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Six field sites in western Oregon were assayed periodically over one year for arylsulfatase, dehydrogenase and urease activities as well as a variety of chemical and physical parameters. Two of the sites were under forest vegetation, one was under native grasses and three were under clover/grass pasture. The interactions between the enzymatic activities and the variations in the environmental parameters were determined using a variety of computerized statistical methods including multiple regression and principal component analysis. Urease and arylsulfatase activities were most highly correlated with soil organic matter content ($r = 0.58$ for both), and dehydrogenase activity was most highly correlated with exchangeable sodium ($r = 0.52$). Multiple regression analysis of the

data showed the variability in arylsulfatase, dehydrogenase, and urease activities were partially accounted for by the soil parameters monitored (r^2 = 0.657, 0.509, 0.738, respectively). No predictable seasonal fluctuations were observed for any of the three enzyme systems. All statistical analyses indicated that the availability of nutrients in the soil was an important factor associated with level of the enzymatic activity.

Analysis of Variations in Selected Enzymatic Activities with Environmental Parameters in the Soil

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ANALYSIS OF VARIATIONS IN SELECTED ENZYMATIC ACTIVITIES WITH ENVIRONMENTAL PARAMETERS IN THE SOIL

1. INTRODUCTION

It is difficult to determine how soil microorganisms interrelate with environmental parameters because of the problems associated with isolating and identifying the microbes present in soil. Only an estimated 1% of the total bacterial population in the soil can be recovered by present isolation methods. The extracellular enzymes secreted into the soil by microorganisms apparently survive in the soil for certain periods of time and may provide an index to the biological activity in the soil. Although enzymatic activities cannot be attributed to any particular group of organisms, measurement of these activities represents a method of assessing the metabolic activity of the total soil biomass.

Three enzyme systems were included in this study. Dehydrogenase was chosen as an indicator of overall metabolic activity in the soil since dehydrogenases are possessed by both micro and macroorganisms. Urease was included because of its role in nitrogen conversions, and arylsulfatase was chosen because of its potential importance in making sulfates available to plants.

Reliable methodology exists for the measurement of the activities of these three enzyme systems.

The objectives of this study were, firstly, to increase the fundamental knowledge concerning the interaction of soil micro-organisms (as expressed by enzymatic activities) with parameters in the soil environment, and secondly, to examine how the distribution and degree of activity over time of three soil enzyme systems are related to, and to what extent controlled by, characteristics of the soil environment.

2. LITERATURE REVIEW

2.1 Introduction

Soil is a complex biological system which has been compared to a living matrix with soil enzymes corresponding to the enzymes in animal tissue (22). However, the biological transformations that occur in the soil are generally mediated by extracellular rather than intracellular enzymes. By some mechanism, the extracellular enzymes are stabilized and protected in the soil, and it now appears that at least some of the stability of the enzymes is derived by being covalently bound to soil organic matter (24).

Thorton and McLaren (37) have ascertained that many soil enzymes maintain nearly constant levels in the soil and the basal level of each enzyme varies among different soils. The levels of a variety of enzyme activities in a soil can, in some cases, serve to characterize a soil and to identify the location from which a sample of a given soil was obtained. Even soils located in close proximity to each other could be distinguished from each other by the patterns of enzymatic activities which they possessed. The reasons for these individual patterns, as well as the fluctuations of a given enzyme's activity in soils, remains unknown.

2.2 Urease

Urease is one of the most studied extracellular soil enzymes due to the importance of its substrate, urea, which is widely used as a nitrogen fertilizer. Urea is hydrolyzed by urease, and ammonium is released ($NH_2CONH_2 + 2H_2O \rightarrow 2NH_4^+ + CO_3^{-2}$). Urease is produced by a large number of microorganisms as well as by higher plants (24). Conrad (6) was among the first to recognize the enzymatic nature of urea hydrolysis when he hypothesized that the rise in the catalytic activity in soil samples after incubation originated from the increased growth of microorganisms.

A number of different methods have been developed to determine the amount of urease present in the soil. Most are based upon measuring the amount of ammonium released from toluene-treated, urea amended, buffered soils (13, 24, 35). Several non-buffered methods have also been developed (6, 8, 21) as well as some that do not employ toluene (14, 19, 21, 25). Other methods measure the amount of carbon dioxide released rather than ammonium (6, 25). The choice of method depends upon the purpose. According to Zantua and Bremner (39), if the purpose is to detect the urease present in the soil, then a buffered method is preferred. The non-buffered method is more suitable to ascertain the ability of soils to hydrolyze urea under natural conditions. In later work (41)

they concluded that, although the buffer method for determining urease activity yields higher values than the non-buffered method, the results of either method lead to similar conclusions. One widely used buffer method (35) makes use of tris(hydroxymethyl)amino-methane buffer, a solution of urea, and toluene. The toluene is used to halt microbial growth and incorporation of enzymatic reaction products during the assay. The incubation is carried out at 37°C for a two hour period, and, after incubation, a KCl-$AgSO_4$ solution is added to the soil solution in order to stop the enzymatic reaction. Urease activity is reported as the ammonium nitrogen released per unit of soil over a set time.

The stability of urease activity in soil has been the subject of many studies. Soil urease activity is resistant to irradiation, proteolysis, temperature fluctuations, storage and lyophilization (17, 20, 38, 41). Urease activity persists in the soil at a basal level for long periods of time without the addition of substrate, that level being different for each separate soil (40, 41).

Much has been done in the effort to inhibit urease activity in the soil. Upon addition of urea fertilizer, the rapid hydrolysis of the urea causes an accumulation of ammonium and a subsequent rise in soil pH. This can cause several problems such as damage to germinating seeds and young plants, nitrite toxicity, and gaseous loss of nitrogen as ammonia (9). Both organic and inorganic

compounds have been evaluated (1, 3, 8, 10, 12) and many are in commercial use. One study determined that a variety of trace elements that are found in sewage sludge can inhibit urease activity (36).

