 UNDISTURBED	*
	*
4 REVEG SP 77	*
	*
3 REVEG SP 76	*
	*

(b)

	AVERAGE
REVEG SP 75	8.6375
REVEG SP 76	9.0063
UNDISTURBED	9.4938
REVEG SP 77	9.9063
2 REVEG SP 75	*
	*
3 REVEG SP 76	*
	*
1 UNDISTURBED	*
	*
4 REVEG SP 77	*
	*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

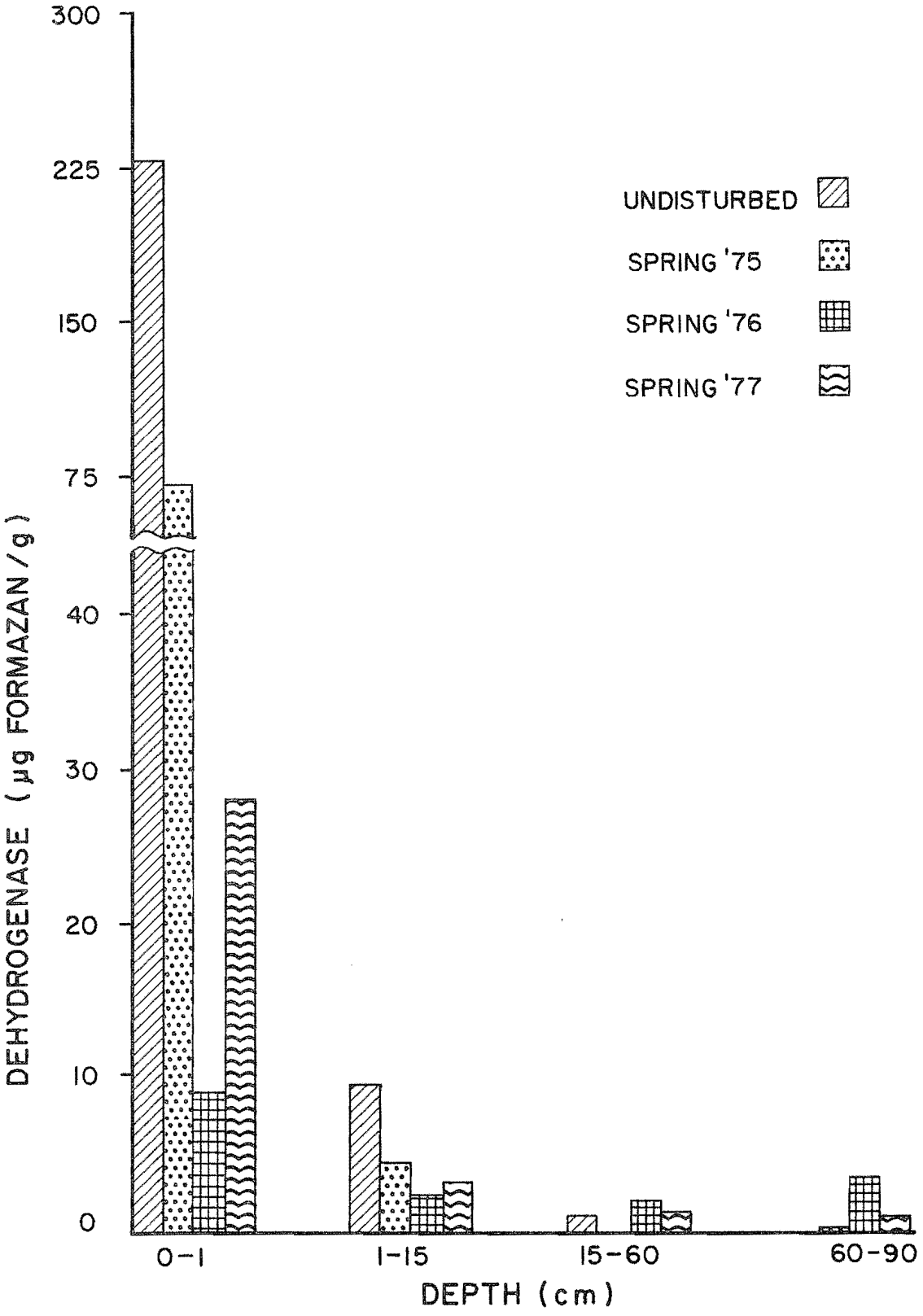


Figure 9. Comparison of dehydrogenase activity by site and depth.

Interrelationships between chemical and biological parameters

In an attempt to explain the large disparity in dehydrogenase activity between the undisturbed site and the revegetated sites a simple linear regression analysis was performed. This analysis relates the functional dependence of dehydrogenase activity on the magnitude of each of the various chemical parameters measured. The calculated coefficient of determination (r^2) explains the proportion of the total variation in dehydrogenase activity accounted for by the fitted regression (Table 18).

Variation in dehydrogenase activity in the UD 1 cm sample was determined to be dependent, in decreasing order of importance, on the following parameters: N:P > C:N > TN. It has previously been shown that there was no significant difference in either total organic carbon or total phosphorus concentrations among sites. The total nitrogen concentration in the 1 cm depth of the UD site was significantly higher than the concentration found in the revegetated sites. This higher nitrogen concentration, and the subsequent effects on the N:P and C:N ratios, in the undisturbed sites appears to be the major factor influencing microbial activity.

Figure 10 is a summary of the total nitrogen, total phosphorus, total organic carbon concentrations and their ratios for each site by depth. The total nitrogen concentration at UD 01 is twice as high as any other site. This clearly has an effect on the nutrient balance in the soil, subsequently affecting microbial growth and activity.

Although, from Table 13 it appears that the dehydrogenase activity at other sites is dependent upon the total nitrogen concentration (e.g. Sp 76 1 cm), from Figure 10 it can be seen that the total nitrogen concentration at Sp 76 1 cm is the lowest value among all four sites and, as seen in Figure 9, dehydrogenase for Sp 76 1 cm is also the lowest among the sites at this depth.

Table 18. Coefficients of determination (r^2) relating dehydrogenase activity to various chemical parameters at the 1 and 15 cm sampling depths.

Site	Depth (cm)	TN	NO_3-N	NH_4-N	Ext P	TP	TOC	pH	$\%H_2O$	C:N	N:P
UD	1	0.91407	0.00001	0.11056	0.14312	0.76738	0.00158	0.19461	0.02882	0.92566	0.95544
UD	15	0.11273	0.09450	0.07463	0.00335	0.89036	0.82795	0.47418	0.30979	0.91239	0.68506
Sp 75	1	0.42650	0.64114	0.18750	0.32864	0.32153	0.09764	0.86594	0.38767	0.02028	0.31897
Sp 75	15	0.87051	0.51004	0.58077	0.51372	0.03279	0.51142	0.39418	0.09906	0.02675	0.65392
Sp 76	1	0.85935	0.56331	0.25927	0.98647	0.31951	0.19044	0.46421	0.98841	0.52665	0.76868
Sp 76	15	0.80079	0.57531	0.04637	0.21574	0.62068	0.66300	0.00057	0.21452	0.88612	0.97555
Sp 77	1	0.20353	0.68820	0.09940	0.10044	0.07545	0.75148	0.07089	0.04702	0.66659	0.35524
Sp 77	15	0.59033	0.25971	0.60709	0.00134	0.04224	0.11925	0.07435	0.00973	0.80608	0.39908

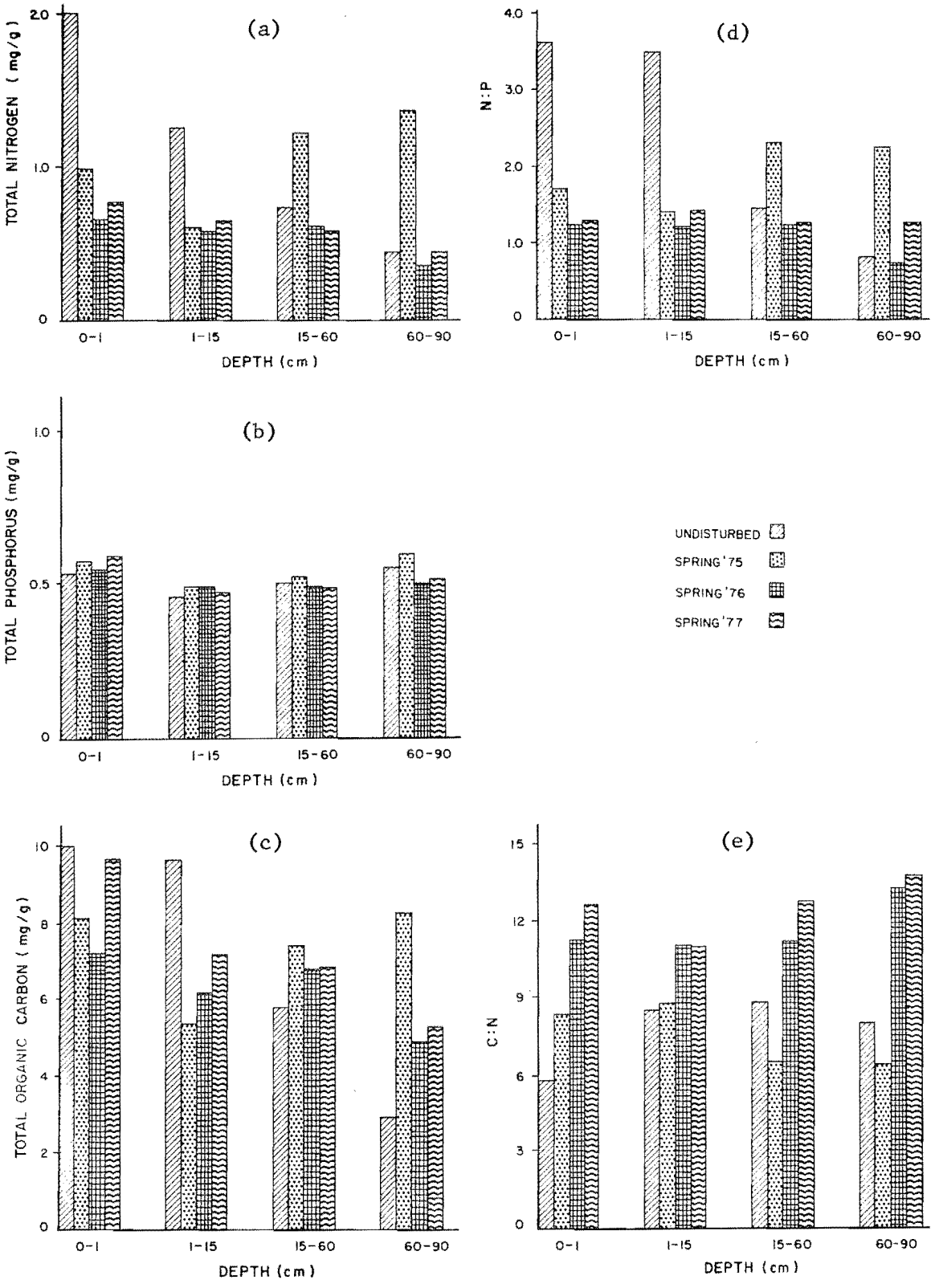


Figure 10. Summary of the total nitrogen (a), total phosphorus (b), and total organic carbon (c) concentrations; and their ratios (d, e) for each site.

Bioassay

Treatment levels for the bioassay study, as determined from the field study, are reviewed in Table 19. Initial nutrient levels of the soil used in the bioassay are summarized in Table 20. The tabulated data for the analyses performed at various stages throughout the bioassay are listed in Appendix B.

Initial dehydrogenase activity, measured on each treatment, 24 hours following setup ranged from 10.7 to 21.4 μg formazan/g soil (Table 21). The Duncan's multiple range test was used to determine differences in the initial dehydrogenase activity among the treatments. Although some differences did exist among treatments it was determined that these differences were not great enough to interfere with later analyses.

Growth responses

The results of three week and four week measurements of dehydrogenase activity show substantial variation with time and treatment (Figure 11). Analysis of variance indicates statistically significant differences at the 95 percent level in the three week samples but not all of the four week samples (Table 22). The three week samples were also, in general, higher in dehydrogenase activity than the four week samples. This may indicate that the algae were responsible for a large percentage of the activity. However, the coefficient of determination, assessing the dependence of dehydrogenase activity on chlorophyll-a concentration is only 0.39044 (Figure 12).

Determination of differences in dehydrogenase activity among treatments for the three week samples was accomplished using the Duncan's multiple range test (Table 23). The treatments which included the addition

Table 19. Summary of treatments.

Treatment Number	Treatment
1	Control, no treatment
2	0.04 mg/g NO_3-N, 0.08 mg/g P, 1.1 mg/g wood fiber
3	Same as 2 + 0.01 mg/g S
4	0.04 mg/g NO_3-N
5	0.08 mg/g NO_3-N
6	0.04 mg/g NH_4-N
7	0.08 mg/g NH_4-N
8	0.08 mg/g P
9	Same as 4 + 8
10	Same as 5 + 8
11	Same as 6 + 8
12	Same as 7 + 8
13	Same as 4 + 8 + 0.01 mg/g S
14	Same as 6 + 8 + 0.01 mg/g S

Table 20. Initial nutrient levels in soil before treatment.[a]

Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Total P mg/g	Extractable P µg/g	TOC mg/g	pH[b]
0.80	0.23	3.71	8.58	0.58	3.27	4.52	8.47

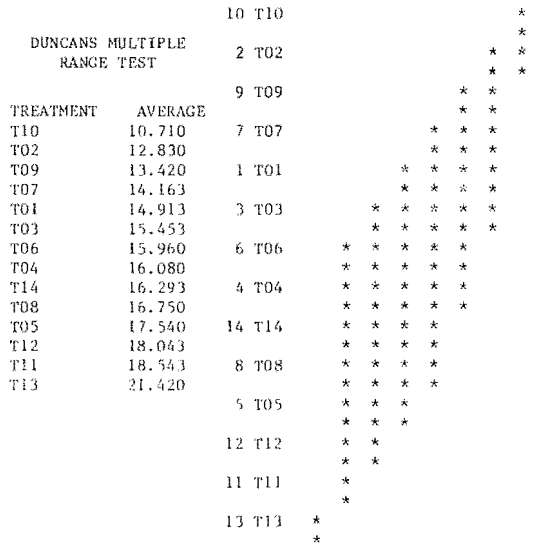
[a]All values are the average of three unless indicated.

[b]No replicates.

Table 21. Initial dehydrogenase concentrations and results from the Duncan's multiple range analysis.[a,b]

DUNCANS MULTIPLE RANGE TEST

TREATMENT	AVERAGE
T10	10.710
T02	12.830
T09	13.420
T07	14.163
T01	14.913
T03	15.453
T06	15.960
T04	16.080
T14	16.293
T08	16.750
T05	17.540
T12	18.043
T11	18.543
T13	21.420



[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For treatment descriptions see Table 19.

Table 22. Summary of the results from the analysis of variance of the bioassay data.

Parameter	Three Weeks	Four Weeks
Dehydrogenase activity	2.223*	1.221
Chlorophyl-a	2.277*	
ATP		2.339*
Nitrate-N		2.982**
Ammonium-N		1.455
Extractable-P		2.962**
pH		2.648**
Critical F values **1%	2.61	
*5%	1.97	

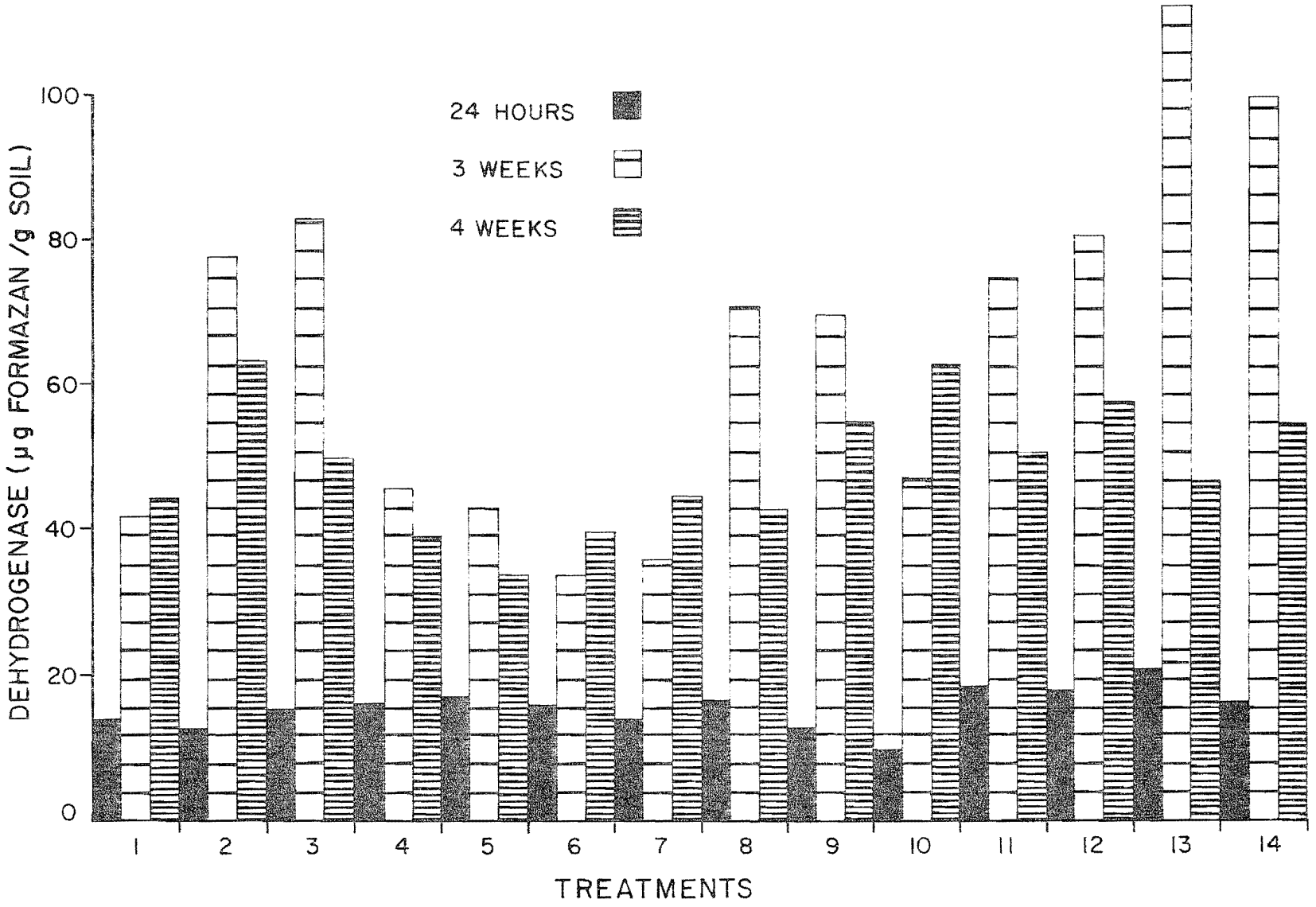


Figure 11. Summary of dehydrogenase activity measured at different times.