Little research has been performed to determine the interactions between urease activity and environmental parameters in the soil. Urease activity was found to be significantly correlated with organic carbon (13, 36, 42), but the degree of correlation varied for different soils. Urease activity was found to decrease with depth and it was thought that this was probably associated with the decrease of organic carbon with depth (15). Total nitrogen and cation exchange capacity have also been found to be positively correlated with urease activity (42); however, there are conflicting reports on whether or not urease activity is correlated with soil texture (13, 42). It has been reported that the Michaelis constant (K_M value) for urease activity varies in different soils (31). The K_M value was ascertained to be significantly correlated with organic carbon and total nitrogen was not correlated with pH or texture.

Pancholy and Rice (18) studied the relationship of urease activity to old field succession for three types of vegetation: tall grass prairie, post oak-blackjack forest, and oak-pine forest. They found that for all vegetation types urease activity was the lowest

in the first successional stage (pioneer plants), intermediate in the second stage and highest in the climax stage. Urease activity increased during the spring and summer months and decreased during the fall and winter. However, their study did not take into account that many of the sampling areas contained different soil types.

2.3 Arylsulfatase

Only recently has the potential importance of the microbial hydrolysis of sulfate esters in making inorganic sulfate available to plants in aerobic soils been recognized. Of the sulfatases, arylsulfatase has received the most attention. Tabatabai and Bremner (32) were the first to detect arylsulfatase activity in the soil. They developed a method of measuring arylsulfatase levels by the colorimetric determination of the p-nitrophenol released by the enzyme after adding potassium p-nitrophenyl sulfate, buffer, and toluene to a soil sample and incubating it for one hour at 37°C. Afterwards, the arylsulfatase activity is stopped and a stable color is developed upon the addition of a $NaOH\text{-}CaCl_2$ solution.

Although arylsulfatase activity is potentially important in the soil environment, there have been very few studies dealing with it. It has been found that arylsulfatase activity declines markedly with

depth and this decrease has been associated with a reduction in soil organic matter. Also, in soils with different chemical and physical properties, arylsulfatase activity was significantly correlated with soil organic matter levels (33). The K_M value for soil arylsulfatase has been investigated and it was found to vary for different soils (34).

2.4 Dehydrogenase

Dehydrogenase activity is thought to be an index of total aerobic biological activity in the soil. Stevenson (26) obtained significant correlations between oxygen uptake and dehydrogenase activity while Skujins (23) reported that dehydrogenase activity was highly correlated with soil respiration as well as proteolysis and nitrification.

Dehydrogenases are involved in the transfer of electrons from a wide variety of substrates to numerous acceptors. Many different dehydrogenase systems are included in the total soil dehydrogenase activity. The biochemical properties of dehydrogenases precludes the presence of free dehydrogenases in the soil (22), and, therefore, assays for these enzymes do not include bacteriostatic agents. Casida, Klein and Santoro (5) have developed a routinely used assay which consists of adding 2, 3, 5-triphenyltetrazolium chloride (TTC), $CaCO_3$ and an energy source (optional) to a soil sample and followed by incubation for 24 hours at 37°C.

Dehydrogenases reduce TTC to triphenyl formazan, and with the addition of methanol, a red color is formed. The concentration of the triphenyl formazan is determined colorimetrically. This procedure is subject to interference by nitrate (2) while the use of an energy source depends upon the purpose of the study. No energy source is needed if the actual dehydrogenase activity is to be measured while one is usually added for the measurement of total potential activity (4).

Stevenson (27) studied the effect of decomposition of crop plants on dehydrogenase activity and determined that increased dehydrogenase activity during the initial stages of decomposition was partially due to a preferential stimulation of certain microorganisms and not to the effects of a general increase of the soil microbial population.

Pancholy and Rice (18) included dehydrogenase in their study of soil enzymes in relation to old field succession of tall grass prairie, oak-blackjack forest, and oak-pine forest. For all vegetation types, dehydrogenase activity was lowest in the pioneer plant stage, intermediate in the second stage and highest in the climax stage. There were some inconsistencies to these findings and no seasonal trend was observed. This study was limited because of differing soil types at the sampling locations.

2.5 Use of Factor Analysis in Microbial Ecology

The use of factor analysis in recent years in microbial ecology has helped to shift research emphasis from reliance on identification of the microbial inhabitants to an ecosystem approach based on biological activities (16, 29). Factor analysis has been used as an aid in determining how environmental parameters affect microorganisms. Hagedorn and Holt (11) studied the ecology of soil arthrobacters and incorporated factor analysis to group the environmental parameters into four factors (soil structure, acidity, fertility, and moisture). The factor analysis indicated that the arthrobacters in the soils studied were acid sensitive and their numbers decreased with decreasing pH in a cause-and-effect relationship.

Factor analysis has become a general term for a variety of statistical procedures which were developed to aid the analysis of intercorrelations within a set of variables. A major type of factor analysis is principal components analysis (PCA). The initial data used for the PCA is the outcome of n tests for N observations. The first step in the computerized procedure for PCA is to determine the correlations between each pair of tests which results in an n matrix. The correlation matrix is used as the actual input for the PCA program.