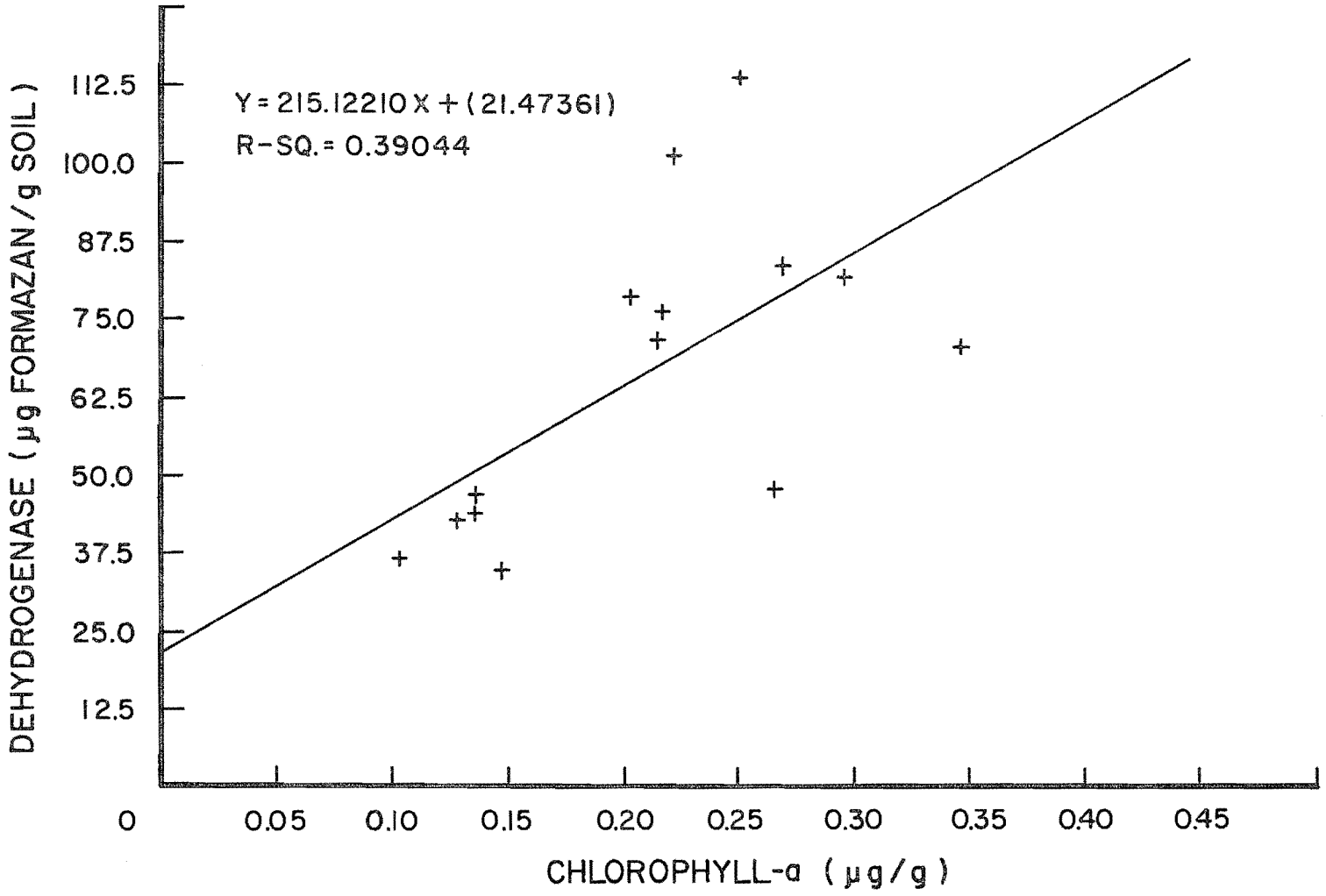
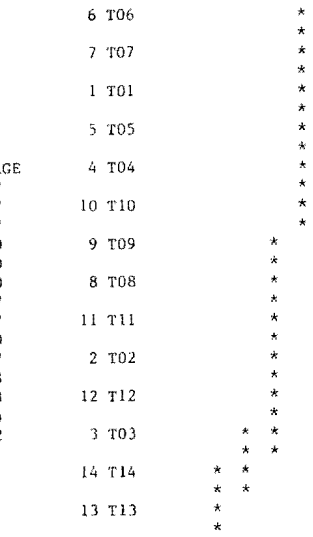


Figure 12. Relationship of dehydrogenase activity to chlorophyll-a content.

Table 23. Dehydrogenase activity (µg formazan/g) in the three week samples and comparison among treatments.[a,b]

DUNCANS MULTIPLE RANGE TEST

TREATMENT	AVERAGE
T06	34.247
T07	36.167
T01	42.297
T05	43.380
T04	46.380
T10	47.330
T09	70.247
T08	71.337
T11	75.960
T02	78.297
T12	81.543
T03	83.333
T14	100.84
T13	113.42



[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see

of nitrogen, phosphorus and sulfur had the highest levels of dehydrogenase activity.

Chlorophyll-a concentrations also varied among treatments (Figure 13) although differences among those treatments which included both nitrogen and phosphorus additions are not well defined (Table 24).

ATP concentrations in the four week samples exhibited less variation among treatments than did chlorophyll-a concentrations or dehydrogenase activity in the three week samples (Figure 14). Treatments 9 and 14 had significantly higher ATP concentrations but the reason for this is unclear (Table 25).

Chemical parameters

As would be expected, those treatments which included phosphorus and/or nitrogen additions had significantly higher levels of these

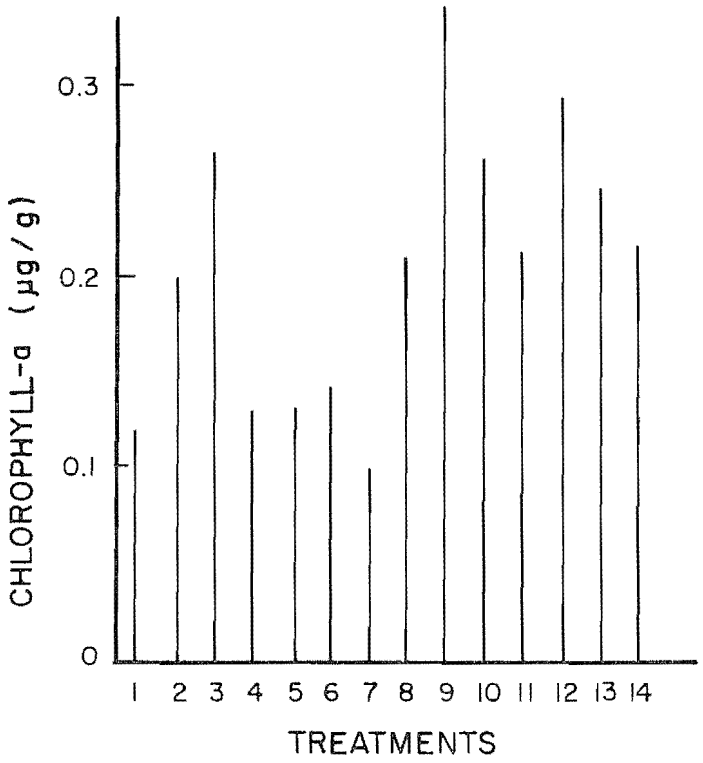
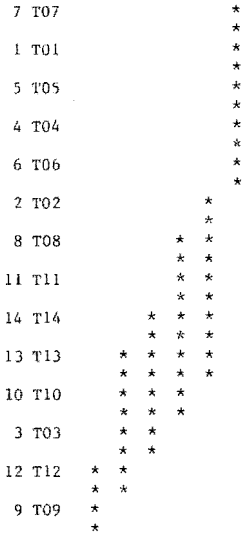


Figure 13. Chlorophyll-a concentration at three week analysis.

Table 24. Summary of Duncan's multiple range analysis of chlorophyll-a concentrations (µg/g) at the three week sample period.

DUNCANS MULTIPLE RANGE TEST

TREATMENT	AVERAGE
T07	0.10067
T01	0.12567
T05	0.13300
T04	0.13367
T06	0.14500
T02	0.20100
T08	0.21267
T11	0.21467
T14	0.21933
T13	0.24867
T10	0.26300
T03	0.26667
T12	0.29333
T09	0.34400



[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For treatment descriptions see Table 19.

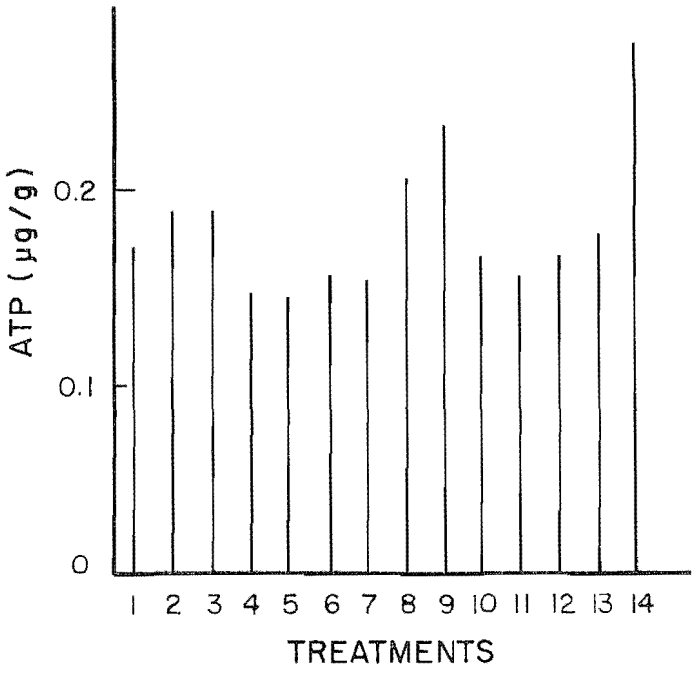
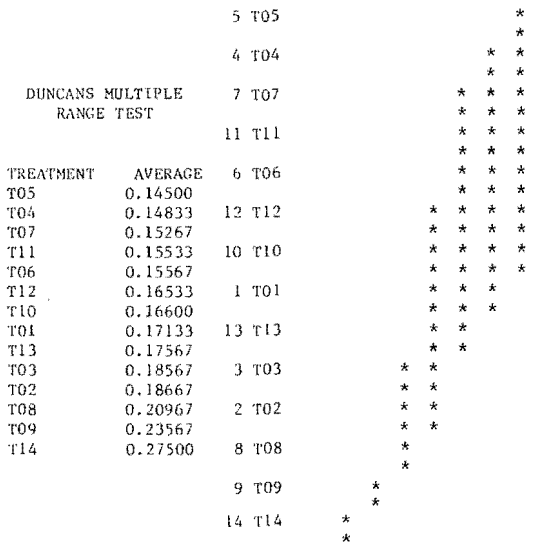


Figure 14. ATP concentration at four week analysis.

Table 25. Summary of Duncan's multiple range analysis of ATP concentrations (µg/g) at the four week analysis period.

DUNCANS MULTIPLE RANGE TEST

TREATMENT	AVERAGE
T05	0.14500
T04	0.14833
T07	0.15267
T11	0.15533
T06	0.15567
T12	0.16533
T10	0.16600
T01	0.17133
T13	0.17567
T03	0.18567
T02	0.18667
T08	0.20967
T09	0.23567
T14	0.27500



[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For treatment descriptions see Table 19.

nutrients at the four week sampling period in proportion to the level of nutrient added (Table 26). The effect of nutrient addition on pH is readily apparent from the results of the Duncan's multiple range test (Table 26). Those treatments which included the addition of either ammonium-N or elemental sulfur had significantly lower pH values at the four week sample period than those treatments which did not include readily oxidizable material.

Coefficients of determination for the microbial activity measurements are summarized in Table 27. The microbial activity showed little dependence on the chemical parameters measured.

Figure 15 is a summary of the relationship between ATP concentrations and the inorganic nitrogen to extractable phosphorus ratio (N:P). It appears that those treatments with N:P ratios greater than 3 are phosphorus limited but the data are insufficient to make conclusive statements.

Summary of bioassay results

In order to determine which treatments resulted in the optimum balance between microbial activity and nutrient status, each treatment was ranked according to the results of the Duncan's multiple range analyses (Table 28). Based on the following criteria: high microbial activity, increased nutrient availability and decreased pH; two treatments were selected to be used in the lysimeter study. These two treatments, numbers 13 nd 14, utilized the same level of nutrients currently being used in the mine with the addition of elemental sulfur.

Lysimeters

In 1978 the mining company began to segregate A horizon soils from other "topsoils" during the mining process. These A horizon soils, with

Table 26. Summary of Duncan's multiple range analysis[a] of data from four week samples.[b]

(a) nitrate-N (µg/g)

DUNCANS MULTIPLE RANGE TEST

TREATMENT	AVERAGE
T01	2.3833
T08	3.0000
T11	3.9667
T14	5.5000
T09	5.6000
T03	9.2333
T13	9.4833
T02	10.883
T12	16.700
T06	23.467
T04	25.533
T10	31.667
T07	50.267
T05	70.400

(b) extractable-P (µg/g)

DUNCANS MULTIPLE RANGE TEST

TREATMENT	AVERAGE
T12	7.8400
T13	7.8900
T14	7.9267
T07	7.9467
T05	8.0333
T06	8.1100
T10	8.2367
T04	8.2667
T08	8.2667
T11	8.2667
T03	8.3233
T09	8.3567
T01	8.3733
T02	8.3733

(c) pH

DUNCANS MULTIPLE RANGE TEST

TREATMENT	AVERAGE
T06	2.7700
T05	2.8733
T07	2.9333
T04	2.9433
T01	3.4233
T03	5.5400
T13	5.7333
T09	5.8033
T02	5.8600
T10	6.1500
T11	6.3667
T14	6.6433
T08	6.9167
T12	7.0900

[a] Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b] For treatment descriptions see Table 19.

Table 27. Coefficients of determination (r^2) relating microbial population parameters to chemical parameters.

	NO_3-N + NH_4-N	Ext. P	pH	Four Week Dehydrogenase Activity	Chlorophyl-A
ATP	0.29158	0.30733	0.00153	0.15652	
Four Week Dehydrogenase Activity	0.17881	0.50637	0.01213		
Three Week Dehydrogenase Activity					0.39044

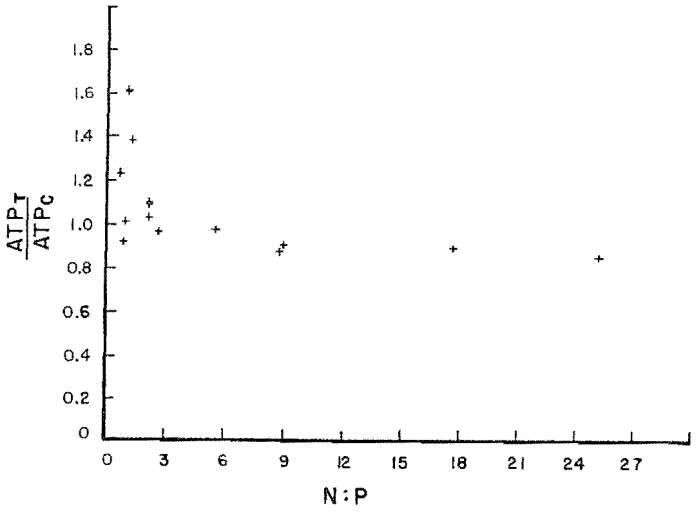


Figure 15. N:P ratio in relation to the ratio between ATP concentrations in the treatments and the ATP concentration in the control.

Table 28. Ranking of treatments from lowest to highest for each parameter measured.

	Treatment Ranking
Autotrophic population Estimate	
Chlorophyll-a	$7 = 1 = 5 = 4 = 6 < 2 = 8 = 11 = 14 < 13 = 10 = 3 < 12 \neq 9$
Dehydrogenase	$6 = 7 = 1 = 5 = 4 = 10 < 9 = 8 = 11 = 2 = 12 = 3 < 14 = 13$
Heterotrophic population Estimate	
ATP	$5 = 4 < 7 = 11 = 6 = 12 = 10 = 1 = 13 < 3 = 2 < 8 < 9 < 14$
Nutrient status	
Nitrate-N	$1 = 8 = 11 = 14 = 9 < 3 = 13 = 2 < 12 < 6 = 4 < 10 < 7 < 5$
Extractable-P	$6 = 5 = 7 = 4 < 1 < 3 = 13 = 9 = 2 < 10 < 11 < 14 < 8 = 12$
pH	$12 < 13 = 14 = 7 < 5 < 6 < 10 < 4 = 8 = 11 = 3 < 9 = 1 = 2$

higher nutrient levels than lower horizons, are now placed as the upper soil layer on the revegetation sites. In the lysimeter study, this same layering of soils (overburden, B horizon and A horizon) was used. Initial nutrient levels in each of these soil layers and in the manure, together with the tabulated data from the analyses performed throughout the study, are summarized in Appendix C. The treatments evaluated in the lysimeter study are presented in Table 29.

Effects of time, treatment and depth

A summary of the results from a three factor analysis of variance is tabulated in Table 30. Analysis of the data by sampling dates shows a significant difference among dates at the 99 percent level of confidence for both ATP and nitrite-N concentrations, and at the 95 percent level of

Table 29. Review of treatments used in the lysimeters.

Lysimeter	Treatment
1	Control, no treatment
2	$\frac{81\ kg}{ha}$ NO_3-N, $\frac{162\ kg}{ha}$ P
3	Manure $\frac{9\ mt}{ha}$
4	Same as 2 + $\frac{33\ kg}{ha}$ S
5	$\frac{81\ kg}{ha}$ NH_4-N, $\frac{162\ kg}{ha}$ P, $\frac{33\ kg}{ha}$ S

Table 30. Summary of analysis of variance from lysimeter data.

	Sampling Period	Treatment	Depth	Period x Treatment	Period x Depth	Treatment x Depth
DF	3	4	2	12	6	8
TN	1.70	1.46	55.75**	0.64	1.79	1.19
NO_2-N	5.29**	4.83**	7.09**	1.65	2.21*	3.62**
NO_3-N	3.17*	14.01**	13.73**	1.05	1.14	2.82**
NH_4-N	2.80*	5.24**	6.01**	2.07*	2.77*	5.10**
Ext P	2.54	8.37**	29.82**	0.35	0.92	3.01*
TP	3.03*	5.54**	23.16**	0.75	0.30	4.86**
TOC	1.11	0.91	4.93**	1.04	0.88	0.77
DE	0.70	5.23**	15.67**	0.92	0.75	3.46**
ATP	12.12**	4.34**	20.64**	1.38	3.25**	2.11*

Denominator DF = 179

Critical Values		1%**	5%*
DF numerator	2	4.73	3.05
	3	3.89	2.65
	4	3.43	2.42
	6	2.90	2.15
	8	2.61	1.99
	12	2.28	1.81
	24	1.90	1.58

*,** Significant at the 0.05 and 0.01 levels, respectively.

confidence for nitrate-N, ammonium-N and total phosphorus concentrations. There was no difference among sampling dates in total nitrogen, extractable phosphorus or total organic carbon concentrations, nor in dehydrogenase activity.

Analysis of the data by treatments shows a significant difference at the 99 percent level of confidence, among treatments for nitrite-N, nitrate-N, ammonium-N, extractable phosphorus, and total phosphorus concentrations and also for dehydrogenase activity and ATP concentrations. There was no significant difference among treatments in total nitrogen or total organic carbon concentrations.

Analysis of the data by depth showed a significant difference at the 99 percent level of confidence among sampling depths for all parameters measured.

Analysis of interactions between factors showed that differences among sampling dates and differences among treatments were, in general, dependent upon differences among depths. Differences in ammonium-N concentrations among sampling dates were dependent, not only upon differences among depths but, also upon differences among treatments.

Biological and chemical parameters

Total nitrogen. No interactions affected differences in total nitrogen concentrations with depth. A Duncan's multiple range analysis of the total nitrogen data by sampling depth indicated that the total nitrogen concentration in the 60 cm sampling depth was significantly less than the concentration found in either the 1 cm or 15 cm sampling depths (Table 31).

Nitrite-N. Results from the analysis of variance test indicated that there was a statistically significant difference at the 99 percent

Table 31. Summary of Duncan's multiple range analysis[a] of total nitrogen (mg/g) by depth, for all treatments and sampling dates.[b]

	AVERAGE
DEPTH 60CM	0.87167
DEPTH 01CM	1.3665
DEPTH 15CM	1.4337
3 DEPTH 60CM	
1 DEPTH 01CM	*
	*
2 DEPTH 15CM	*
	*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

confidence level, in nitrite-N concentrations among sampling dates. The Duncan's multiple range test however, was unable to detect these differences (Table 32a).

Differences in nitrite-N concentrations among treatments were dependent upon differences among depths. At the 1 cm sampling depth there was no significant difference among treatments. However, differences among treatments at lower depths were not great (Table 32b). The manure treatment was the only treatment which had significant differences in nitrite-N concentrations among depths (Table 32c).

Nitrate-N. Although results from the analysis of variance test indicated that there was a statistically significant difference in nitrate-N concentrations among sample dates, the Duncan's multiple range test was unable to detect these differences (Table 33a).

Differences in nitrate-N concentration treatments are dependent upon differences among depths. At all depths the manure treatment and the control have the lowest nitrate-N concentrations. At the 60 cm depth there is no difference among treatments (Table 33b).

Table 32. Summary of Duncan's multiple range analysis[a] of nitrite-N (µg/g), period x depth for all treatments (a), treatment x depth for all periods (b) and depth x treatment for all periods (c).[b,c]

(a)

1 cm

	AVERAGE
9WEEKS	0.11000
3WEEKS	0.11200
6WEEKS	0.12800
13WEEKS	0.14333

3 9WEEKS	*
	*
1 3WEEKS	*
	*
2 6WEEKS	*
	*
4 13WEEKS	*
	*

15 cm

	AVERAGE
3WEEKS	.73333E-02
13WEEKS	.73333E-01
9WEEKS	.73333E-01
6WEEKS	0.22667

1 3WEEKS	*
	*
4 13WEEKS	*
	*
3 9WEEKS	*
	*
2 6WEEKS	*
	*

60 cm

	AVERAGE
3WEEKS	0.14000
9WEEKS	0.17667
13WEEKS	0.18333
6WEEKS	0.20667

1 3WEEKS	*
	*
3 9WEEKS	*
	*
4 13WEEKS	*
	*
2 6WEEKS	*
	*

(b)

1 cm

TREATMENT	AVERAGE
MINING CO + S (4)	.83333E-01
MINING CO (2)	.97500E-01
CONTROL (1)	0.10667
MANURE (3)	0.11833
NH4,PO4,S (5)	0.21083

4 MINING CO + S (4)	*
	*
2 MINING CO (2)	*
	*
1 CONTROL (1)	*
	*
3 MANURE (3)	*
	*
5 NH4,PO4,S (5)	*
	*

15 cm

TREATMENT	AVERAGE
MANURE (3)	.16667E-01
MINING CO + S (4)	.33333E-01
NH4,PO4,S (5)	.70833E-01
CONTROL (1)	0.14667
MINING CO (2)	0.20833

3 MANURE (3)	*
	*
4 MINING CO + S (4)	*
	*
5 NH4,PO4,S (5)	* *
	* *
1 CONTROL (1)	* *
	* *
2 MINING CO (2)	*
	*

60 cm

TREATMENT	AVERAGE
CONTROL (1)	.91667E-01
NH4,PO4,S (5)	0.13083
MINING CO (2)	0.19583
MANURE (3)	0.22000
MINING CO + S (4)	0.24500

1 CONTROL (1)	*
	*
5 NH4,PO4,S (5)	* *
	* *
2 MINING CO (2)	* *
	* *
3 MANURE (3)	*
	*
4 MINING CO + S (4)	*
	*

(c)

Control (1)

	AVERAGE
DEPTH 60CM	.91667E-01
DEPTH 01CM	0.10667
DEPTH 15CM	0.14667

3 DEPTH 60CM	*
	*
1 DEPTH 01CM	*
	*
2 DEPTH 15CM	*
	*

NO_3-N+P (2)

	AVERAGE
DEPTH 01CM	.97500E-01
DEPTH 15CM	0.14583
DEPTH 60CM	0.19583

1 DEPTH 01CM	*
	*
2 DEPTH 15CM	*
	*
3 DEPTH 60CM	*
	*

Manure (3)

	AVERAGE
DEPTH 15CM	.16667E-01
DEPTH 01CM	0.11833
DEPTH 60CM	0.22000

2 DEPTH 15CM	*
	*
1 DEPTH 01CM	* *
	* *
3 DEPTH 60CM	*
	*

NO_3-N+P+S (4)

	AVERAGE
DEPTH 01CM	.83333E-01
DEPTH 15CM	.95833E-01
DEPTH 60CM	0.24500

1 DEPTH 01CM	*
	*
2 DEPTH 15CM	*
	*
3 DEPTH 60CM	*
	*

NH_4-N+P+S (5)

	AVERAGE
DEPTH 15CM	.70833E-01
DEPTH 60CM	0.13083
DEPTH 01CM	0.21083

2 DEPTH 15CM	*
	*
3 DEPTH 60CM	*
	*
1 DEPTH 01CM	*
	*

[a] Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b] For definition of terms see page xii.

[c] For treatment descriptions see Table 29.

Table 33. Summary of Duncan's multiple range analysis[a] for nitrate-N (µg/g), by period for all treatments and depths (a), treatment x depth for all periods (b), and depth x treatments for all periods (b).[b,c]

(a)

	AVERAGE
3WEEKS	31.863
13WEEKS	39.604
6WEEKS	40.608
9WEEKS	49.152

1 3WEEKS	*
	*
4 13WEEKS	*
	*
2 6WEEKS	*
	*
3 9WEEKS	*
	*

(b)

1 cm		15 cm		60 cm	
TREATMENT	AVERAGE	TREATMENT	AVERAGE	TREATMENT	AVERAGE
MANURE (3)	19.283	MANURE (3)	29.171	MANURE (3)	20.042
CONTROL (1)	22.983	CONTROL (1)	31.867	CONTROL (1)	20.283
MINING CO + S (4)	41.175	NH4,PO4,S (5)	56.533	NH4,PO4,S (5)	23.913
NH4,PO4,S (5)	56.229	MINING CO + S (4)	63.263	MINING CO + S (4)	31.383
MINING CO (2)	89.502	MINING CO (2)	65.983	MINING CO (2)	32.992
3 MANURE (3)	*	3 MANURE (3)	*	3 MANURE (3)	*
	*		*		*
1 CONTROL (1)	*	1 CONTROL (1)	*	1 CONTROL (1)	*
	*		*		*
4 MINING CO + S (4)	*	5 NH4,PO4,S (5)	*	5 NH4,PO4,S (5)	*
	*		*		*
5 NH4,PO4,S (5)	* *	4 MINING CO+S (4)	*	4 MINING CO+S (4)	*
	* *		*		*
2 MINING CO (2)	*	2 MINING CO (2)	*	2 MINING CO (2)	*
	*		*		*

(c)

Control (1)		NO_3-N+P (2)		Manure (3)		NO_3-N+P+S (4)		NH_4-N+P+S (5)	
	AVERAGE		AVERAGE		AVERAGE		AVERAGE		AVERAGE
DEPTH 60CM	20.283	DEPTH 60CM	32.992	DEPTH 01CM	19.283	DEPTH 60CM	31.383	DEPTH 60CM	23.913
DEPTH 01CM	22.983	DEPTH 15CM	66.712	DEPTH 60CM	20.042	DEPTH 01CM	41.175	DEPTH 01CM	56.229
DEPTH 15CM	31.867	DEPTH 01CM	89.502	DEPTH 15CM	29.171	DEPTH 15CM	62.533	DEPTH 15CM	56.533
3 DEPTH 60CM	*	3 DEPTH 60CM	*	1 DEPTH 01CM	*	3 DEPTH 60CM	*	3 DEPTH 60CM	*
	*		*		*		*		*
1 DEPTH 01CM	*	2 DEPTH 15CM	* *	3 DEPTH 60CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	*
	*		* *		*		*		*
2 DEPTH 15CM	*	1 DEPTH 01CM	*	2 DEPTH 15CM	*	2 DEPTH 15CM	*	2 DEPTH 15CM	*
	*		*		*		*		*

[a] Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b] For definition of terms see page xii.

[c] For treatment descriptions see Table 29.

Examination was made for differences in nitrate-N concentrations among depths for each treatment. Treatment 2 was the only treatment in which the multiple range test indicated a significant difference among depths (Table 33c).

Ammonium-N. Differences in ammonium-N concentrations among sampling dates were dependent upon differences among treatments and among sampling depths. However, the Duncan's multiple range test was unable to detect any difference in ammonium-N concentrations among dates when analyzed by treatments or by depths (Table 34a,b). It does appear however, that the treatment which included the addition of ammonium-N had significantly higher ammonium-N concentration (44.5 mg/g) at the three week sampling date than at the nine week sampling date (3.6 mg/g).

Differences in ammonium-N concentrations among treatments were dependent upon differences among sampling dates and depths. Although the multiple range test did not detect differences among treatments when analyzed by sampling dates (Table 34c) it does appear that the treatment which included the addition of ammonium-N had a higher ammonium-N concentration at the three and six week sampling dates than did the other treatments.

Determination of differences in ammonium-N concentrations among treatments by depth indicates that at the 1 cm sampling depth the treatment which included the addition of ammonium-N had a significantly higher ammonium-N concentration than did the other samples (Table 34d). In addition differences in ammonium-N concentrations among depths were not detected by the multiple range tests when analyzed by sampling date or treatment (Table 34e,f). It does appear however, that the ammonium-N concentration in the 1 cm samples are higher than in the lower depths

Table 34. Summary of Duncan's multiple range analysis[a] of ammonium-N (µg/g), period x treatment for all depths (a), period by depth for all treatments (b), treatment x period for all depths (c), treatment x depth for all periods (d), depth x period for all treatments (e) and depth x treatment for all periods (f).[b,c]

(a)

Control (1)	AVERAGE	NO_3-N+P (2)	AVERAGE	Manure (3)	AVERAGE	NO_3-N+P+S (4)	AVERAGE	NH_4-N+P+S (5)	AVERAGE
9WEEKS	2.1667	6WEEKS	1.7667	6WEEKS	1.8667	6WEEKS	1.7444	13WEEKS	2.3667
3WEEKS	2.1889	9WEEKS	2.1833	9WEEKS	2.0111	9WEEKS	1.7889	9WEEKS	3.5611
6WEEKS	2.6167	13WEEKS	2.3389	13WEEKS	2.3389	13WEEKS	2.5056	6WEEKS	21.028
13WEEKS	2.9167	3WEEKS	2.4889	3WEEKS	2.6222	3WEEKS	7.8056	3WEEKS	44.544
3 9WEEKS	*	2 6WEEKS	*	2 6WEEKS	*	2 6WEEKS	*	4 13WEEKS	*
	*		*		*		*		*
1 3WEEKS	*	3 9WEEKS	*	3 9WEEKS	*	3 9WEEKS	*	3 9WEEKS	*
	*		*		*		*		*
2 6WEEKS	*	4 13WEEKS	*	4 13WEEKS	*	4 13WEEKS	*	2 6WEEKS	*
	*		*		*		*		*
4 13WEEKS	*	1 3WEEKS	*	1 3WEEKS	*	1 3WEEKS	*	1 3WEEKS	*
	*		*		*		*		*

(b)

1 cm	AVERAGE	15 cm	AVERAGE	60 cm	AVERAGE
13WEEKS	2.3733	6WEEKS	2.0067	6WEEKS	2.2000
9WEEKS	2.4500	3WEEKS	2.1567	9WEEKS	2.4033
6WEEKS	13.207	9WEEKS	2.1733	13WEEKS	2.6267
3WEEKS	30.810	13WEEKS	2.4800	3WEEKS	2.8233
4 13WEEKS	*	2 6WEEKS	*	2 6WEEKS	*
	*		*		*
3 9WEEKS	*	1 3WEEKS	*	3 9WEEKS	*
	*		*		*
2 6WEEKS	*	3 9WEEKS	*	4 13WEEKS	*
	*		*		*
1 3WEEKS	*	4 13WEEKS	*	1 3 WEEKS	*
	*		*		*

(c)

P1		P2		P3		P4	
TREATMENT	AVERAGE	TREATMENT	AVERAGE	TREATMENT	AVERAGE	TREATMENT	AVERAGE
CONTROL (1)	2.1889	MINING CO + S (4)	1.7222	MINING CO + S (4)	1.7889	MINING CO (2)	2.3389
MINING CO (2)	2.4889	MINING CO (2)	1.7889	MANURE (3)	2.0111	MANURE (3)	2.3389
MANURE (3)	2.6222	CONTROL (1)	2.6167	CONTROL (1)	2.1667	NH4,PO4,S (5)	2.3667
MINING CO + S (4)	7.8056	MANURE (3)	7.9278	MINING CO (2)	2.1833	MINING CO + S (4)	2.5056
NH4,PO4,S (5)	44.544	NH4,PO4,S (5)	14.967	NH4,PO4,S (5)	3.5611	CONTROL (1)	2.9167
1 CONTROL (1)	*	4 MINING CO + S (4)	*	4 MINING CO + S (4)	*	2 MINING CO (2)	*
	*		*		*		*
2 MINING CO (2)	*	2 MINING CO (2)	*	3 MANURE (3)	* *	3 MANURE (3)	*
	*		*		* *		*
3 MANURE (3)	*	1 CONTROL (1)	*	1 CONTROL (1)	* *	5 NH4,PO4,S (5)	*
	*		*		* *		*
4 MINING CO + S (4)	*	3 MANURE (3)	*	2 MINING CO (2)	* *	4 MINING CO + S (4)	*
	*		*		* *		*
5 NH4,PO4,S (5)	*	5 NH4,PO4,S (5)	*	5 NH4,PO4,S (5)	*	1 CONTROL (1)	*
	*		*		*		*

[a] Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b] For definition of terms see page xii.

[c] For treatment descriptions see Table 29.

Table 34. Continued.

(d)

1 cm		15 cm		60 cm	
TREATMENT	AVERAGE	TREATMENT	AVERAGE	TREATMENT	AVERAGE
MINING CO (2)	1.9167	MANURE (3)	1.9167	MINING CO + S (4)	1.8292
CONTROL (1)	2.0083	MINING CO (2)	2.0542	MANURE (3)	2.3542
MANURE (3)	2.3583	NH4,PO4,S (5)	2.0583	MINING CO (2)	2.6292
MINING CO + S (4)	6.2250	MINING CO + S (4)	2.3125	CONTROL (1)	2.7292
NH4,PO4,S (5)	48.542	CONTROL (1)	2.6792	NH4,PO4,S (5)	3.0250
2 MINING CO (2)	*	3 MANURE (3)	*	4 MINING CO + S (4)	*
1 CONTROL (1)	*	2 MINING CO (2)	*	3 MANURE (3)	*
3 MANURE (3)	*	5 NH4,PO4,S (5)	*	2 MINING CO (2)	*
4 MINING CO + S (4)	*	4 MINING CO + S (4)	*	1 CONTROL (1)	*
5 NH4,PO4,S (5)	*	1 CONTROL (1)	*	5 NH4,PO4, S (5)	*

(e)

P1		P2		P3		P4	
	AVERAGE		AVERAGE		AVERAGE		AVERAGE
DEPTH 15CM	2.1567	DEPTH 15CM	2.0067	DEPTH 15CM	2.1733	DEPTH 01CM	2.3733
DEPTH 60CM	2.8233	DEPTH 60CM	2.2D00	DEPTH 60CM	2.4033	DEPTH 15CM	2.4800
DEPTH 01CM	30.810	DEPTH 01CM	13.207	DEPTH 01CM	2.4500	DEPTH 60CM	2.6267
2 DEPTH 15CM	*	2 DEPTH 15CM	*	2 DEPTH 15CM	*	1 DEPTH 01CM	*
3 DEPTH 60CM	*	3 DEPTH 60CM	*	3 DEPTH 60CM	*	2 DEPTH 15CM	*
1 DEPTH 01CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	*	3 DEPTH 60CM	*

(f)

Control (1)		NO_3-N+P (2)		Manure (3)		NO_3-N+P+S (4)		NH_4-N+P+S (5)	
	AVERAGE		AVERAGE		AVERAGE		AVERAGE		AVERAGE
DEPTH 01CM	2.0083	DEPTH 01CM	1.9167	DEPTH 15CM	1.9167	DEPTH 60CM	1.8292	DEPTH 15CM	2.0583
DEPTH 15CM	2.6792	DEPTH 15CM	2.0375	DEPTH 60CM	2.3542	DEPTH 15CM	2.3292	DEPTH 60CM	3.0250
DEPTH 60CM	2.7292	DEPTH 60CM	2.6292	DEPTH 01CM	2.3583	DEPTH 01CM	6.2250	DEPTH 01CM	48.542
1 DEPTH 01CM	*	1 DEPTH 01CM	*	2 DEPTH 15CM	*	3 DEPTH 60CM	*	2 DEPTH 15CM	*
2 DEPTH 15CM	*	2 DEPTH 15CM	*	3 DEPTH 60CM	*	2 DEPTH 15CM	*	3 DEPTH 60CM	*
3 DEPTH 60CM	*	3 DEPTH 60CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	*	1 DEPTH 01CM	*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

[c]For treatment descriptions see Table 29.

at the three week and six week sampling dates. It also appeared that the 1 cm sampling depth of treatment 5 has a higher ammonium-N concentration than the lower depths of that treatment.