Data derived from measurements of different natural variables is often intercorrelated. The purpose of the principal components analysis is to transform such information into a set of uncorrelated, independent factors. Using the correlation matrix, the PCA program treats the variables as vectors and condenses the test vectors to a number of j factors capable of expressing the data. Geometrically, factorization can be described as a process through which j number of axes are rotated in such a way to maximize the variance between the variables. The factor loadings generated by the program are defined as the relation between the factor space and the test space. Factor loadings can be expressed by the equation $y_j = b_1x_1 + b_2x_2 + \ldots + b_nx_n$, where the factor score coefficients are represented by b, the variables by x, and the factor by y. Eigenvalues are generated for each factor, and a general rule for selecting the number of factors for further research is to retain those factors which have an eigenvalue greater than 1.00. The number of factors to consider can also be decided in terms of the interpretability of the factor solution or by the amount of variability which is to be retained.

The factors are sometimes difficult to interpret in terms of the original tests. Therefore, after eliminating the factors of no significance, those remaining are subjected to rotation. The

factors are rotated to positions that enable as many factor loadings as possible to approach zero. This procedure aids in the interpretation of the factors as they relate to the original variables (7, 30).

In terms of an ecological study, it appears that factor analytical techniques will enable researchers to ascertain in more detail the manner in which ecological parameters interact with each other.

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3. MANUSCRIPT: ANALYSIS OF VARIANCE OF ARYLSULFATASE ACTIVITY AND ENVIRONMENTAL PARAMETERS IN SOIL

3.1 Introduction

The potential importance of arylsulfatase in making inorganic sulfate available to plants by hydrolysis of aromatic sulfate esters present in the soil has only recently been recognized. To date only one report has addressed the relationship of arylsulfatase activity with parameters in the soil environment (8). It was shown that arylsulfatase activity declined markedly with depth and that this decrease was associated with a reduction in soil organic matter. Also, in soils with different chemical and physical properties, arylsulfatase activity was significantly correlated with soil organic matter levels, but not with pH, total sulfur or nitrogen, or proportions of silt and clay. In the work reported here the variations in arylsulfatase activity over time were analyzed in relation to environmental parameters in western Oregon soils.

3.2 Material and Methods

Site Descriptions

The six sites used in this study are located in Benton County, Oregon, in the foothills of the Coastal Mountains. Area A (sites

1-3) was a toposequence with a southwest aspect (Table 3.1). The soils were poorly to moderately well drained with a heavy clay layer in the B-horizon, well formed profiles, and abundant mottles. The area is predominately under pasture and is grazed by sheep in the fall and spring. Site 1 (Hazelair series) is on the toeslope and site 3 (Steiwer series) is on the summit; both are under grass-clover pasture. Site 2 (Hazelair/Steiwer intergrade) is on the backslope with an old growth oak canopy and a grass-clover understory. Area B (sites 4-6) is a biosequence with an eastern aspect located on the Dixonville soil series. This soil is well drained with fractured saprolite in the C-horizon, and contains a highly developed profile with few mottles. Site 4 was on the toeslope, adjacent to a stream with a mixed fir and hardwood forest, and a substory of native grasses and small shrubs. Site 5 was on the footslope with native grasses and small shrubs while site 6 was on the backslope with a 20 year old Douglas/Silver fir forest and a substory of native grasses (Table 3.1).

Sampling Procedure and Soil Analyses

At each site, a nine square meter plot was marked out and each plot was then divided into nine 3.0 m^2 subplots. At every sampling period, two composite samples were taken from the upper

10 cm of the A1 horizon of each site. Composite samples consisted of soil cores taken from four randomly chosen subplots so that eight of the nine subplots were sampled each period. The enzymes and certain soil characteristics were determined at each of the fourteen sampling periods over one year, 1977-78. Some soil characteristics were measured six times (periods 4, 8, 10, 12, 13, 14), while other, relatively constant soil characteristics (such as those in Table 3.1), were measured only once.

Established procedures were used for all analyses, and each was performed in duplicate. Ammonia-N (1, 5), nitrate-N (2, 6), soil temperature, % moisture, soil pH (using a 2:1 water to soil solution), soil conductivity (10), and total exchangeable hydrogen (10) were determined at every sampling interval. Assays for Kjeldahl-N, exchangeable K, Ca, Na, Mg, sulfate-S, organic matter content, and available phosphorus were determined for six sampling periods, while cation exchange capacity, texture analysis (% clay, silt, and sand), and degree of porosity were measured once. Those assays performed once or for six sampling periods were done either by or using the facilities of the Oregon State University Soil Testing Laboratory (4). The % gravimetric moisture was expressed relative to field capacity, degree of soil saturation, and air-dried soil. Organic-N was calculated as Kjeldahl-N minus NH_3-N, and for all

but one observation, organic-N was equal to Kjeldahl-N within two significant figures. Total-N was assumed to be equal to the sum of the concentrations of organic-N, NH_3-N, and NO_3-N. Carbon content was estimated as equal to 58.0% of the total soil organic matter content.

Arylsulfatase activity was determined with the procedure described by Tabatabai and Bremner (7). This involves incubating duplicate soil samples plus a control for one hour at 37°C in the presence of p-nitrophenol sulfate (substrate) and toluene (to prevent additional enzyme production). After incubation, NaOH and $CaCl_2$ solutions are added to stop further enzymatic activity and to form a stable yellow color with the reaction product, p-nitrophenol. After filtration, the concentration of p-nitrophenol is determined colorimetrically, the level of concentration in the control is substracted, and arylsulfatase activity is reported as μg p-nitrophenol released/gm soil/hour.

Statistical Analyses

Forward selection and backward elimination multiple regression analyses were performed using the Statistical Interactive Programing System (SIPS) on the Oregon State University CDC-3300 computer. All other analyses were carried out on the OSU Cyber Model 73 computer. The correlation and principal components

analysis programs were based on those developed by Cooley and Lohnes (3). The correlation coefficients were determined using all available data; the multiple regression and the multivariate analyses included principally the data obtained from the six sampling periods for which complete soil analyses were made (72 observations).