Extractable phosphorus. Differences in extractable phosphorus concentrations among treatments were dependent upon differences among depths (Table 35). At the 1 cm sampling depth, the control treatment had a significantly lower extractable phosphorus concentration than the other treatments while the manure treatment had the highest concentration. At the 15 cm sampling depth, the manure treatment had a significantly higher extractable phosphorus concentration than the other treatments. At the 60 cm sampling depth there was no difference in extractable phosphorus concentrations among sites.

Total phosphorus. Comparisons of total phosphorus concentrations among sampling dates for all treatments and depths were accomplished using the Duncan's multiple range test (Table 36a). The test, however, was not powerful enough to detect differences among the means.

Only in the 1 cm sampling depth were there any differences in total phosphorus concentrations among treatments (Table 36b) at different depths. At this depth, the control and the manure treatment had significantly lower total phosphorus concentrations than the other treatments.

Total organic carbon. Results of the analysis of variance test indicated that there was a statistically significant difference, at the 99 percent level of confidence, in total organic carbon concentrations among depths. The Duncan's multiple range test was unable to detect these differences (Table 37).

Dehydrogenase activity. Differences in dehydrogenase activity among treatments were dependent upon differences among depths (Figure 16a). At

Table 35. Summary of Duncan's multiple range analysis[a] of extractable phosphorus (µg/g), treatments x depth for all periods.[b,c]

1 cm		15 cm		60 cm	
TREATMENT	AVERAGE	TREATMENT	AVERAGE	TREATMENT	AVERAGE
CONTROL (1)	4.4650	CONTROL (1)	4.6567	NH4,PO4,S (5)	2.7167
MINING CO (2)	9.6067	MINING CO (2)	4.7408	MINING CO + S (4)	2.8867
NH4,PO4,S (5)	10.043	MINING CO + S (4)	4.8825	CONTROL (1)	3.3000
MINING CO + S (4)	10.390	NH4,PO4,S (5)	5.1558	MINING CO (2)	3.5108
MANURE (3)	12.263	MANURE (3)	13.001	MANURE (3)	4.1183
1 CONTROL (1)	*	1 CONTROL (1)	*	5 NH4,PO4,S (5)	*
	*		*		*
2 MINING CO (2)	* *	2 MINING CO (2)	*	4 MINING CO + S (4)	*
	* *		*		*
5 NH4,PO4,S (5)	*	4 MINING CO + S (4)	*	1 CONTROL (1)	*
	*		*		*
4 MINING CO + S (4)	*	5 NH4,PO4,S (5)	*	2 MINING CO (2)	*
	*		*		*
3 MANURE (3)	*	3 MANURE (3)	*	3 MANURE (3)	*
	*		*		*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

[c]For treatment descriptions see Table 29.

Table 36. Summary of Duncan's multiple range analysis[a] of total phosphorus (mg/g) by periods for all treatments and depths (a) and treatment x depth for all periods (b).[b,c]

(a)

	AVERAGE
13WEEKS	0.50531
3WEEKS	0.51289
6WEEKS	0.56058
9WEEKS	0.61904
4 13 WEEKS	*
	*
1 3WEEKS	*
	*
2 6WEEKS	*
	*
3 9WEEKS	*
	*

(b)

1 cm		15 cm		60 cm	
TREATMENT	AVERAGE	TREATMENT	AVERAGE	TREATMENT	AVERAGE
CONTROL (1)	0.46158	CONTROL (1)	0.44392	MINING CO (2)	0.46117
MANURE (3)	0.46583	MINING CO + S (4)	0.46767	CONTROL (1)	0.48192
MINING CO (2)	0.75083	MANURE (3)	0.46817	MANURE (3)	0.49717
NH4,PO4,S (5)	0.80792	MINING CO (2)	0.47233	MINING CO + S (4)	0.49750
MINING CO + S (4)	0.98300	NH4,PO4,S (5)	0.47908	NH4,PO4,S (5)	0.50375
1 CONTROL (1)	*	1 CONTROL (1)	*	2 MINING CO (2)	*
	*		*		*
3 MANURE (3)	*	4 MINING CO + S (4)	*	1 CONTROL (1)	*
	*		*		*
2 MINING CO (2)	* *	3 MANURE (3)	*	3 MANURE (3)	*
	* *		*		*
5 NH4,PO4,S (5)	*	2 MINING CO (2)	*	4 MINING CO + S (4)	*
	*		*		*
4 MINING CO + S (4)	*	5 NH4,PO4,S (5)	*	5 NH4,PO4,S (5)	*
	*		*		*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

[c]For treatment descriptions see Table 29.

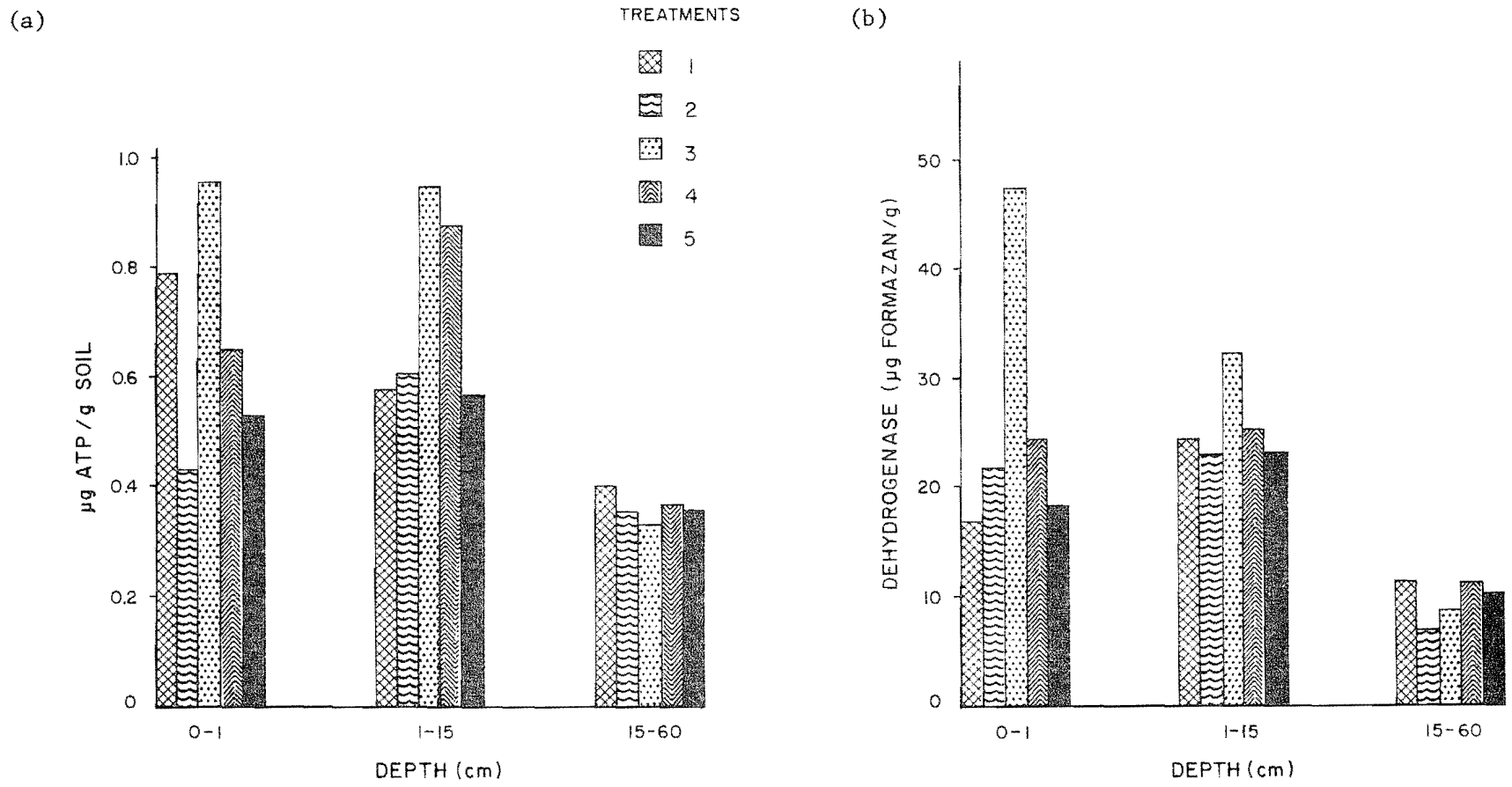


Figure 16. ATP concentrations (a) and dehydrogenase activity (b) for each treatment.

Table 37. Summary of Duncan's multiple range analysis[a] of total organic carbon (mg/g) by depth, for all treatments and sampling dates.[b]

	AVERAGE
DEPTH 60CM	7.3565
DEPTH 01CM	9.1567
DEPTH 15CM	10.951
3 DEPTH 60CM	*
	*
1 DEPTH 01CM	*
	*
2 DEPTH 15CM	*
	*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

the 1 cm sampling depth, the manure treatment had significantly higher dehydrogenase activity than did the other treatments. The manure treatment also had the highest activity at the 15 cm sampling depth. There was no difference in dehydrogenase activity among treatments at the 60 cm sampling depth (Table 38).

ATP. At both the 1 and 15 cm sampling depth, ATP concentrations were highest at the nine week sampling date but, not significantly higher than the concentrations found at the 13 week sampling date (Table 39a). At the 60 cm sampling depth there was no difference in ATP concentrations among sampling dates.

Determination of differences in ATP concentrations among treatments by depth indicated that the manure treatment had the highest concentration in both the 1 and 15 cm sampling depths (Figure 16b). These higher concentrations however, were not significantly different from the other treatments (Table 39b).

Grass yields. By the six week sampling period, all of the grasses which had germinated in the control (1) and the ammonium-N treatment (5) had died. In the control it appeared that adverse moisture conditions, as evidenced by a cracked soil surface, may have been responsible for

Table 38. Summary of Duncan's multiple range analysis[a] of dehydrogenase activity (µg formazan/g), treatment x depth for all periods.[b,c]

1 cm		15 cm		60 cm	
TREATMENT	AVERAGE	TREATMENT	AVERAGE	TREATMENT	AVERAGE
CONTROL (1)	16.803	NH4,PO4,S (5)	23.328	MINING CO (2)	7.0917
NH4,PO4,S (5)	18.345	MINING CO (2)	23.589	MANURE (3)	8.9433
MINING CO (2)	21.942	CONTROL (1)	25.048	NH4,PO4,S (5)	10.392
MINING CO + S (4)	24.459	MINING CO + S (4)	25.113	MINING CO + S (4)	11.438
MANURE (3)	64.338	MANURE (3)	32.428	CONTROL (1)	11.600
1 CONTROL (1)	*	5 NH4,PO4,S (5)	*	2 MINING CO (2)	*
	*		*		*
5 NH4,PO4,S (5)	*	2 MINING CO (2)	*	3 MANURE (3)	*
	*		*		*
2 MINING CO (2)	*	1 CONTROL (1)	* *	5 NH4,PO4,S (5)	*
	*		* *		*
4 MINING CO + S (4)	* *	4 MINING CO + S (4)	* *	4 MINING CO + S (4)	*
	* *		* *		*
3 MANURE (3)	*	3 MANURE (3)	*	1 CONTROL (1)	*
	*		*		*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

[c]For treatment descriptions see Table 29.

Table 39. Summary of Duncan's multiple range analysis[a] of ATP (µg/g), period x depth for all treatments (a) and treatment x depth for all periods (b).[b,c]

(a)

1 cm		15 cm		60 cm	
	AVERAGE		AVERAGE		AVERAGE
3WEEKS	0.39287	6WEEKS	0.43087	13WEEKS	0.33593
6WEEKS	0.46547	3WEEKS	0.69033	3WEEKS	0.34200
13WEEKS	0.85080	13WEEKS	0.76547	6WEEKS	0.35160
9WEEKS	0.97793	9WEEKS	0.98420	9WEEKS	0.41653
1 3WEEKS	*	2 6WEEKS	*	4 13WEEKS	*
	*		*		*
2 6WEEKS	* *	1 3WEEKS	* *	1 3WEEKS	*
	* *		* *		*
4 13WEEKS	* *	4 13WEEKS	*	2 6WEEKS	*
	* *		*		*
3 9WEEKS	*	3 9WEEKS	*	3 9WEEKS	*
	*		*		*

(b)

1 cm		15 cm		60 cm	
TREATMENT	AVERAGE	TREATMENT	AVERAGE	TREATMENT	AVERAGE
MINING CO (2)	0.42758	NH4,PO4,S (5)	0.56917	MANURE (3)	0.32783
NH4,PO4,S (5)	0.53283	CONTROL (1)	0.58033	MINING CO (2)	0.35458
MINING CO + S (4)	0.65108	MINING CO (2)	0.59958	NH4,PO4,S (5)	0.35692
CONTROL (1)	0.78883	MINING CO + S (4)	0.88817	MINING CO + S (4)	0.36658
MANURE (3)	0.95850	MANURE (3)	0.95133	CONTROL (1)	0.40167
2 MINING CO (2)	*	5 NH4,PO4,S (5)	*	3 MANURE (3)	*
	*		*		*
5 NH4,PO4,S (5)	* *	1 CONTROL (1)	*	2 MINING CO (2)	*
	* *		*		*
4 MINING CO + S (4)	* *	2 MINING CO (2)	*	5 NH4,PO4,S (5)	*
	* *		*		*
1 CONTROL (1)	* *	4 MINING CO + S (4)	*	4 MINING CO + S (4)	*
	* *		*		*
3 MANURE (3)	*	3 MANURE (3)	*	1 CONTROL (1)	*
	*		*		*

[a]Those groups within a comparison which are not significantly different from each other at the 95% level of confidence are connected by a line of stars to the right of the ranking list.

[b]For definition of terms see page xii.

[c]For treatment descriptions see Table 29.

the death of the plants. The amount and timing of water applications were closely controlled to simulate snow melt and storm events typical of the growing season in southeastern Montana. This means that over the 13 week study period, a total of only 35 cm of water was added to each lysimeter. The control treatment did not include a wood fiber mulch application which reduces evaporation and therefore, lost most of its moisture to evaporation soon after watering took place.

In treatment 5, incipient aqueous ammonia toxicity may possibly have caused the death. In soils of pH ranging from 7.2 to 8.0, approximately 0.5 to 2.0 percent of the molar concentration of NH_4-N in the solution is in the nonionic ammonia form. Incipient toxicity levels are those concentration levels at which plant growth inhibition is just noticeable. Those concentrations then, are not lethal to the plants affected but may cause damage which leads to serious complications that result in death.

Seven weeks into the experiment, lysimeters one and five were reseeded. The reseeding of the control did not result in any germination, apparently due to the continued adverse moisture conditions. The reseeding of the ammonium-N treatment resulted in germination and growth without adverse effects. This new growth is probably the result of decreased ammonium-N concentrations due to the process of nitrification.

When the lysimeter experiment was terminated the grass from each treatment was harvested. After air drying the samples, dry weights and the total nitrogen contents were determined (Table 40). Treatment three, the manure treatment, had a yield of more than twice the amount of grass of any other treatment while treatment one, the control, had no yield at all, in spite of two seedings. There appears to be no difference, in total nitrogen concentrations in the grass, among treatments.

Table 40. Grass yields.

Treatment	Dry Weight of Grass (g)	%N
1	0.	-
2	0.6	4.3
3	2.0	4.4
4	0.2	4.6
5	0.3	3.8

Interrelationships between chemical and biological parameters

From the Duncan's multiple range analyses it was found that both dehydrogenase activity and ATP concentrations were highest in the 1 cm sampling depth of the manure treatment. This treatment also gave a higher grass yield than the other treatments. To determine the dependence of the microbial activity on the magnitude of each of the various chemical parameters measured, a simple linear regression analysis was performed. The resulting coefficients of determination are tabulated in Table 41.

Dehydrogenase activity in the manure sample shows a high degree of dependence on total nitrogen concentrations ($r^2 = 0.93993$), total phosphorus concentrations ($r^2 = 0.80004$) and the N:P ratios ($r^2 = 0.99246$). The total phosphorus concentrations in the manure samples were no different from the control and less than the concentrations found in the other treatments.

The ATP concentrations in the manure samples at the 1 cm sampling depth were determined to be dependent upon the extractable phosphorus concentrations ($r^2 = 0.92862$). These concentrations were higher, although not significantly different, than the concentrations found in the other treatments.

Table 41. Coefficients of determination (r^2) relating dehydrogenase activity (a) and ATP (b) to various chemical parameters at the 1 cm sampling depth for each treatment.

	C/N	Ext P	TP	NO_3-N	TN	pH	$\%H_2O$	TOC	N/P	NH_4-N
(a) Dehydrogenase activity										
T1 1 cm	0.26066	0.00797	0.84714	0.00060	0.11185	0.12899	0.03078	0.00001	0.26665	0.45879
T2	0.96882	0.57565	0.11628	0.09227	0.88401	0.00177	0.36577	0.01879	0.69650	0.09831
T3	0.40553	0.00574	0.80004	0.13749	0.93993	0.68738	0.08067	0.21432	0.99246	0.79481
T4	0.41370	0.54162	0.44444	0.03206	0.36884	0.16899	0.00965	0.89050	0.53087	0.99966
T5	0.08061	0.03155	0.58358	0.01641	0.01035	0.00001	0.28645	0.21099	0.20504	0.35137
(b) ATP concentrations										
T1 1 cm	0.28936	0.02661	0.89448	0.00882	0.19190	0.01710	0.03796	0.05315	0.37194	0.18261
T2	0.60106	0.11865	0.14517	0.11277	0.82502	0.20563	0.21075	0.11169	0.59970	0.12468
T3	0.31720	0.92862	0.06415	0.02818	0.00124	0.01185	0.54489	0.61329	0.02067	0.06203
T4	0.09493	0.05545	0.88692	0.78205	0.96507	0.03552	0.15251	0.19275	0.55067	0.24063
T5	0.25362	0.00176	0.37689	0.16148	0.19739	0.00013	0.88549	0.14197	0.02888	0.35575

Linear correlation of dehydrogenase activity with ATP concentrations was not significant at the 95th percentile (Table 42). This indicated that dehydrogenase activity and ATP concentrations did not estimate the same microbial communities.

Table 42. Linear relation between different estimates of biomass for lysimeter study.

	y Variable	Number of Data Points	Correlation Coefficient
dehydrogenase activity	ATP	20	0.406

Critical value for n = 20 in 0.423 at the P > 0.95.

DISCUSSION

Observations in Field Study

Seasonal variations

Seasonal variation had little influence on stable nutrient pools such as total nitrogen, total phosphorus and total organic carbon. Although there are fluxes in the availability of these nutrients with changes in season, the total pools themselves are unaffected. This does not mean that over long periods of time these pools will not change. In the absence of any outside perturbation a decrease in organic carbon could be expected and, depending on environmental conditions, a decrease in total nitrogen also.

Seasonal variations had the greatest effect on the availability of ammonium-N. Ammonium-N concentrations were highest in the summer and lowest in the winter. The summer high was due, perhaps, to water stress inhibiting the nitrifying population of the soil. The low concentrations found in the winter were probably the result of markedly reduced rates of ammonification in cold soils.

Variations with sampling depth

Only the undisturbed site showed decreases in carbon and nitrogen concentrations from the surface to lower depths. The undisturbed site receives the majority of its organic matter input through litter fall on the surface and to a lesser extent from root material. The revegetated sites are the products of a drastically disturbed system in which surface soils, rich in nitrogen and carbon, are intermixed with other soil

horizons so there is no longer any differences between depths. Organic matter which may be placed at the lower depths, due to this mixing, will be slow to decompose due to low partial pressures of oxygen.

Differences in total phosphorus concentrations with depth were not dependent upon differences among sites. Since the source of soil phosphorus is the parent mineral rock, large variations in total phosphorus concentrations would not be expected over a localized area. Differences in total phosphorus concentrations with depth were small. The 15 cm depth had a significantly lower concentration than did the 1 cm depth but the difference was less than 0.1 mg/g.

Nitrate-N decreased in concentration from the surface down in the undisturbed site but not in the revegetated sites. In all three revegetated sites, the lowest depth sampled (90 cm) had the highest NO_3-N concentration. These concentrations however, were not statistically significantly different from the other depths sampled.

Extractable phosphorus concentrations were significantly higher in the surface samples than any other depth. This higher concentration of extractable phosphorus found in the 1 cm samples probably results from the mineralization of organic matter.

In all sites, dehydrogenase activity was greatest in the 1 cm samples. In the undisturbed, Sp 75 and Sp 77 sites, the dehydrogenase activity decreased sharply in the 15 cm samples and was not significantly different from the lower depths. The Sp 76 site showed no significant difference in dehydrogenase activity among depths.

There was no significant difference in respiration activity among depths in the three sampling periods in which this parameter was measured. Instead of being representative of the system however, these

results may be an indication of problems with the test. During sampling, contamination of lower depths with some surface soil may have occurred. Samples returned to the lab were air dried, wet to -5 bars water potential and incubated for 24 hrs before analysis. This may have allowed any contaminating organisms to establish themselves. All sampling depths were incubated under the same partial pressure of oxygen. The samples were placed in sealed sampling chambers and the chambers were evacuated for 1.5 hrs before being flushed with a gas mixture of 22 percent O_2, balance helium. This evacuation step, intended for the removal of atmospheric CO_2 from the soil pores, may have had a detrimental affect on the microbial population, many obligate aerobes do not have the capacity to grow or the ability to sporulate at low partial pressures of O_2. Whether any of these procedural steps effected the respiration activities in the samples is unsubstantiated. However, because analysis of the data showed there was no difference in activity with depths, and all of these potential problems did exist, it was determined that this parameter did not adequately represent the system and its measurement was eliminated from the fourth sampling period.

Differences among sites

There was no difference among sites in either ammonium-N or nitrite-N concentrations. Although there was an increase in ammonium-N concentrations in the summer samples over the winter samples, all sites showed this same trend. This may indicate that the nitrification potential in the revegetated sites is equivalent to the potential found in the natural soil systems in the area.

There was no difference among sites in total phosphorus concentrations. Since the parent mineral material is the major source of soil phosphorus, large variations in total phosphorus concentrations in a localized area would not be expected.

Although the undisturbed site was the only site which exhibited a gradation in total organic carbon concentration with depth, there was no significant difference in organic carbon concentrations among sites. Aridsols, typical of this area, are characterized by low concentrations of organic carbon. None of the sites studied had concentrations higher than 1 percent. In the absence of a rich A horizon in the undisturbed site, and with the addition of a wood fiber mulch on the surface of the revegetated sites, the organic carbon concentration in the revegetated sites is not seriously affected by the mining process.

Total nitrogen concentrations in the undisturbed site were higher than in the revegetated sites in the 1 and 15 cm sampling depths. The Sp 75 site, in the 1 cm sampling depth, was the only revegetated site in which the total nitrogen concentration was not significantly lower than the undisturbed site.

Dehydrogenase activity in the 1 cm depth of the undisturbed site exhibits a high degree of dependence on the total nitrogen concentration as indicated by the linear regression analysis. This dependence of microbial activity in the undisturbed site on total nitrogen is also apparent in the relationship between dehydrogenase activity and the C:N and N:P ratios.

The major source of these higher nitrogen levels, and consequently higher biological activity is plant litter. Although the litter is

sparse, as evidenced by low levels of organic carbon, the contribution to the total nitrogen pool is significant.

The differences in nitrate-N concentrations among sites was largely dependent upon differences among depths and to a lesser extent, on differences among seasons. In general, the concentration of nitrate-N in the lower depths (60 and 90 cm) of the undisturbed site were less than the concentrations found at the same depths in the revegetated sites. In a semi-arid region such as this, leaching is not considered a major factor in the movement of nitrate-N below the root zone. The high nitrate-N concentration in these lower depths was probably due to the mixing of soil horizons during the mining process.

The reclamation practices of the mining company at the times the Sp 75, Sp 76 and Sp 77 sites were revegetated, although including the segregation of "topsoil," did not include the separation of the A horizon from the B horizon. The mining company now separates these two horizons and this practice may improve the nitrogen status and therefore the microbial activity of their revegetated areas.

In the upper depths sampled (1 and 15 cm) there is little difference in nitrate-N concentrations among sites. The 1 cm depth of the Sp 75 site was however, significantly higher at the winter and spring sampling periods. The Sp 75 site at the 1 cm depth is also the only site in which the dehydrogenase activity, although significantly lower than the undisturbed site, is at elevated levels. The microbial activity in this site, however, does not appear to have a strong dependence on total nitrogen concentrations, although there appears to be some dependence on nitrate-N.

There was no nitrogen fixation activity detected in any of the regular samples brought in from the field. However, surface samples from

an undisturbed area, specifically chosen because of the presence of an algal crust, did exhibit nitrogen fixation activity. West and Skujins (1978) have indicated that these crusts are closely correlated with the clay content in arid soils, apparently because clays retain more water and nutrients and bind soil particles together. This heterogeneous spatial distribution of nitrogen fixation activity however, makes it difficult to estimate the importance of this parameter to the system.

There was no significant difference in extractable phosphorus concentrations among sites. Since the availability of phosphorus is inextricably linked to soil pH, the best way to make native phosphorus more available would be to lower the soil pH.

Microbial Bioassay

It was determined from the field study that the factor which had the greatest effect on microbial activity was total nitrogen, and consequently, C:N and N:P ratios. The microbial bioassay allowed for rapid screening of several nutrient additions at different levels, which otherwise would have been too costly and time consuming to carry out.

Those treatments which involved the addition of nitrogen only were no different or lower than the controls in the three week measurements of chlorophyll-a and dehydrogenase activity, and in the four week measurements of ATP. This indicates that the addition of nitrogen alone is not enough to increase microbial activity in the soil.

Those treatments which involved the addition of phosphorus only had significantly higher microbial activity than did the controls but had, in general lower microbial activity than did those treatments which included both phosphorus and nitrogen additions. The higher activity in the

phosphorus treatments may have been made possible by the presence of nitrogen fixing algae. Some heterocystic blue-green algae were observed on the soil surface. However, since no nitrogen fixation measurements were made its occurrence is purely conjectural.

The three week analyses were basically intended to measure the response of the autotrophic community to the different soil treatments. Chlorophyll-a concentrations were highest in those treatments which included both nitrogen and phosphorus with little difference among these treatments. Dehydrogenase activity was highest in those treatments which included nitrogen, phosphorus and sulfur. Heterotrophic activity as determined by ATP concentrations in the samples was highest in treatments 14 and 9 with little difference among the other treatments.

Those treatments with the highest nitrogen levels did not stimulate microbial activity any more than the lower nitrogen concentrations did. These was no significant difference in microbial response to the two different nitrogen sources.

After four weeks of incubation there was no difference in ammonium-N concentrations among treatments indicating that those samples receiving ammonium-N had adequate nitrifying populations to oxidize the ammonium-N to nitrate-N. The greatest difference in nitrogen source was the affect of the nitrogen source on the soil pH. The oxidation of ammonium-N resulted in a decrease in pH to a value significantly less than the pH found in samples receiving nitrate-N.

Only two of the three samples receiving elemental S had significantly lower pH values after four weeks of incubation.

Linear regression analysis, to determine the dependence of microbial responses on chemical parameters did not reveal any strong relationships.

This however, is probably a result of the chemical parameters measured. From the field study it was determined that microbial activity depended more on total nutrient pools rather than available nutrients. However, in the bioassy study, only available nutirents were measured primarily to determine what would be available for plant growth.

Results from the bioassay indicate that the addition of nitrogen and phosphorus at levels currently used by the mine, plus the addition of elemental S for decreasing pH, should provide a suitable environment for important microbial communities to establish themselves. This coupled with the new practice of the mining company of segregating A horizon soils from deeper horizons should reduce the amount of time it takes to alleviate the great disparity in microbial activity between undisturbed and revegetated sites.

Lysimeter Study

Unlike the field study, in which the replicate samples were composited and then subsampled for analysis, the samples from the lysimeters were not composited but, analyzed separately. The initial reason for compositing the field samples was to eliminate problems associated with horizontal heterogeneity of soil systems. Because the lysimeter samples were not composited, this horizontal heterogeneity resulted in large degrees of variation in the analytical results. This large amount of variation in the data occasionally made it difficult to obtain statistically significant differences at the 5 percent level.

Nutrients

Total nitrogen, found to be so important in the field study, was not the major nutrient of interest in the lysimeter study. Due to the

replacement of the A horizon soils to the top 15 cm of the soil column, the indigenous nitrogen is no longer lost to lower depths. This eliminated the problems encountered in the revegetated areas of the mine, of lower nitrogen concentrations in the upper soil column than those found in the undisturbed areas.

Differences in available nitrogen among treatments indicated that the soils have an active nitrifying population. The ammonium-N concentrations in the four treatments not receiving any ammonium-N additions did not change throughout the experiment. The concentration found in the lysimeter receiving an ammonium-N addition decreased from 44.5 µg NH_4-N/g at three weeks to 2.4 µg NH_4-N/g at 13 weeks. This value was comparable to the concentrations found in the other treatments. At no time was the nitrite-N concentration greater than 0.5 µg/g in any of the treatments.

The nitrate-N concentrations found in the lysimeter receiving ammonium-N were approximately equal to the concentrations found in the lysimeter receiving nitrate-N fertilizer. This indicated that an active nitrifying population was present in the soil. The control and manure treatment had significantly lower nitrate-N concentrations than did the lysimeters receiving inorganic nitrogen fertilizer.

The addition of inorganic phosphorus fertilizer to the soils increased the total phosphorus concentrations in the surface samples to levels almost twice as high as the total phosphorus concentrations found in the manure treatment and the control. The manure treatment however, had higher levels of extractable phosphorus in both the 1 and 15 cm sampling depths. Meek et al. (1979) have also found that manure applications increase the $NaHCO_3$-extractable phosphorus in calcareous soils

and they report that this increase may persist for many years after applications have stopped.

The lysimeters which received elemental sulfur had lower extractable phosphorus levels than did the manure treatment but, these levels were higher than the treatment not including sulfur and the control. Also, the sulfur additions did not decrease the overall the soil pH. This was probably due to the production of insufficient quantities of H_2SO_4 to overcome the buffering capacity of the soil.

Manure

The manure treatment resulted in higher grass yields and greater microbial activity in the soil than did any of the other treatments. The higher dehydrogenase activity appeared to be associated with total phosphorus, total nitrogen and N:P ratios: the higher ATP concentrations appeared dependent upon extractable phosphorus concentrations.

Goss and Stewart (1979) have shown that phosphorus from manure sources is more efficient in producing a unit of yield than are equal rates of commercial phosphorus forms. They also suggest that the availability, rate of availability, and mobility of phosphorus are functions of microbial activity.

The benefits associated with manure application are unfortunately, frequently exceeded by the costs involved with transporting and spreading the manure. Based on a hauling cost of $0.43/km ($.70/mile) and a spreading cost of $370/ha ($150/acre), it would cost about $494/ha ($200/acre) to apply manure (Larry Bond, personal communication). This is based on the assumption that the mining company is able to procure adequate manure supplies from within a 25 kilometer (40 mile) radius.

Revegetation of the Decker Mine

The major factors contributing to high levels of microbial activity in the undisturbed area of the Decker Mine were the total nitrogen concentration and consequently the N:P and C:N ratios. In this semi-arid region, most of the total nitrogen was concentrated in the upper few centimeters of the soil profile. Removal of this horizon greatly reduces soil fertility.

Previous revegetation techniques used at the mine did not conserve this nutrient rich A horizon but mixed it with other less fertile horizons. This left the surface soils of the revegetated areas deplete in nutrients and consequently with limited microbial activity. Segregation and replacement of the A horizon soils to the surface of the revegetated areas eliminated the need for increased use of inorganic nitrogen fertilizers.

Addition of manure to the soil system increased extractable phosphorus concentrations and increased microbial activity. The manure addition had an added advantage of higher grass yields also.

Due to the hazards of pollution from excessive use of nitrogen and phosphorus fertilizers, and the high cost, in terms of fossil energy, of chemical fertilizers, the simultaneous use of chemical and physical practices which enhance microbial growth should dramatically improve revegetation.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The field study

1. Seasonal variations had minimal effect on the nutrient sinks of total nitrogen, total phosphorus and total organic carbon.

2. Gradation in nutrient concentrations with depth were apparent only in the undisturbed site. In the undisturbed areas the surface contained the highest concentrations of nutrients.

3. In the revegetated sites, due to mixing of different soil horizons, there was no nutrient pool at the surface. Total nitrogen and total carbon levels were significantly lower than those found at the surface of the undisturbed site.

4. High nitrate-N concentrations in the lower sampling depths of the revegetated areas are a potential groundwater pollution problem.

5. There was little difference in total phosphorus concentrations with depth. This was attributed to the fact that the major source of soil phosphorus is the parent mineral rock.

6. Microbial activity was highest in the surface samples and decreased sharply at lower depths.

7. The microbial activity in the surface of the undisturbed site was significantly higher than the activity found in the other sites.

8. The higher microbial activity in the undisturbed site was apparently dependent upon total nitrogen concentrations and C:N and N:P ratios.

9. The failure to detect nitrogen fixation activity in any of the samples was presumably the result of the heterogenous spatial distribution of nitrogen fixing algal crusts.

The bioassay study

10. The microbial bioassay provided a fast, inexpensive technique for the rapid screening of several nutrient additions at different levels.

11. The addition of nitrogen or phosphorus alone was not enough to improve soil fertility.

The lysimeter study

12. The replacement of A horizon soils to the top 15 cm of the soil column eliminated the problem of loss of soil nitrogen due to mixing of soil horizons and maintained soil fertility.

13. The surface applications of wood fiber mulch were effective barriers to moisture movement out of the soil.

14. The addition of ammonium-N to the soil resulted in rapid nitrification to nitrate-N with no build up of nitrite-N.

15. The addition of inorganic phosphorus fertilizers resulted in higher total phosphorus concentrations but the addition of manure as a source of phosphorus resulted in higher extractable phosphorus concentrations.

16. The levels of elemental sulfur used in this experiment were not high enough to reduce soil pH and therefore did not affect extractable phosphorus concentrations.

17. The manure treatment resulted in higher grass yields and greater microbial activity in the soil than did any of the other treatments.

18. Because of the availability of adequate manure supplies in close proximity to the mine, manure as a soil amendment is an economically feasible alternative.

Overall

19. The technique developed to measure respiration activity in soils had too many potential problems to be considered a reliable estimate of microbial activity.

Recommendations for Further Research

1. A field study is needed to substantiate the positive results obtained from the addition of manure to the soil. This study should include the determination of optimum application rates based on biological response, nutrient availability and economic alternatives.

2. Another laboratory study is needed to further evaluate the use of elemental sulfur as a soil amendment. Application rates which will overcome the soil buffer capacity need to be determined and the resulting sulfate concentrations should be examined.

3. More work is needed to determine nitrogen fixation potentials of algal crusts and legumes in the undisturbed and revegetated areas.

4. A continuation of the field research done in this project should include a comparison of the old revegetated sites with the undisturbed sites and with new revegetated sites which have A horizon soils at the top 15 cm.

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APPENDICES

Appendix A

Summary of the Field Results

Table A-1. Summary of analytical results from the spring sampling date.