3.3 Results and Discussion

Figure 3.1 depicts the variations in arylsulfatase activity over the sampling year. The trends seen in each site were markedly similar; however, the alterations in the enzymatic activity did not appear to be related to seasonal changes. The greatest decrease in activity was seen at period 7 (July 1977) and may have been due to the effects of the drought conditions that were prevalent at that time, although the activities had increased again by period 8 (August).

Of the environmental variables measured, the content of soil organic matter was most highly correlated with arylsulfatase activity ($r = 0.58$, Table 3.2). Multiple regression analysis of the data showed that the soil variables accounted for 67.1% of the observed variation in arylsulfatase activity; however, ten of the variables alone accounted for 65.7% of the variability (Table 3.3). It is interesting to note that, although seven of the parameters in the best fit regression equation had r-values that were among the ten highest, the parameters with the second and third highest r-values

(Ca content and cation exchange capacity) were excluded. This was probably due to their high correlation with several environmental variables including Mg ($r = 0.93$, $r = 0.85$, respectively), % clay + silt ($r = 0.91$, $r = 0.87$, respectively) and Na ($r = 0.76$ for both). Therefore the variability seen over time in the concentration of Ca and the differences in the cation exchange capacity for each site were expressed in the equation by a combination of other parameters. The reason that the moisture parameter was excluded was not immediately apparent. Phosphorus, ammonia-N, and total exchangeable hydrogen were included in the equation, despite their extremely low r-values, perhaps because they were not significantly correlated with other environmental parameters. Of the ten parameters included in the best fit equation (Table 3.3), only one was a physical parameter of the soil (% clay + silt), while seven variables were related to soil nutrient aspects which included Mg, Na, P, C:N, NH_3-N, organic-N, and organic matter content.

A principal components analysis of the environmental data was performed to aid in interpreting the relationships between the parameters. Twenty factors were originally generated and four factors, representing 65.1% of the variability seen in the environmental parameters, were retained for further examination (Table 3.4). The first factor expressed 31.9% of the original variability and appeared to represent soil fertility since the parameters that

had high factor loadings included Ca, Mg, Na, and C:N. Cation exchange capacity and % clay + silt were also significantly expressed in the first factor and this may be due to the effects that these two variables could have on the availability of cations to microorganisms which might lead to increased enzyme production. Sulfate-S had a high negative factor loading, perhaps because of its anionic nature based on the manner in which the factor was constructed around soil cations. Arylsulfatase activity was moderately correlated with the first factor ($r = 0.456$).

The second factor expressed 15.3% of the environmental variability and was insignificantly correlated with arylsulfatase activity ($r = 0.206$) even though the highest factor loadings for soil organic matter, organic-N and NH_3-N were contained in this factor (Table 3.4). This is an indication that perhaps the C:N ratio is more critical to the level of activity rather than the actual concentrations of C and N. The third factor, containing 9.7% of the variation, appeared to express seasonal temperature shifts and was also negatively correlated with arylsulfatase activity ($r = -.381$). This could indicate that a lack of moisture may be responsible for decreased enzymatic activity. The fourth factor expressed 8.1% of the variation, was insignificantly correlated with arylsulfatase activity ($r = 0.128$), and did not reflect any particular cluster of soil parameters.

Thorton and McLaren (9) have ascertained that many soil enzymes maintain a constant, minimum level of activity in soil over a long period of time with no addition of substrate. The significant correlation of cation exchange capacity with arylsulfatase activity and the inclusion of % clay + silt in the regression equation could mean that these parameters affect the basal level of arylsulfatase activity in a particular soil.

Of the environmental parameters examined, soil organic matter, Ca, Mg, Na, cation exchange capacity, and % moisture were most highly correlated with arylsulfatase activity over time. Even though these r-values were not very high, it becomes difficult when dealing with a system containing so many variables to set limits as to what degree of association is significant. All of the statistical analyses indicated that nutrient availability in the soil was an important factor associated with arylsulfatase activity. Additional research is needed to determine if enzymatic activities in soil can be related to specific environmental parameters and whether or not any such associations are meaningful. This will be essential if soil enzymes are ever to be used as an expression of the metabolic capabilities of any particular group of organisms within the soil biomass.

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Figure 3.1: Variations in arylsulfatase activity over fourteen sampling periods, 1977-78.

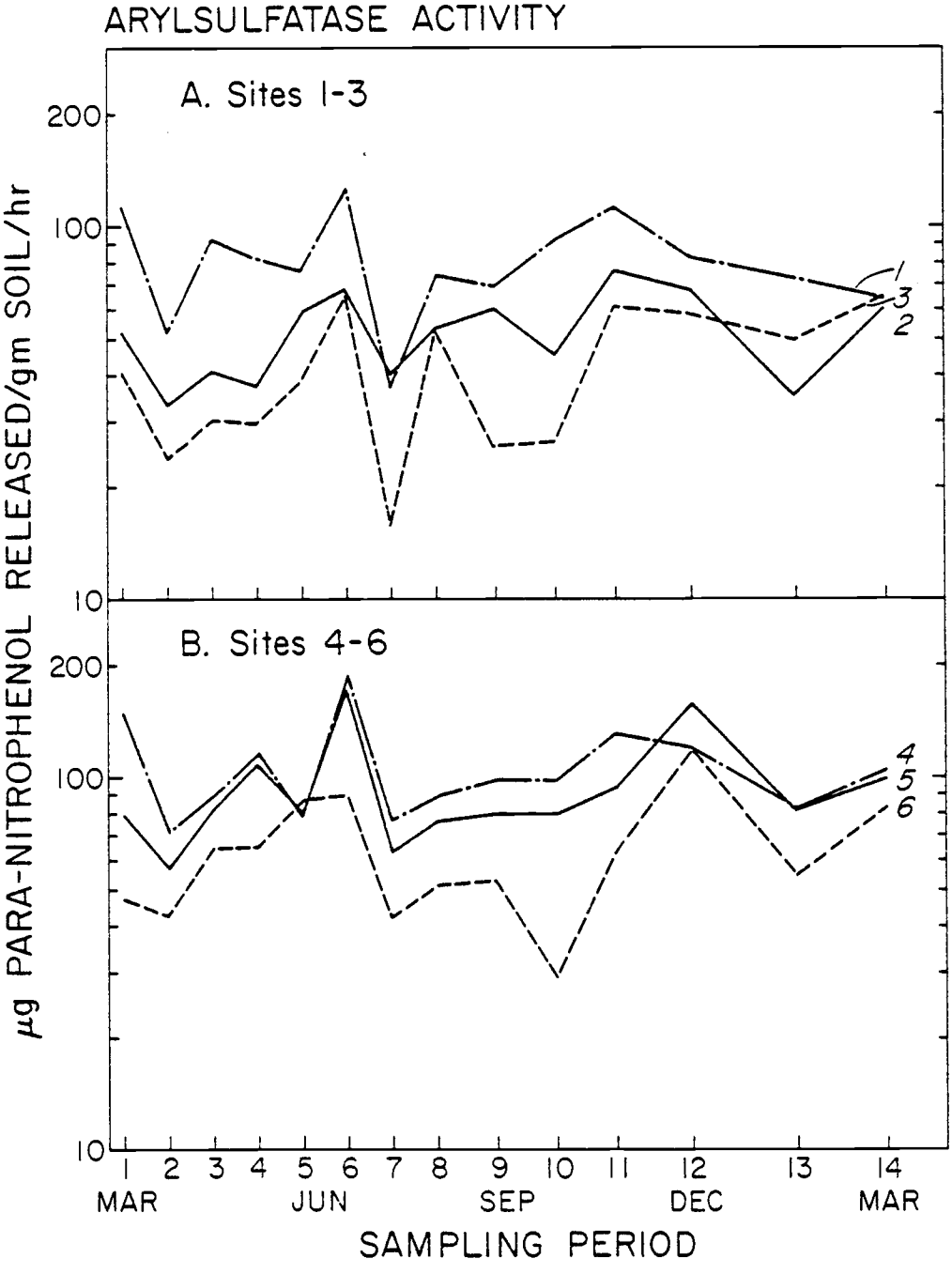
ARYLSULFATASE ACTIVITY
A. Sites 1-3
B. Sites 4-6
200
100
10
1
2
3
4
5
6
μg PARA-NITROPHENOL RELEASED/gm SOIL/hr
1 2 3 4 5 6 7 8 9 10 11 12 13 14
MAR
JUN
SEP
DEC
MAR
SAMPLING PERIOD

Table 3.1: Characteristics of experimental sites

Area	Site	Soil Series	Relief (%)	Texture (%)			Porosity (%)	Cation exchange capacity*
				Clay	Silt	Sand		
A	1	Hazelair	12	25.29	53.13	21.58	46.72	20.61
	2	Hazelair/ Steiwer Transition	19	29.87	52.93	17.20	45.62	26.39
	3	Steiwer	9	29.10	52.57	18.33	49.72	26.69
B	4	Dixonville	8	44.30	42.55	13.15	50.62	50.64
	5	Dixonville	10	40.75	47.00	12.25	45.91	47.97
	6	Dixonville	14	44.15	46.38	9.47	53.56	53.34

* CEC in meq. /100 gms soil

Table 3.2: Correlations between soil arylsulfatase activity and environmental parameters over time.*

Environmental parameters	Correlation coefficients (r-value)
Soil organic matter (SOM)‡	0.58
Calcium (Ca)	0.50
Cation exchange capacity (CEC)	0.47
Sodium (Na)	0.45
% Moisture relative to field capacity (FC)	0.43
Magnesium (Mg)	0.40
Carbon:Nitrogen (CN)	0.37
Organic nitrogen (ORGN)	0.32
% Clay + silt (CPS)	0.26
Soil conductivity (COND)	0.23
Nitrate-N (NO_3)	-0.23
pH	0.19
Phosphorus (P)	-0.19
Potassium (K)	-0.17
Organic-N:Sulfate-S (NS)	0.14
Sulfate sulfur (SO_4)	-0.14
Total exchangeable hydrogen (TEH)	0.12
Ammonia-N (NH_3)	-0.11
Soil temperature (STEMP)	-0.11
Porosity (POR)	0

* Based on periodic sampling over a one year period.

‡ Abbreviations of soil characteristics used in computer programs.

Table 3.3: Best fit equation and analysis of variance for soil characteristics and arylsulfatase activity.*

Best fit equation

$$\text{Arylsulfatase activity} = 594.28 + 0.053\,(\text{COND})^{\ddagger} - 2.18\,(NH_3) - 375.37\,(\text{ORGN}) + 41.77\,(\text{SOM}) - 6.64\,(\text{CN}) + 0.016\,(\text{TEH}) + 6.19\,(\text{Mg}) + 78.26\,(\text{Na}) - 0.728\,(\text{P}) - 6.79\,(\text{CPS})$$

Analysis of variance

Source	Degrees of freedom	Sum of Squares	Mean square
Total	71	7.69×10^4	1.08×10^3
Regression	10	5.06×10^4	5.06×10^3
Residual	61	2.64×10^4	4.32×10^2

$r^2 = 0.657$

* The "best" set of independent variables that expressed arylsulfatase activity was determined by finding the combination of variables with the lowest mean square residual.

‡ Explanations of abbreviations of soil properties are listed in Table 2.

Table 3.4: Factors retained from the principal components analysis of the environmental parameters.