Season	Sampling Site	Depth		Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	Total P mg/g	TOC mg/g	pH[a]	Respiration µg C x 10^{-4}	Field[a] Moisture %	Dehydrogenase µg formazan/g	C/N	Nitrogen Fixation	N/P
SP	CD	01	$\bar{X}$	1.52	0.043	1.43	13.42	23.17	0.480	7.75	8.28	8.31	7.3	224.74	5.11		3.17
			SD	0.006	0.0076	0.097	5.90	4.44	0.0135	0.647		5.32		13.26			
			CV	0.004	0.176	0.068	0.440	0.192	0.028	0.083		0.640		0.0590			
		15	$\bar{X}$	1.23	0.037	0.98	9.08	5.97	0.423	11.93	8.22	1.15	15.3	2.32	9.73		5.84
			SD	0.064	0.0029	0.110	1.66	0.115	0.0080	1.77		0.54		2.85			
			CV	0.052	0.079	0.112	0.183	0.019	0.019	0.148		0.471		1.228			
		60	$\bar{X}$	0.79	0.065	0.54	11.92	2.70	0.487	6.74	8.87	10.65	16.2	2.27	8.54		1.62
			SD	0.030	0.005	0.055	5.25	0.346	0.013	1.91		4.72		2.23			
			CV	0.038	0.077	0.103	0.441	0.128	0.027	0.283		0.443		0.982			
		90	$\bar{X}$	0.42	0.077	0.54	6.67	5.73	0.531	2.23	8.69	10.79	15.1	0	5.35		0.79
			SD	0.074	0.0029	0.023	0.629	1.06	0.0242	0.439		2.63		0			
			CV	0.177	0.038	0.043	0.094	0.185	0.046	0.197		0.244		0			
	Sp 75	01	$\bar{X}$	0.72	0.047	4.74	16.83	20.23	0.599	5.74	8.38	11.23	9.6	130.58	8.01		1.20
			SD	0.093	0.0029	0.411	12.27	1.16	0.0176	0.481		3.04		6.87			
			CV	0.130	0.062	0.087	0.729	0.057	0.029	0.084		0.271		0.053			
		15	$\bar{X}$	0.56	0.07	0.50	16.92	4.13	0.480	4.19	8.81	12.78	12.6	0	7.44		1.17
			SD	0.047	0	0.029	6.99	0.306	0.0259	0.096		6.48		0			
			CV	0.084	0	0.058	0.413	0.074	0.054	0.023		0.508		0			
		60	$\bar{X}$	1.73	0.043	3.89	21.67	2.13	0.613	8.78	8.2	13.77	10.4	0	3.84		2.82
			SD	0.251	0.003	0.454	6.92	2.48	0.027	2.85		6.37		0			
			CV	0.119	0.067	0.116	0.319	1.16	0.045	0.324		0.462		0			
		90	$\bar{X}$	2.32	0.053	2.75	14.58	2.53	0.61	13.54	8.23	16.77	12.1	0	5.87		3.80
			SD	0.528	0.003	0.218	5.99	2.50	0.019	0.257		2.90		0			
			CV	0.228	0.054	0.079	0.411	0.987	0.032	0.019		0.173		0			
	Sp 76	01	$\bar{X}$	0.86	0.055	2.30	11.58	10.77	0.530	8.06	8.41	13.76	11.0	18.39	9.37		1.62
			SD	0.087	0.005	0.393	4.13	0.115	0.051	0.552		6.59		0.866			
			CV	0.101	0.091	0.171	0.356	0.011	0.097	0.068		0.479		0.047			
		15	$\bar{X}$	0.67	0.058	0.66	25.5	2.77	0.467	5.15	8.81	9.74	13.5	3.26	7.66		1.43
			SD	0.106	0.008	0.071	13.67	1.23	0.028	0.450		3.35		1.88			
			CV	0.158	0.099	0.107	0.536	0.446	0.060	0.087		0.344		0.576			
		60	$\bar{X}$	0.56	0.052	0.98	8.0	3.9	0.487	7.08	8.23	14.17	14.7	1.39	12.72		1.15
			SD	0.075	0.003	0.144	1.32	0.656	0.003	1.77		7.53		1.21			
			CV	0.135	0.056	0.147	0.165	0.168	0.006	0.250		0.532		0.872			
		90	$\bar{X}$	0.36	0.062	2.61	8.17	0.73	0.469	3.18	8.47	10.65	13.6	0.08	8.91		0.77
			SD	0.038	0.003	0.247	0.577	0.416	0.024	0.538		8.59		0.138			
			CV	0.106	0.047	0.095	0.071	0.568	0.051	0.169		0.807		1.73			
	Sp 77	01	$\bar{X}$	0.64	0.047	4.14	8.17	31.07	0.648	6.58	8.65	6.59	19.4	33.76	10.28		0.99
			SD	0.087	0.008	0.061	2.50	3.92	0.015	0.970		2.74		3.84			
			CV	0.136	0.164	0.053	0.307	0.126	0.023	0.148		0.417		0.114			
		15	$\bar{X}$	0.73	0.043	0.73	11.58	5.3	0.482	7.50	8.56	10.69	16.5	2.34	10.27		1.51
			SD	0.02	0.003	0.076	2.36	0.458	0.004	2.60		3.97		1.66			
			CV	0.027	0.067	0.105	0.204	0.086	0.008	0.347		0.372		0.708			
		60	$\bar{X}$	0.54	0.055	1.18	9.17	2.30	0.494	4.71	8.72	6.23	14.3	1.59	8.72		1.09
			SD	0.01	0.01	0.030	0.382	0.529	0.055	1.12		1.32		1.72			
			CV	0.018	0.182	0.026	0.042	0.230	0.112	0.239		0.211		1.08			
		90	$\bar{X}$	0.47	0.082	1.66	8.33	1.43	0.509	4.48	8.84	3.86	13.8	3.65	11.02		2.13
			SD	0.075	0.021	0.211	2.27	0.586	0.021	0.677		2.58		4.34			
			CV	0.158	0.255	0.127	0.272	0.409	0.042	0.151		0.670		1.19			

[a] No replicates.

Note: Mean of 3 replicates unless otherwise indicated.

None Detected

Table A-2. Summary of analytical results from the summer sampling date.

Season	Site	Sampling Depth		Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	Total P mg/g	TOC mg/g	pH[a]	Respiration µg C x 10^{-4}	Field[a] Moisture %	Dehydrogenase µg formazan/g	C/N	Nitrogen Fixation	N/P
S	UD	01	$\bar{X}$	1.37	0.048	1.40	23.67	12.73	0.490	10.00	7.88	13.82	1.4	169.57	7.32		2.80
			SD	0.115	0.003	0.050	17.61	1.17	0.019	1.09		4.53		77.93			
			CV	0.084	0.060	0.036	0.744	0.092	0.039	0.109		0.328		0.460			
		15	$\bar{X}$	1.43	0.037	1.13	20.75	6.33	0.446	11.32	8.08	8.95	7.5	8.63	7.92		3.21
			SD	0.270	0.019	0.083	12.80	0.677	0.009	3.69		3.41		6.16			
			CV	0.189	0.516	0.073	0.617	0.107	0.020	0.326		0.381		0.713			
		60	$\bar{X}$	0.73	0.083	0.51	24.5	4.54	0.491	5.05	7.87	2.37	9.4	0	6.92		1.49
			SD	0.030	0.024	0.380	6.26	1.02	0.008	0.165		0.469		0			
			CV	0.041	0.283	0.750	0.256	0.224	0.017	0.033		0.198		0			
		90	$\bar{X}$	0.60	0.093	1.03	19.33	4.88	0.558	2.72	8.56	5.10	9.6	0	4.54		1.07
			SD	0.056	0.020	0.025	2.75	0.508	0.024	0.390		3.31		0			
			CV	0.093	0.216	0.024	0.142	0.104	0.042	0.143		0.649		0			
	Sp 75	01	$\bar{X}$	0.90	0.057	1.23	13.67	7.00	0.552	7.50	7.46	13.40	1.5	34.8	8.33		1.63
			SD	0.044	0.017	0.044	2.08	0.418	0.029	0.185		4.52		3.30			
			CV	0.048	0.310	0.035	0.152	0.060	0.052	0.025		0.338		0.095			
		15	$\bar{X}$	0.55	0.04	0.90	11.83	2.65	0.513	6.02	8.61	4.17	5.0	2.73	10.95		1.07
			SD	0.03	0.006	0.023	0.577	0.587	0.006	0.242		0.428		1.52			
			CV	0.054	0.157	0.026	0.049	0.222	0.013	0.040		0.102		0.555			
		60	$\bar{X}$	0.65	0.04	1.18	14.33	2.15	0.543	5.94	8.24	7.16	9.6	0	8.97		1.20
			SD	0.110	0	0.029	1.76	0.537	0.010	0.560		1.95		0			
			CV	0.169	0	0.024	0.122	0.250	0.018	0.094		0.273		0			
		90	$\bar{X}$	0.69	0.065	2.31	23.75	0.53	0.664	6.52	8.27	4.43	11.4	0	9.41		1.04
			SD	0.118	0	1.12	5.25	0.918	0.073	0.675		2.85		0			
			CV	0.171	0	0.486	0.221	1.73	0.110	0.103		0.645		0			
	Sp 76	01	$\bar{X}$	0.45	0.087	1.30	37.0	4.71	0.631	6.96	8.24	11.71	1.7	3.37	15.58		0.71
			SD	0.035	0.008	0.121	15.88	0.423	0.031	1.02		4.68		1.27			
			CV	0.079	0.088	0.093	0.429	0.090	0.050	0.147		0.399		0.378			
		15	$\bar{X}$	0.45	0.055	0.76	16.0	3.26	0.585	6.95	8.67	3.93	5.7	1.4	15.32		0.77
			SD	0.101	0.005	0.032	3.04	0.335	0.015	0.205		5.78		0.173			
			CV	0.222	0.091	0.042	0.190	0.103	0.026	0.029		1.45		0.124			
		60	$\bar{X}$	0.50	0.022	0.58	50.67	6.05	0.507	5.99	8.56	3.52	7.6	1.07	11.99		0.99
			SD	0.026	0.008	0.010	42.12	4.69	0.015	0.549		1.09		0.321			
			CV	0.053	0.352	0.017	0.831	0.775	0.029	0.092		0.310		0.301			
		90	$\bar{X}$	0.36	0.122	2.70	15.17	1.06	0.585	6.80	8.72	3.69	12.1	4.77	19.06		0.61
			SD	0.011	0.019	0.112	1.04	0.934	0.010	1.87		0.990		4.61			
			CV	0.032	0.156	0.042	0.069	0.881	0.017	0.276		0.268		0.967			
	Sp 77	01	$\bar{X}$	1.38	0.092	1.23	18.5	12.90	0.653	15.04	7.77	5.44	1.7	22.03	11.12		2.11
			SD	0.072	0.011	0.017	6.5	3.38	0.024	0.907		1.00		4.80			
			CV	0.052	0.126	0.014	0.351	0.262	0.036	0.060		0.184		0.045			
		15	$\bar{X}$	0.69	0.04	2.25	17.17	4.04	0.456	5.90	8.04	3.92	4.7	2.30	8.56		1.51
			SD	0.036	0.009	0.148	7.69	1.21	0.038	1.22		0.967		1.11			
			CV	0.052	0.216	0.066	0.448	0.299	0.084	0.207		0.247		0.484			
		60	$\bar{X}$	0.55	0.052	9.62	18.33	4.15	0.486	5.38	8.32	2.36	9.1	0	9.84		1.13
			SD	0.021	0.003	0.257	5.53	1.02	0.030	0.944		0.555		0			
			CV	0.038	0.056	0.027	0.302	0.246	0.062	0.175		0.235		0			
		90	$\bar{X}$	0.54	0.097	9.79	16.83	3.43	0.563	3.64	8.41	2.72	12.3	0	6.74		0.96
			SD	0.061	0.013	0.130	5.13	0.835	0.011	0.350		0.776		0			
			CV	0.113	0.130	0.013	0.305	0.244	0.019	0.096		0.285		0			

None Detected

[a] No replicates.

Note: Mean of 3 replicates unless otherwise indicated.

Table A-3. Summary of analytical results from the fall sampling date.

Season	Site	Depth		Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	Total P mg/g	TOC mg/g	pH[a]	Respiration µg C x 10^{-4}	Field Moisture %	Dehydrogenase µg formazan/g	C/N	Nitrogen Fixation	N/P
F	UD	01	$\bar{X}$	3.57	0.072	1.72	7.42	18.25	0.656	9.46	7.44	3.64	1.4	336.0	2.65	None Detected	5.44
			SD	0.813	0.006	0.345	2.67	4.59	0.012	0.563		3.62		118.91			
			CV	0.228	0.081	0.200	0.360	0.251	0.019	0.060		0.995		0.354			
		15	$\bar{X}$	1.17	0.052	0.83	4.83	5.34	0.590	6.64	7.65	7.80	9.0	18.64	5.68		2.39
			SD	0.425	0.003	0.147	1.010	0.173	0.003	1.75		2.70		13.54			
			CV	0.363	0.056	0.177	0.209	0.032	0.007	0.263		0.346		0.726			
		60	$\bar{X}$	0.79	0.082	0.55	5.42	1.02	0.578	4.69	8.66	4.89	8.2	0	5.94		1.37
			SD	0.193	0.008	0.106	0.382	0.372	0.033	0.928		2.03		0			
			CV	0.244	0.093	0.192	0.070	0.123	0.056	0.198		0.414		0			
		90	$\bar{X}$	0.27	0.078	0.39	5.17	3.17	0.609	3.11	8.46	4.81	8.3	0	11.52		0.44
			SD	0.061	0.003	0.058	0.289	0.476	0.015	0.728		0.60		0			
			CV	0.225	0.037	0.149	0.056	0.150	0.024	0.234		0.125		0			
	Sp 75	01	$\bar{X}$	1.29	0.067	0.93	6.50	10.12	0.560	7.64	7.97	5.56	3.3	65.04	5.94		2.30
			SD	0.410	0.003	0.10	0.75	0.224	0.005	0.852		3.08		7.14			
			CV	0.319	0.043	0.107	0.115	0.022	0.009	0.111		0.553		0.110			
		15	$\bar{X}$	0.61	0.042	0.54	8.08	7.90	0.513	4.92	8.48	3.31	6.6	5.64	8.02		1.19
			SD	0.021	0.008	0.052	2.02	3.67	0.006	1.25		0.869		3.29			
			CV	0.034	0.183	0.096	0.250	0.464	0.011	0.253		0.262		0.583			
		60	$\bar{X}$	0.76	0.027	1.02	8.50	2.19	0.527	6.29	8.33	7.13	8.5	0	8.32		1.44
			SD	0.015	0.003	0.350	0.25	0.530	0.009	0.627		3.88		0			
			CV	0.020	0.108	0.343	0.029	0.242	0.017	0.100		0.545		0			
		90	$\bar{X}$	1.04	0.028	4.20	10.58	0.087	0.549	5.14	7.80	8.04	11.0	0	4.94		1.89
			SD	0.208	0.003	0.407	0.764	0.150	0.021	1.27		0.235		0			
			CV	0.200	0.102	0.097	0.072	1.732	0.039	0.257		0.029		0			
	Sp 76	01	$\bar{X}$	0.68	0.108	1.11	10.75	6.32	0.528	7.89	8.03	2.98	3.0	6.80	11.54		1.29
			SD	0.042	0.008	0.070	0.901	0.768	0.015	0.746		1.36		2.50			
			CV	0.061	0.070	0.063	0.084	0.121	0.029	0.094		0.457		0.368			
		15	$\bar{X}$	0.69	0.037	0.71	9.58	2.38	0.474	5.45	8.39	3.80	6.3	3.06	7.86		1.46
			SD	0.055	0.008	0.078	0.520	0.173	0.014	0.190		0.647		0.376			
			CV	0.079	0.208	0.110	0.054	0.073	0.029	0.035		0.170		0.123			
		60	$\bar{X}$	0.67	0.027	0.75	7.33	2.48	0.523	5.53	8.52	7.17	6.7	2.37	8.25		1.28
			SD	0.070	0.003	0.029	0.382	0.295	0.014	0.463		0.962		0.554			
			CV	0.104	0.108	0.038	0.052	0.119	0.027	0.084		0.134		0.234			
		90	$\bar{X}$	0.34	0.045	0.97	8.37	1.10	0.508	4.99	8.88	6.24	8.2	10.42	14.69		0.67
			SD	0.026	0.017	0.127	0.375	0.224	0.002	1.32		0.318		10.00			
			CV	0.078	0.385	0.131	0.045	0.203	0.003	0.265		0.051		0.959			
	Sp 77	01	$\bar{X}$	0.45	0.103	1.33	8.33	8.74	0.562	4.55	8.13	4.2	2.1	44.81	10.19		0.80
			SD	0.105	0.003	0.055	0.144	1.04	0.019	1.08		0.730		9.52			
			CV	0.235	0.028	0.041	0.017	0.119	0.034	0.237		0.174		0.212			
		15	$\bar{X}$	0.54	0.025	0.91	7.83	3.96	0.501	6.62	8.21	6.88	7.1	4.95	12.25		1.08
			SD	0.128	0.005	0.141	0.629	0.645	0.041	1.83		0.543		2.38			
			CV	0.236	0.20	0.156	0.080	0.163	0.082	0.277		0.079		0.480			
		60	$\bar{X}$	0.34	0.022	0.96	7.58	1.84	0.542	7.52	8.54	4.93	7.5	0	22.37		0.63
			SD	0.038	0.003	0.026	0.382	0.224	0.025	0.826		0.614		0			
			CV	0.112	0.133	0.028	0.050	0.121	0.047	0.110		0.124		0			
		90	$\bar{X}$	0.21	0.036	1.64	7.42	3.17	0.574	5.36	8.59	5.31	8.8	0	25.13		0.37
			SD	0.086	0.003	0.076	0.520	2.75	0.005	1.14		1.27		0			
			CV	0.404	0.079	0.046	0.070	0.866	0.009	0.212		0.239		0			

[a]No replicates.

Note: Mean of 3 replicates unless otherwise indicated.

Table A-4. Summary of analytical results from the winter sampling date.