Factor loadings $\geq |0.30|$ shown

Test	Factors				Communiality‡
	1	2	3	4	
STEMP			.697*		.662
FC	.496		-.718*		.846
COND		.651*		.511	.695
pH	.597*	.413	.374	-.427	.849
NO_3				-.693*	.616
NH_3	-.531	.620*			.712
ORGN		.552*	-.548		.651
SOM		.541*	-.510		.680
SO_4	-.624*				.489
CN	.675*				.483
NS	.373				.199
TEH					.144
K		.628*			.629
Ca	.944*				.921
Mg	.930*				.883
Na	.758*				.662
P		.680*			.562
CEC	.904*				.886
CPS	.915*				.908
POR	.393	-.368		.471*	.523
r-value (arylsulfatase vs. factor)	.456	.206	-.381	.128	

* Highest factor loading for a given test.

‡ Communiality = the proportion of the variation retained in each test by the first four factors

4. INTERACTIONS BETWEEN UREASE ACTIVITY AND SELECTED SOIL ENVIRONMENTAL PARAMETERS

4.1 Introduction

Urease is one of the most thoroughly studied extracellular soil enzymes due to the agricultural importance of its substrate, urea, although little research has been performed to determine the interactions between urease activity and environmental parameters in the soil. Urease activity has been shown to be significantly correlated with organic carbon (5, 10, 14), but the degree of correlation appears to vary for different soils. Total nitrogen and cation exchange capacity have also been found to be positively correlated with urease activity (14) while there are conflicting reports on whether or not urease activity is correlated with soil texture (5, 14). Pancholy and Rice (6) examined urease levels in nine surface soils over one year and found that the enzymatic activity did not correlate with organic matter content or pH and concluded that perhaps the type of vegetation governed the level of activity. A seasonal variation in urease activity was described, and this included increases during the spring and summer months and decreased activity during the fall and winter. In the work reported here, the variations in urease activity

over time were analyzed in relation to a wide selection of environmental parameters in western Oregon soils.

4.2 Material and Methods

Site Descriptions

The six sites used in this study are located in Benton County, Oregon, in the foothills of the Coastal Mountains. Area A (sites 1-3) was a toposequence with a southwest aspect (Table 4.1). The soils were poorly to moderately well drained with a heavy clay layer in the B-horizon, well formed profiles, and abundant mottles. The area is predominately under pasture and is grazed by sheep in the fall and spring. Site 1 (Hazelair series) is on the toeslope and site 3 (Steiwer series) is on the summit; both are under grass-clover pasture. Site 2 (Hazelair/Steiwer intergrade) is on the backslope with an old growth oak canopy and a grass-clover understory. Area B (sites 4-6) is a biosequence with an eastern aspect located on the Dixonville soil series. This soil is well drained with fractured saprolite in the C-horizon, and contained a highly developed profile with few mottles. Site 4 was on the toeslope, adjacent to a stream with a mixed fir and hardwood forest, and a substory of native grasses and small shrubs. Site 5 was on the footslope with native grasses and small shrubs while site 6 was on the backslope with a

20 year old Douglas/Silver fir forest and a substory of native grasses (Table 3.1).

Sampling Procedure and Soil Analyses

At each site, a nine square meter plot was marked out and each plot was then divided into nine 3.0 m^2 subplots. At every sampling period, two composite samples were taken from the upper 10 cm of the A1 horizon of each site. Composite samples consisted of soil cores taken from four randomly chosen subplots so that eight of the nine subplots were sampled each period. The enzymes and certain soil characteristics were determined at each of the fourteen sampling periods over one year, 1977-78. Some soil characteristics were measured six times (periods 4, 8, 10, 12, 13, 14), while other, relatively constant soil characteristics (such as those in Table 4.1), were measured only once.

Established procedures were used for all analyses, and each was performed in duplicate. Ammonia-N (1, 7), nitrate-N (2, 8), soil temperature, % moisture, soil pH (using a 2:1 water to soil solution), soil conductivity (12), and total exchangeable hydrogen (12) were determined at every sampling interval. Assays for Kjeldahl-N, exchangeable K, Ca, Na, Mg, sulfate-S, organic matter content, and available phosphorus were determined for six sampling periods, while cation exchange capacity, texture analysis

(% clay, silt, and sand), and degree of porosity were measured once. Those assays performed once or for six sampling periods were done either by using the facilities of the Oregon State University Soil Testing Laboratory (4). The % gravimetric moisture was expressed relative to field capacity, degree of soil saturation, and air-dried soil. Organic-N was calculated as Kjeldahl-N minus NH_3-N, and for all but one observation, organic-N was equal to Kjeldahl-N within two significant figures. Total-N was assumed to be equal to the sum of the concentrations of organic-N, NH_3-N, and NO_3-N. Carbon content was estimated as equal to 58.0% of the total soil organic matter content.

Urease activity was determined with an assay developed by Tabatabai and Bremner (9). It consists of adding tris(hydroxymethyl) aminomethane buffer, a solution of urea (substrate), and toluene (to halt metabolic activity) to duplicate samples and a control (without substrate added), which are then incubated at 37°C for a two hour period. After incubation, a KCl-$AgSO_4$ solution is added to the soil solution in order to stop the urease activity. The ammonia released by the urease was measured by using an ammonium electrode. After subtracting the amount of ammonia in the control, urease activity is reported as μg ammonia released/gm soil/hour.

Statistical Analyses

Forward selection and backward elimination multiple regression analyses were performed using the Statistical Interactive Programing System (SIPS) on the Oregon State University CDC-3300 computer. All other analyses were carried out on the OSU Cyber Model 73 computer. The correlation and factor analysis programs were based on those developed by Cooley and Lohnes (3). The correlation coefficients were determined using all available data; the multiple regression and the multivariate analyses included principally the data obtained from the six sampling periods for which complete soil analyses were made (72 observations).