Season	Site	Depth		Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	Total P mg/g	TOC mg/g	pH[a]	Respiration µg C × 10^{-4}	Field[a] Moisture %	Dehydrogenase µg formazan/g	C/N	Nitrogen Fixation	N/P
W	UD	01	$\bar{X}$	1.60	0.055	2.13	1.90	16.87	0.529	12.85	7.62		3.6	180.93	8.01		3.02
			SD	0.050	0.009	0.452	0.973	1.19	0.038	0.706				32.16			
			CV	0.031	0.157	0.213	0.512	0.071	0.072	0.055				0.178			
		15	$\bar{X}$	1.25	0.063	1.50	1.54	6.60	0.469	8.68	8.54		16.0	9.14	6.97		2.66
			SD	0.067	0.011	0.117	0.794	2.31	0.022	0.055				6.84			
			CV	0.053	0.182	0.078	0.515	0.349	0.048	0.006				0.749			
		60	$\bar{X}$	0.67	0.067	0.83	1.37	6.07	0.491	6.73	8.14		10.8	2.37	10.10		1.36
			SD	0.122	0.011	0.081	0.596	2.18	0.001	0.699				3.12			
			CV	0.183	0.173	0.098	0.436	0.360	0.003	0.104				1.32			
		90	$\bar{X}$	0.53	0.053	0.68	1.13	7.30	0.541	3.74	8.56		10.8	0	7.01		0.98
			SD	0.015	0.006	0.035	0.431	1.28	0.021	0.638				0			
			CV	0.029	0.108	0.051	0.380	0.175	0.039	0.171				0			
	Sp 75	01	$\bar{X}$	1.06	0.050	3.38	3.17	22.80	0.608	11.77	7.97		10.4	59.33	11.07		1.74
			SD	0.038	0.010	0.120	0.506	2.05	0.048	3.42				8.41			
			CV	0.036	0.200	0.035	0.160	0.090	0.079	0.291				0.142			
		15	$\bar{X}$	0.70	0.037	1.20	1.33	7.00	0.491	6.42	8.61		15.0	9.52	9.13		1.43
			SD	0.119	0.006	0.103	0.340	0.889	0.003	0.731				2.35			
			CV	0.170	0.157	0.086	0.255	0.127	0.006	0.114				0.246			
		60	$\bar{X}$	1.73	0.043	1.02	2.43	2.87	0.453	8.80	7.56		12.0	0	5.08		3.82
			SD	0.287	0.006	0.071	0.104	0.252	0.039	0.615				0			
			CV	0.166	0.133	0.069	0.043	0.088	0.086	0.070				0			
		90	$\bar{X}$	1.49	0.056	1.43	6.28	2.03	0.623	7.87	8.32	Not Measured	10.6	0	5.29	None Detected	2.39
			SD	0.182	0.011	0.142	1.27	0.551	0.065	0.228				0			
			CV	0.122	0.204	0.099	0.202	0.271	0.104	0.029				0			
	Sp 76	01	$\bar{X}$	0.68	0.050	0.73	2.23	7.63	0.511	6.05	8.27		5.4	9.02	8.85		1.33
			SD	0.032	0	0.029	0.202	1.03	0.008	0.505				2.02			
			CV	0.047	0	0.039	0.090	0.134	0.017	0.083				0.224			
		15	$\bar{X}$	0.53	0.040	0.78	1.02	4.30	0.442	7.25	8.42		11.3	2.39	13.69		1.20
			SD	0.036	0	0.058	0.202	1.78	0.012	0.410				0.600			
			CV	0.068	0	0.074	0.199	0.413	0.027	0.057				0.251			
		60	$\bar{X}$	0.73	0.093	0.27	0.73	3.40	0.481	8.67	8.46		11.4	3.57	11.93		1.52
			SD	0.083	0.021	0.030	0.029	1.13	0.017	0.651				6.18			
			CV	0.115	0.223	0.112	0.039	0.331	0.035	0.075				1.73			
		90	$\bar{X}$	0.43	0.033	3.10	1.33	2.80	0.472	4.64	9.20		9.9	0	10.88		0.89
			SD	0.021	0.006	0.316	0.375	0.360	0.036	0.346				0			
			CV	0.049	0.173	0.102	0.281	0.129	0.076	0.074				0			
	Sp 77	01	$\bar{X}$	0.66	0.070	0.93	2.00	5.47	0.510	12.68	8.19		3.4	11.6	19.11		1.29
			SD	0.025	0.035	0.270	0.826	1.79	0.021	5.20				1.08			
			CV	0.038	0.495	0.290	0.413	0.327	0.041	0.410				0.093			
		15	$\bar{X}$	0.69	0.040	0.74	1.75	5.67	0.441	8.84	8.15		13.6	4.37	12.75		1.56
			SD	0.015	0	0.202	0.312	0.551	0.007	1.22				0.907			
			CV	0.022	0	0.272	0.178	0.097	0.016	0.138				0.207			
		60	$\bar{X}$	0.93	0.040	1.19	1.75	2.27	0.446	9.72	8.50		10.2	4.37	10.49		2.08
			SD	0.050	0.010	0.170	0.458	0.577	0.030	0.532				0.346			
			CV	0.054	0.250	0.142	0.262	0.255	0.067	0.055				0.079			
		90	$\bar{X}$	0.64	0.073	1.08	1.82	3.10	0.438	7.87	8.47		14.0	2.28	12.36		1.46
			SD	0.072	0.011	0.081	0.388	1.67	0.007	0.361				0.343			
			CV	0.114	0.157	0.076	0.214	0.539	0.015	0.046				0.150			

[a]No replicates.

Note: Mean of 3 replicates unless otherwise indicated.

Table A-5. Summary table for the characterization of the soils at each sampling site.

Sampling Site	Sampling Depth (cm)	CEC meq/100 g	EC_e mmhos/cm	Ca^{+2} meq/ℓ	Mg^{+2} meq/ℓ	Na^{+} meq/ℓ	SAR $(mmole/\ell)^{\frac{1}{2}}$	K^{+} meq/ℓ	SO_4^{-2} meq/ℓ	Cl^{-} meq/ℓ	$CaCO_3$ eq., %	Soil Type
UD	01	18.5	0.5	4.4	0	0.58	0.39	0.46	0.85	0.3	0.039	clay
UD	15	21.5	0.9	5.2	1.6	1.51	0.82	0.24	0.83	0.1	0.112	clay
UD	60	19.7	0.6	2.0	0.4	3.97	3.62	2.49	38.63	0.1	4.23	clay
UD	90	14.8	5.6	8.0	17.7	25.05	6.99	0.22	61.52	0.1	a	clay
75	01	17.3	0.5	3.2	1.2	0.74	0.50	0.49	1.27	0.1	2.44	sandy clay
75	15	14.8	0.5	3.4	0	2.28	1.75	0.20	1.75	0.1	2.92	sandy clay
75	60	14.8	3.1	4.8	6.8	10.60	4.40	0.40	25.85	0.1	3.47	clay
75	90	13.2	5.9	15.2	19.7	20.87	5.00	0.81	67.20	0.2	a	clay
76	01	16.5	0.4	2.8	1.6	0.40	0.27	0.29	1.37	0.1	3.78	sandy clay loam
76	15	16.5	0.4	2.0	0.8	1.45	1.22	0.14	0.83	0.1	3.03	sandy loam
76	60	16.1	0.6	2.0	0.4	3.62	3.30	0.14	2.58	0.1	3.64	sandy loam
76	90	15.3	0.8	1.2	1.2	5.79	5.28	0.13	3.77	0.1	a	sandy loam
77	01	12.6	0.5	3.2	1.2	0.79	0.53	0.81	42.06	0.1	3.68	sandy loam
77	15	17.3	0.3	2.8	0.4	0.56	0.44	0.17	0.62	0.1	3.24	sandy clay loam
77	60	15.2	0.7	2.0	0.8	4.16	3.51	0.17	3.56	0.1	3.83	loamy sand
77	90	11.6	3.4	6.0	4.8	14.52	6.25	0.33	27.68	0.1	a	sandy clay loam

[a]Measurement not taken.

Appendix B

Summary of the Bioassay Results

Table B-1. Summary of analyses performed at 24 hours, 3 weeks, and 4 weeks in the bioassay study.

Sample Time	24 Hours	3 Weeks		4 Weeks						
Treatment	Dehydrogenase µg Formazan/g	Dehydrogenase µg Formazan/g	Chlorophyll-a µg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	pH[a]	Dehydrogenase µg Formazan/g	ATP µg/g
01 $\bar{X}$	14.91	42.30	0.126	<0.01	2.38	0.52	2.42	8.37	44.75	0.171
SD	4.94	13.70	0.006		0.333	0.06	1.01		2.66	0.003
CV	0.332	0.324	0.052		0.140	0.112	0.416		0.059	0.019
02 $\bar{X}$	12.83	78.46	0.198	<0.01	10.88	1.05	5.86	8.37	63.88	0.187
SD	1.37	19.59	0.033		3.70	0.25	0.02		16.71	0.018
CV	0.107	0.250	0.167		0.340	0.238	0.003		0.262	0.099
03 $\bar{X}$	15.45	83.33	0.267	<0.01	9.23	1.80	5.54	8.32	50.75	0.186
SD	1.61	22.68	0.071		2.76	0.28	0.09		16.14	0.030
CV	0.104	0.272	0.268		0.299	0.155	0.016		0.318	0.160
04 $\bar{X}$	16.08	46.38	0.134	<0.01	25.53	1.58	2.94	8.27	39.04	0.148
SD	1.74	10.54	0.005		2.27	0.86	0.11		7.43	0.015
CV	0.108	0.227	0.034		0.089	0.544	0.036		0.190	0.102
05 $\bar{X}$	17.54	43.38	0.133	<0.01	70.4	1.53	2.87	8.03	34.25	0.145
SD	4.46	20.25	0.027		2.4	1.23	0.44		5.99	0.006
CV	0.254	0.469	0.202		0.034	0.804	0.155		0.175	0.038
06 $\bar{X}$	15.96	34.25	0.145	<0.01	23.47	1.12	2.77	8.11	40.58	0.156
SD	0.97	9.53	0.015		2.16	0.33	0.26		3.09	0.005
CV	0.061	0.278	0.104		0.092	0.298	0.096		0.076	0.032
07 $\bar{X}$	14.16	36.17	0.101	<0.01	50.27	1.23	2.93	7.95	45.30	0.153
SD	1.46	3.18	0.034		16.06	0.84	0.19		7.23	0.007
CV	0.103	0.088	0.334		0.319	0.680	0.065		0.160	0.044
08 $\bar{X}$	16.75	71.34	0.213	<0.01	3.0	0.65	6.92	8.27	43.88	0.210
SD	1.0	15.81	0.083		0.68	0.05	0.32		4.07	0.038
CV	0.060	0.222	0.388		0.225	0.077	0.046		0.093	0.183
09 $\bar{X}$	13.42	70.25	0.344	<0.01	5.60	0.98	5.80	8.36	55.29	0.236
SD	0.44	24.20	0.054		0.346	0.37	0.17		9.63	0.053
CV	0.033	0.344	0.158		0.062	0.382	0.030		0.174	0.225
10 $\bar{X}$	10.71	47.33	0.263	<0.01	31.67	1.93	6.15	8.24	63.25	0.166
SD	2.29	12.63	0.059		5.26	0.75	0.48		26.78	0.007
CV	0.214	0.267	0.224		0.166	0.389	0.078		0.423	0.042
11 $\bar{X}$	18.54	75.96	0.215	<0.01	3.97	0.72	6.37	8.27	51.30	0.155
SD	1.46	25.26	0.061		0.97	0.06	0.69		16.00	0.035
CV	0.079	0.333	0.284		0.246	0.081	0.108		0.312	0.225
12 $\bar{X}$	18.04	81.54	0.293	<0.01	16.70	0.98	7.09	7.84	58.50	0.165
SD	1.82	25.56	0.064		10.19	0.82	0.63		22.21	0.013
CV	0.101	0.314	0.219		0.610	0.839	0.090		0.380	0.079
13 $\bar{X}$	21.42	113.42	0.249	<0.01	9.48	2.08	5.73	7.89	47.50	0.176
SD	6.78	18.77	0.077		4.17	1.15	1.19		13.92	0.017
CV	0.316	0.165	0.308		0.439	0.551	0.208		0.293	0.096
14 $\bar{X}$	16.29	100.84	0.219	<0.01	5.50	0.60	6.64	7.93	55.54	0.275
SD	1.75	25.97	0.063		2.13	0.26	0.18		4.76	0.041
CV	0.107	0.257	0.289		0.387	0.441	0.027		0.086	0.150

[a]No replicates

Note: Mean of 3 replicates unless otherwise indicated.

Appendix C

Summary of the Lysimeter Results

Table C-1. Summary of analytical results from the 3 week sampling date.

		Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	Total P mg/g	TOC mg/g	pH[a]	Field[a] Moisture %	Dehydrogenase µg Formazan/g	$SO_4^{=}$[a] µg/g	ATP µg/g	EC[a] µmhos/cm	C/N	C/P	N/P
101	$\bar{X}$	0.99	0.062	32.4	2.10	3.58	0.48	8.71	8.22	5.8	11.6	29.95	0.323	820	8.80	18.15	2.06
	SD	0.095	0.010	7.59	0.250	0.157	0.13	0.106			5.39		0				
	CV	0.096	0.169	0.234	0.119	0.044	0.279	0.012			0.464						
115	$\bar{X}$	1.37	0.042	26.6	2.00	3.80	0.41	8.39	8.20	10.4	15.5	97.55	0.418	1328	6.12	20.46	3.34
	SD	0.02	0.006	1.44	0.265	2.37	0.02	1.08			4.99		8.0				
	CV	0.015	0.139	0.054	0.132	0.624	0.049	0.129			0.322		0.019				
160	$\bar{X}$	0.59	0.085	11.80	2.47	1.74	0.46	6.52	8.23	5.7	0.2	807	0.323	2725	11.05	14.17	1.28
	SD	0.087	0.043	3.13	0.161	0.415	0.026	1.47			0.35		0				
	CV	0.147	0.503	0.265	0.065	0.238	0.058	0.226			1.73						
201	$\bar{X}$	1.67	0.075	74.20	1.68	7.18	0.83	8.93	8.12	9.7	18.5	21.75	0.438	1334	5.35	10.76	2.01
	SD	0.166	0.130	31.99	0.289	0.326	0.15	2.26			3.06		25.7				
	CV	0.099	1.73	0.431	0.017	0.045	0.182	0.253			0.166		0.059				
215	$\bar{X}$	1.21	0	35.2	1.72	4.65	0.46	9.68	8.09	12.9	26.3	83.22	0.409	1352	8.00	21.04	2.63
	SD	0.046	0	4.51	0.161	1.68	0.02	0.410			3.25		22.5				
	CV	0.038		0.128	0.094	0.362	0.043	0.042			0.124		0.055				
260	$\bar{X}$	0.91	0.117	16.55	4.07	1.74	0.47	9.33	8.20	6.8	1.8	291.5	0.323	3999	10.25	19.85	1.94
	SD	0.084	0.012	1.021	2.31	0.469	0.015	0.496			3.1		0				
	CV	0.092	0.099	0.062	0.567	0.269	0.033	0.053			1.73						
301	$\bar{X}$	1.00	0.075	20.93	3.37	7.66	0.41	7.03	8.27	9.3	41.3	164.0	0.508	1614	7.03	17.15	2.44
	SD	0.090	0.066	12.29	1.50	4.00	0.031	1.19			14.4		28.0				
	CV	0.090	0.882	0.587	0.447	0.522	0.074	0.169			0.350		0.055				
315	$\bar{X}$	1.66	0	22.9	2.20	16.39	0.47	12.30	8.10	13.3	32.6	101.35	0.919	1638	7.41	26.17	3.53
	SD	0.108	0	2.43	0.557	1.53	0.015	0.805			7.16		45.6				
	CV	0.065		0.106	0.253	0.093	0.032	0.065			0.220		0.050				
360	$\bar{X}$	1.24	0.165	11.98	2.30	2.51	0.48	8.72	8.27	7.6	3.5	268.5	0.323	4321	7.03	18.17	2.58
	SD	0.101	0.030	2.80	0.427	1.02	0.038	1.37			6.00		0				
	CV	0.082	0.184	0.234	0.186	0.404	0.079	0.157			1.73						
401	$\bar{X}$	1.24	0	38.37	18.57	6.46	0.82	6.50	7.77	8.6	14.5	129.5	0.372	1426	5.24	7.93	1.51
	SD	0.006	0	0.625	16.68	1.19	0.265	0.441			4.54		58.9				
	CV	0.005		0.016	0.898	0.184	0.322	0.068			0.31		0.158				
415	$\bar{X}$	1.54	0	70.3	2.47	4.80	0.41	10.12	7.96	13.4	18.1	111.0	0.983	1466	6.57	24.68	3.76
	SD	0.252	0	8.74	0.525	1.68	0.015	0.626			2.04		62.5				
	CV	0.016		0.124	0.213	0.351	0.037	0.062			0.11		0.064				
460	$\bar{X}$	0.73	0.250	19.92	2.38	2.34	0.47	6.84	8.31	8.5	8.1	505	0.418	3306	9.37	14.55	1.55
	SD	0.045	0.061	3.25	0.804	0.520	0.03	2.35			2.59		95				
	CV	0.061	0.242	0.163	0.337	0.222	0.638	0.344			0.32		0.227				
501	$\bar{X}$	1.54	0.358	60.05	128.5	8.79	0.82	10.18	7.90	7.2	14.7	186.0	0.323	2214	6.61	12.41	1.88
	SD	0.107	0.176	31.06	21.50	0.368	0.115	1.529			8.94		0				
	CV	0.069	0.490	0.517	0.167	0.042	0.140	0.150			0.61						
515	$\bar{X}$	1.41	0	42.42	2.40	4.39	0.38	8.59	8.02	13.0	22.5	143.5	0.723	1476	6.09	22.61	3.71
	SD	0.074	0	2.40	0.805	2.16	0.015	1.26			7.14		36.9				
	CV	0.052		0.057	0.335	0.493	0.040	0.147			0.32		0.051				
560	$\bar{X}$	0.73	0.093	12.08	2.90	1.99	0.45	6.12	8.40	6.8	0	714	0.323	3974	8.38	13.6	1.62
	SD	0.074	0.020	1.01	0.823	0.229	0.026	0.341			0		0				
	CV	0.101	0.217	0.084	0.284	0.115	0.059	0.557									

[a]No replicates.

Note: Mean of 3 replicates unless otherwise indicated.

Table C-2. Summary of analytical results from the 6 week sampling date.

		Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	Total P mg/g	TOC mg/g	pH[a]	Field[a] Moisture %	Dehydrogenase µg Formazan/g	$SO_4^{=}$[a] µg/g	ATP µg/g	EC[a] µmhos/cm	C/N	C/P	N/P
101	$\bar{X}$	1.24	0.067	13.27	1.65	5.48	0.47	9.48	7.87	5.6	10.14	295.0	0.323	1129	7.65	20.17	2.64
	SD	0.055	0.029	3.23	0.132	1.14	0.027	0.737			1.27		0				
	CV	0.045	0.433	0.243	0.080	0.208	0.058	0.078			0.126						
115	$\bar{X}$	1.35	0.367	35.97	3.23	5.58	0.46	9.58	7.84	7.9	29.17	29.84	0.323	1152	7.10	20.83	2.93
	SD	0.045	0.058	11.67	1.79	0.101	0.007	0.739			8.08		0				
	CV	0.033	0.157	0.325	0.554	0.018	0.014	0.077			0.277						
160	$\bar{X}$	0.65	0.117	21.05	2.97	4.71	0.51	6.91	7.99	6.8	-	248.9	0.466	2791	10.63	13.55	1.27
	SD	0.059	0.029	4.51	0.425	0.939	0.016	0.335					143				
	CV	0.091	0.247	0.214	0.143	0.200	0.031	0.048					0.307				
201	$\bar{X}$	1.54	0.067	96.93	1.77	12.91	0.81	10.19	7.72	8.0	23.57	11.91	0.455	1171	6.62	12.58	1.90
	SD	0.066	0.058	62.69	0.404	5.30	0.024	1.17			5.51		14.5				
	CV	0.043	0.866	0.647	0.229	0.410	0.029	0.114			0.234		0.032				
215	$\bar{X}$	1.58	0.15	63.35	1.62	4.94	0.50	10.36	7.76	12.3	19.30	32.59	0.395	1226	6.56	20.72	3.16
	SD	0.069	0	2.65	0.202	0.231	0.014	1.64			3.29		31.6				
	CV	0.044	0	0.042	0.125	0.047	0.028	0.158			0.171		0.080				
260	$\bar{X}$	0.79	0.217	20.12	1.92	6.27	0.46	7.33	7.97	7.8	7.30	238.65	0.323	2926	9.28	15.93	1.72
	SD	0.135	0.161	4.78	0.666	2.30	0.024	0.180			8.28		0				
	CV	0.172	0.742	0.237	0.347	0.366	0.052	0.025			1.13						
301	$\bar{X}$	1.52	0.067	18.60	1.65	10.19	0.50	10.15	7.83	8.9	56.87	34.88	0.564	899	6.68	20.30	3.04
	SD	0.061	0.029	5.00	0.265	1.91	0.087	0.902			35.80		58.1				
	CV	0.040	0.433	0.269	0.160	0.188	0.174	0.089			0.630		0.103				
315	$\bar{X}$	1.57	0.067	29.1	1.72	13.10	0.46	8.88	7.98	11.6	27.20	27.54	0.790	1105	5.66	19.30	3.41
	SD	0.168	0.029	2.05	0.115	2.94	0.007	0.648			1.76		143				
	CV	0.107	0.433	0.070	0.067	0.225	0.015	0.073			0.065		0.181				
360	$\bar{X}$	0.69	0.217	16.62	2.23	4.01	0.50	6.64	8.11	6.8	0	272.9	0.323	3448	9.62	13.28	1.38
	SD	0.023	0.076	4.06	0.535	0.685	0.045	0.095					0				
	CV	0.034	0.353	0.244	0.239	0.171	0.089	0.014									
401	$\bar{X}$	1.36	0.167	37.83	1.97	13.34	1.02	9.86	7.75	4.9	27.8	34.88	0.453	868	7.25	9.67	1.33
	SD	0.078	0.029	37.54	0.425	0.733	0.235	0.509			4.60		48.5				
	CV	0.057	0.173	0.992	0.216	0.055	0.230	0.052			0.165		0.107				
415	$\bar{X}$	1.52	0.367	57.13	1.68	5.00	0.46	9.65	8.07	17.1	26.1	28.27	0.323	1796	6.35	20.98	3.30
	SD	0.025	0.462	3.04	0.076	0.232	0.018	0.254			0.173		0				
	CV	0.017	1.26	0.053	0.045	0.046	0.039	0.026			0.007						
460	$\bar{X}$	0.76	0.317	33.85	1.58	3.28	0.54	6.31	8.19	8.6	15.9	228.86	0.323	3282	8.30	11.69	1.41
	SD	0.025	0.076	19.68	0.293	1.20	0.052	0.582			1.90		0				
	CV	0.033	0.241	0.581	0.185	0.366	0.096	0.092			0.120						
501	$\bar{X}$	1.75	0.517	85.15	59.00	12.83	0.65	8.87	7.37	7.8	18.3	50.03	0.533	2423	5.07	13.65	2.69
	SD	0.040	0.425	29.91	7.57	2.98	0.068	1.06			8.28		100.9				
	CV	0.023	0.823	0.351	0.128	0.233	0.105	0.120			0.453		0.189				
515	$\bar{X}$	1.36	0.183	57.48	1.78	5.28	0.46	8.95	7.83	11.6	16.3	46.39	0.323	1890	6.58	19.46	2.96
	SD	0.067	0.144	2.50	0.448	0.220	0.041	0.301			7.84		0				
	CV	0.049	0.787	0.044	0.251	0.042	0.090	0.034			0.480						
560	$\bar{X}$	0.80	0.167	22.67	2.30	3.39	0.52	8.83	7.98	6.8	16.1	253.35	0.323	3588	11.04	16.98	1.54
	SD	0.040	0.029	8.50	0.312	0.263	0.037	0.354			3.13		0				
	CV	0.051	0.173	0.375	0.136	0.078	0.070	0.040			0.194						

[a]No replicates.

Note: Mean of 3 replicates unless otherwise indicated.

Table C-3. Summary of analytical results from the 9 week sampling date.

		Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	Total P mg/g	TOC mg/g	pH[a]	Field[a] Moisture %	Dehydrogenase µg Formazan/g	$SO_4^{=a}$ µg/g	ATP µg/g	EC[a] µmhos/cm	C/N	C/P	N/P
101	$\bar{X}$	1.21	0.133	27.40	1.95	3.96	0.45	9.37	8.02	5.23	21.22	17.18	1.378	512	7.74	20.82	2.69
	SD	0.015	0.058	16.1	0.427	0.523	0.050	0.228			2.57		122.5				
	CV	0.013	0.433	0.588	0.219	0.132	0.112	0.024			0.121		0.089				
115	$\bar{X}$	1.50	0.1	18.93	2.15	3.97	0.45	9.81	8.06	7.26	30.29	84.08	0.932	924	6.54	21.8	3.33
	SD	0.030	0	3.10	1.28	0.307	0.038	0.369			1.15		39.5				
	CV	0.020		0.080	0.593	0.077	0.084	0.038			0.038		0.042				
160	$\bar{X}$	1.21	0.117	23.22	2.40	2.46	0.52	6.93	7.96	5.65	24.30	573	0.463	2199	5.73	13.33	2.33
	SD	0.026	0.029	2.24	0.626	0.120	0.034	0.739			5.15		82.5				
	CV	0.022	0.247	0.096	0.261	0.049	0.066	0.107			0.212		0.178				
201	$\bar{X}$	1.63	0.117	131.9	2.32	7.46	0.69	9.00	7.83	7.12	17.5	108.1	0.494	1737	5.52	13.04	2.36
	SD	0.134	0.029	7.08	0.333	1.08	0.055	1.70			4.15		171.5				
	CV	0.083	0.247	0.054	0.144	0.145	0.080	0.188			0.237		0.347				
215	$\bar{X}$	1.55	0.167	87.33	2.23	4.42	0.50	8.43	7.92	9.78	26.96	126.8	0.770	1545	5.44	16.86	3.10
	SD	0.062	0.029	3.94	0.416	0.418	0.036	0.205			1.36		90.5				
	CV	0.040	0.173	0.045	0.186	0.094	0.071	0.024			0.051		0.117				
260	$\bar{X}$	0.91	0.20	39.63	2.00	2.03	0.48	6.03	8.02	7.39	4.60	572	0.449	3104	6.63	12.56	1.90
	SD	0.040	0.05	17.76	0.391	0.801	0.009	0.197			2.93		41.5				
	CV	0.044	0.25	0.448	0.195	0.394	0.019	0.033			0.637		0.092				
301	$\bar{X}$	1.47	0.133	24.27	1.85	16.22	0.51	9.86	8.02	6.41	51.29	14.48	1.358	807	6.71	19.33	2.88
	SD	0.068	0.058	5.44	0.377	6.72	0.055	1.69			7.97		29				
	CV	0.046	0.433	0.224	0.204	0.414	0.107	0.172			0.155		0.021				
315	$\bar{X}$	1.38	0.1	25.17	1.83	12.89	0.50	10.07	8.00	10.39	41.11	126.8	1.081	1272	7.30	20.14	2.76
	SD	0.105	0	1.53	0.076	3.45	0.038	0.532			2.90		151				
	CV	0.076		0.061	0.042	0.268	0.077	0.053			0.071		0.140				
360	$\bar{X}$	0.86	0.267	25.23	2.35	3.31	0.53	7.14	7.94	7.93	19.34	908.5	0.342	2677	8.30	13.47	1.62
	SD	0.097	0.029	13.46	0.229	2.09	0.048	0.762			15.42		19.5				
	CV	0.113	0.108	0.533	0.098	0.631	0.091	0.107			0.797		0.057				
401	$\bar{X}$	1.67	0.1	61.5	1.48	8.58	1.30	9.36	7.86	9.59	28.18	90.85	1.272	1292	5.60	7.20	1.28
	SD	0.197	0	0	0.369	0.496	0.689	0.623			6.03		2.5				
	CV	0.118			0.248	0.058	0.530	0.067			0.214		0.002				
415	$\bar{X}$	1.53	0.1	71.5	2.33	4.60	0.55	9.38	7.95	10.49	32.05	101.8	1.464	1180	6.13	17.05	2.78
	SD	0.012	0	7.76	0.742	0.383	0.028	1.16			3.96		68				
	CV	0.008		0.109	0.318	0.083	0.051	0.123			0.123		0.046				
460	$\bar{X}$	0.98	0.233	34.27	1.55	1.79	0.51	6.63	8.09	9.16	10.38	736.5	0.373	2032	6.77	13.00	1.92
	SD	0.015	0.029	0.895	0.265	0.407	0.036	0.653			3.88		75.0				
	CV	0.016	0.124	0.026	0.171	0.228	0.071	0.098			0.374		0.201				
501	$\bar{X}$	1.47	0.183	44.72	4.65	9.60	1.10	9.34	7.89	6.89	24.32	90.85	0.387	1190	6.35	8.49	1.34
	SD	0.044	0.144	8.19	3.30	0.121	0.196	0.837			5.10		61.5				
	CV	0.030	0.787	0.183	0.710	0.013	0.178	0.090			0.209		0.159				
515	$\bar{X}$	1.47	0.1	71.40	2.32	4.56	0.59	9.66	7.90	8.55	30.92	126.8	0.674	1861	6.57	16.37	2.49
	SD	0.03	0	6.14	0.325	0.171	0.032	0.364			3.73		16.7				
	CV	0.020		0.086	0.140	0.037	0.054	0.038			0.121		0.025				
560	$\bar{X}$	0.78	0.133	30.90	3.72	2.19	0.59	7.34	8.07	6.93	11.17	756.5	0.455	2491	9.41	12.44	1.32
	SD	0.017	0.029	11.00	0.029	1.62	0.048	0.779			6.96		89				
	CV	0.022	0.216	0.356	0.008	0.742	0.081	0.106			0.623		0.196				

[a]No replicates.

Note: Mean of 3 replicates unless otherwise indicated.

Table C-4. Summary of analytical results from the 13 week sampling date.

		Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	Total P mg/g	TOC mg/g	pH[a]	Field[a] Moisture %	Dehydrogenase µg Formazan/g	SO_4[a] µg/g	ATP µg/g	EC[a] µmhos/cm	C/N	C/P	N/P
101	$\bar{X}$	1.19	0.167	18.83	2.33	4.84	0.45	9.01	8.19	6.15	24.23	9.97	1.131	491	7.57	20.02	2.64
	SD	0.017	0.0289	3.55	0.501	0.085	0.0089	0.445			4.04		47.1				
	CV	0.015	0.173	0.188	0.215	0.018	0.020	0.049			0.167		0.042				
115	$\bar{X}$	1.32	0.1	26.0	3.33	5.28	0.45	10.15	8.10	6.36	23.40	90.0	0.649	891	7.69	22.56	2.93
	SD	0.029	0	0.866	0.909	0.686	0.0023	0.885			1.13		118				
	CV	0.022		0.033	0.273	0.130	0.005	0.087			0.048		0.182				
160	$\bar{X}$	0.99	0.083	25.17	3.08	4.29	0.44	7.56	7.98	6.45	21.90	700	0.354	3412	7.64	17.18	2.25
	SD	0.127	0.0577	6.79	0.645	0.776	0.0208	0.21			7.50		31.5				
	CV	0.129	0.693	0.270	0.209	0.181	0.048	0.028			0.343		0.089				
201	$\bar{X}$	1.21	0.15	91.83	1.90	10.88	0.67	8.86	8.00	6.15	28.23	58.0	0.323	1247	7.32	13.22	1.81
	SD	0.225	0	37.52	0.482	3.00	0.1349	0.321			4.04		0				
	CV	0.185		0.409	0.254	0.275	0.201	0.036			0.143						
215	$\bar{X}$	1.37	0.267	81.0	2.58	4.86	0.44	9.89	7.87	8.35	20.10	126.4	0.859	1368	7.22	22.48	3.11
	SD	0.040	0.0289	10.40	0.683	0.439	0.0123	0.552			5.15		20				
	CV	0.030	0.108	0.128	0.264	0.090	0.028	0.056			0.256		0.023				
260	$\bar{X}$	1.29	0.25	55.67	2.53	4.00	0.43	8.85	7.85	10.12	14.67	497	0.323	2223	6.86	20.58	3.00
	SD	0.02	0.10	10.20	0.840	0.356	0.0171	0.306			0.802		0				
	CV	0.016	0.40	0.183	0.332	0.089	0.039	0.035			0.055						
301	$\bar{X}$	1.07	0.217	13.33	2.57	14.98	0.44	10.44	8.04	8.42	41.23	46.55	1.404	1322	9.76	23.73	2.43
	SD	0.108	0.076	5.01	0.900	9.28	0.023	0.315			6.38		88				
	CV	0.101	0.323	0.376	0.351	0.619	0.053	0.030			0.155		0.063				
315	$\bar{X}$	1.33	0	39.50	1.92	9.63	0.44	9.76	7.98	8.98	28.83	126.65	1.015	1232	7.34	22.18	3.02
	SD	0.046	0	0.50	0.208	0.911	0.0317	0.871			4.06		91.5				
	CV	0.034		0.013	0.109	0.095	0.073	0.089			0.141		0.090				
360	$\bar{X}$	0.81	0.23	26.33	2.53	6.64	0.48	7.28	8.05	7.75	12.97	556	0.323	2510	8.99	15.17	1.69
	SD	0.03	0.058	4.48	1.46	0.583	0.0247	0.980			1.53		0				
	CV	0.037	0.247	0.170	0.575	0.088	0.051	0.135			0.118						
401	$\bar{X}$	1.33	0.10	27.0	2.88	13.18	0.92	8.56	8.00	10.04	27.32	36.97	0.508	1118	6.44	9.30	1.45
	SD	0.032	0.05	14.03	0.301	1.63	0.284	0.820			6.77		59				
	CV	0.024	0.5	0.520	0.105	0.124	0.309	0.096			0.248		0.116				
415	$\bar{X}$	1.33	0	51.17	2.83	5.22	0.44	8.93	8.07	11.49	25.87	97.2	0.747	974	6.71	20.30	3.02
	SD	0.036	0	4.25	1.46	0.413	0.0236	0.239			2.37		48.5				
	CV	0.027		0.083	0.517	0.079	0.053	0.027			0.092		0.065				
460	$\bar{X}$	1.06	0.183	37.5	1.80	4.13	0.47	8.91	8.05	10.37	11.37	395	0.353	2238	8.41	18.96	2.26
	SD	0.095	0.115	11.17	0.05	1.56	0.0317	0.465			9.61		29.5				
	CV	0.090	0.630	0.298	0.028	0.378	0.067	0.052			0.845		0.084				
501	$\bar{X}$	1.23	0.083	35.0	2.18	8.95	0.66	9.40	7.89	8.49	16.07	112.25	0.888	1287	7.64	14.24	1.86
	SD	0.182	0.104	9.54	0.535	4.61	0.0244	0.745			0.808		9				
	CV	0.148	1.25	0.273	0.245	0.515	0.037	0.079			0.050		0.010				
515	$\bar{X}$	1.34	0	54.83	1.73	6.39	0.40	9.38	7.96	8.83	23.53	154.6	0.557	1519	7.00	23.45	3.35
	SD	0.021	0	4.75	0.161	4.00	0.0112	0.817			2.41		23.6				
	CV	0.016		0.087	0.093	0.626	0.028	0.087			0.103		0.042				
560	$\bar{X}$	0.69	0.167	30.0	3.18	3.30	0.45	6.89	7.98	8.62	14.3	497	0.327	2808	9.99	15.31	1.53
	SD	0.059	0.076	22.57	2.96	0.967	0.0226	1.191			5.04		6.1				
	CV	0.085	0.458	0.752	0.930	0.293	0.050	0.173			0.353		0.019				

[a]No replicates.

Note: Mean of 3 replicates unless otherwise indicated.

Table C-5. Initial condition of soils.

		Total N mg/g	NO_2-N µg/g	NO_3-N µg/g	NH_4-N µg/g	Ext. P µg/g	Total P mg/g	TOC mg/g	pH[a]	$CaCO_3$ eq., %	CEC[a] meq/100 g	$SO_4^{=a}$ µg/g	EC[a] µmhos/cm	C/N	C/P	N/P
A Horizon	$\bar{X}$	1.23	0.6	18.73	4.53	7.24	0.44	9.80	7.91	0.877	17.7	26.31	702	7.97	22.27	2.80
	SD	0.08	0	1.26	0.355	0.707	0.019	0.463		0.249						
	CV	0.068	0	0.067	0.078	0.098	0.044	0.047		0.284						
B Horizon	$\bar{X}$	0.69	0.283	8.55	14.22	3.55	0.57	7.68	8.12	6.293	12.2	21.86	647	11.13	13.47	1.21
	SD	0.060	0.029	0.563	0.029	0.256	0.034	0.753		0.335						
	CV	0.087	0.102	0.066	0.002	0.072	0.060	0.098		0.053						
Overburden	$\bar{X}$	0.47	0	15.00	14.58	1.73	0.66	4.77	9.22	-	12.4	41.98	1041	10.15	7.23	0.71
	SD	0.05	0	1.32	1.67	1.01	0.019	0.140								
	CV	0.106		0.088	0.114	0.585	0.029	0.029								
Manure	$\bar{X}$	4.47	1.28	6.72	4.47	244.3	6.32	31.52								
	SD	0.993	0.451	5.78	0.993	12.7	0.656	0.297								
	CV	0.222	0.351	0.861	0.222	0.052	0.104	0.009								

[a]No replicates.

Note: Mean of 3 replicates unless otherwise indicated.

VITA

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