4.3 Results and Discussion

The variations in urease activity over time followed similar trends in each of the soils examined (Figure 4.1). The alterations in the activities did not appear to be seasonally related; contrary to the observations of Pancholy and Rice (6) who found that urease activity fluctuated seasonally, increasing during the spring and summer months. The failure of urease activity to generally increase during the summer months may have been due to the drought conditions that were prevalent at the time, although

sampling over a single year may be insufficient to determine any seasonal patterns.

Of the environmental parameters measured, soil organic matter content had the highest correlation with urease activity ($r = 0.58$, Table 4.2). Multiple regression analysis showed that the environmental parameters accounted for 53.0% of the variation in urease activity; however, eight of the variables alone accounted for 50.9% of the observed variability (Table 4.3). For the soil parameters with the eight highest r-values, three were excluded from the best fit regression equation (cation exchange capacity, Ca, and % clay + silt). This may have been due to their high correlations with other variables included in the regression equation, such as the C:N ratio ($r = 0.59, 0.56, 0.54$, respectively), Mg ($r = 0.85, 0.93, 0.93$, respectively) and pH ($r = 0.58, 0.57, 0.58$, respectively). The differences in cation exchange capacity and soil texture for each site, as well as the variation in the Ca concentration in the soils over time, were expressed in the regression equation by a combination of other parameters. Soil temperature, phosphorous, and pH were included in the regression equation despite their insignificant correlations with urease activity. Five of the parameters in the equation were related to the nutritional status of the soil and included soil organic matter content, Mg, P, organic-N, and C:N ratio.

Two of the equation parameters were related to seasonal aspects (soil temperature and % moisture relative to field capacity).

A principal components analysis was performed on the environmental data to aid in determining how these parameters were interrelated over time (Table 4. 4). Originally twenty factors were generated, although only four were retained for detailed examination. The first factor accounted for 31. 9% of the variability seen in the environmental data, and appeared to represent soil fertility since Ca, Mg, Na, and the C:N ratio were among the parameters with high factor loadings. Two physical parameters, cation exchange capacity and soil texture, were also heavily expressed. Factor 2 accounted for 15. 2 % of the environmental variability and contained high factor loadings for the parameters ammonia-N, organic-N, soil organic matter, K, and P, therefore apparently also contained a second set of variables related to soil fertility. The degree of correlation between urease activity and the second factor ($r = 0.305$) was about the same as between urease activity and the first factor ($r = 0.374$). The third factor expressed 9. 7% of the variation and was negatively correlated with urease activity ($r = -0.304$). This factor had a positive factor loading for soil temperature and a negative loading value for percent moisture, indicating that urease activity tended to decrease when moisture was limited. The fourth

factor expressed 8.1% of the variation, was uncorrelated with urease activity, and did not reflect any particular group of soil parameters.

Our study further confirmed that the lack of correlation found between pH and urease activity (6, 14). Zantua et. al. (14) found significant correlations with urease for clay (r = 0.53) and sand (r = -0.47). McGarity and Myers (5) found no correlation between enzymatic activity and soil texture in a study of urease activity in Australian soils during the winter and summer months. Our results also demonstrated that the correlation between soil texture and urease activity over time was insignificant for those sites which we monitored. Urease activity has been shown to persist in soil without the addition of substrate for long periods of time (13), and the minimum level of enzymatic activity established in each soil appears to vary (11). This may indicate that, while soil texture has no obvious role in explaining the variations in urease activity for a given soil, it may be important as a determinant of basal level of enzymatic activity in the soil.

McGarity and Myers (5) found a positive correlation for urease activity with soil organic matter content (r = 0.502), while Zantua et. al. (14), in their survey of 21 Iowa soils, found a high correlation for urease activity with organic-C (r = 0.72) and total-N (r = 0.71). Both of these variables, however, are indices of soil organic matter levels. Tabatabai (10), in 14 Iowa surface soils, also found a

significant association with organic-C ($r = 0.74$). The correlation of soil organic matter with urease activity in our study ($r = 0.58$) closely agreed with that determined by McGarity and Myers (5), perhaps because both sutides were conducted for a relatively long period of time rather than over a wide range of soils sampled only a few times (10, 14). This could indicate that soil organic matter content plays a more important role in establishing the basal level of urease activity in a given soil (along with textural considerations) than in being responsible for the fluctuations or urease activity in a soil over time. Nonetheless, soil organic matter content and the availability of other nutrients (such as Ca, Mg, and P) appeared to be important factors associated with fluctuations of urease activity.

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Figure 4.1: Variations in urease activity over fourteen sampling periods, 1977-78.

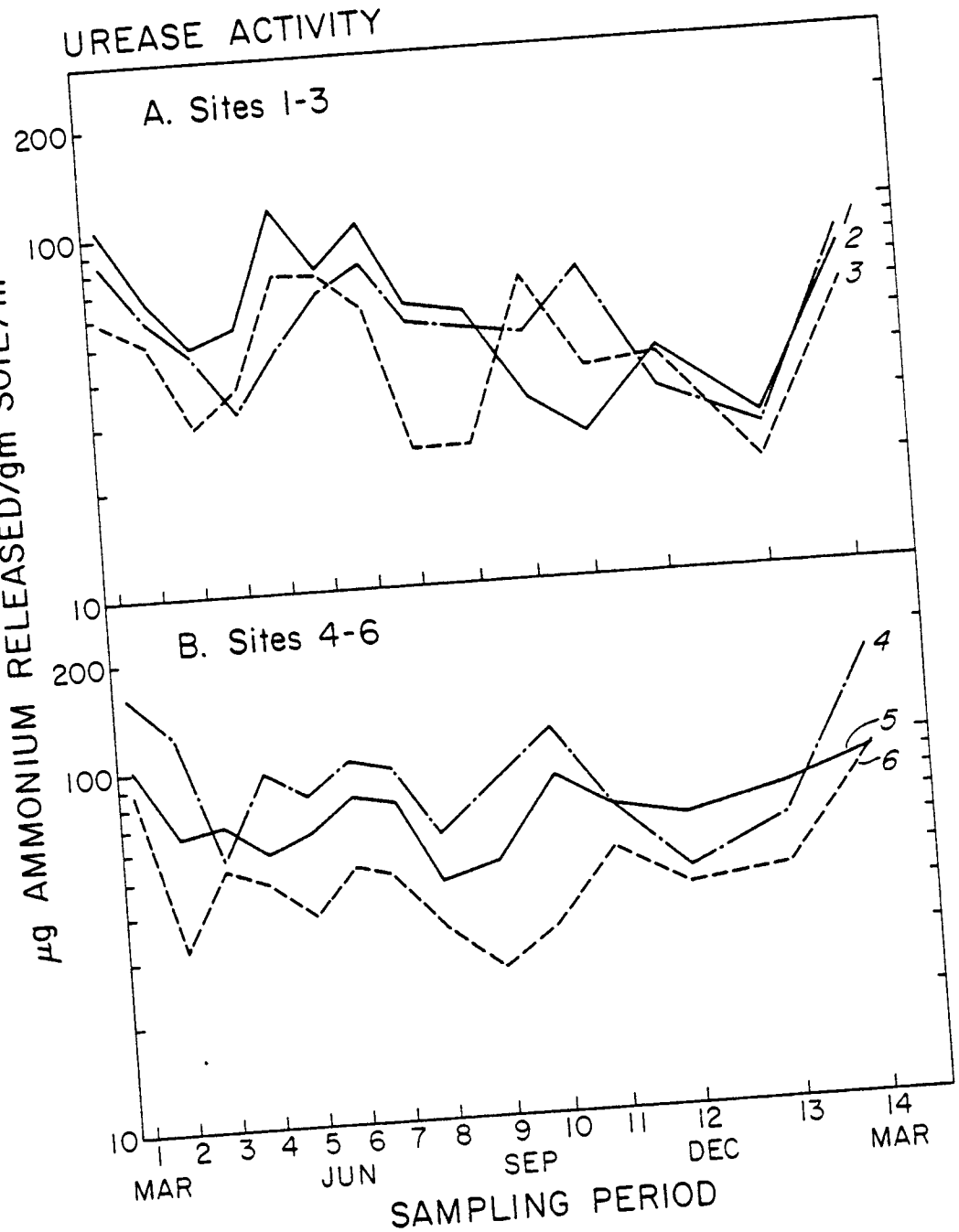
UREASE ACTIVITY
A. Sites 1-3
200
100
10
1
2
3
B. Sites 4-6
200
100
10
4
5
6
μg AMMONIUM RELEASED/gm SOIL/hr
1 2 3 4 5 6 7 8 9 10 11 12 13 14
MAR
JUN
SEP
DEC
MAR
SAMPLING PERIOD

Table 4.1: Characteristics of experimental sites.

Area	Site	Soil Series	Relief (%)	Texture (%)			Porosity (%)	Cation exchange capacity*
				Clay	Silt	Sand		
A	1	Hazelair	12	25.29	53.13	21.58	46.72	20.61
	2	Hazelair/ Steiwer Transition	19	29.87	52.93	17.20	45.62	26.39
	3	Steiwer	9	29.10	52.57	18.33	49.72	26.69
B	4	Dixonville	8	44.30	42.55	13.15	50.62	50.64
	5	Dixonville	10	40.75	47.00	12.25	45.91	47.97
	6	Dixonville	14	44.15	46.38	9.47	53.46	53.34

* CEC in meq. /100 gms soil

Table 4.2: Correlations between soil urease activity and environmental parameters over time.*

Environmental parameters	Correlation coefficients (r-value)
Soil organic matter (SOM)‡	0.58
Calcium (Ca)	0.41
Carbon to nitrogen ratio (CN)	0.40
Cation exchange capacity (CEC)	0.38
% Moisture relative to field capacity (FC)	0.37
Magnesium (Mg)	0.32
% Clay + silt (CPS)	0.28
Organic nitrogen (ORGN)	0.26
Sodium (Na)	0.25
Organic-N to sulfate-S ratio (NS)	0.24
Sulfate sulfur (SO_4)	-0.18
pH	0.15
Phosphorus (P)	0.14
Soil temperature (STEMP)	-0.13
Soil conductivity (COND)	0.13
Potassium (K)	0.11
Nitrate-N (NO_3)	-0.08
Porosity (POR)	0.06
Ammonia-N (NH_3)	0.04
Total exchangeable hydrogen (TEH)	0.00

* Based on periodic sampling over a one year interval.

‡Abbreviations of soil characteristics used in computer programs.

Table 4.3: Best fit equation and analysis of variance for urease activity derived from multiple regression analysis.*

Best fit equation

$$\text{Urease activity} = 191.59 + 3.52\,(\text{STEMP})^{\ddagger} + 0.877\,(\text{FC}) - 23.41\,(\text{pH}) - 781.61\,(\text{ORGN}) + 54.02\,(\text{SOM}) - 9.50\,(\text{CN}) + 1.49\,(\text{Mg}) + 1.09\,(\text{P})$$

Analysis of variance

Source	Degrees of freedom	Sum of Squares	Mean Square
Total	71	7.42×10^4	1.04×10^3
Regression	8	3.78×10^4	4.73×10^3
Residual	63	3.64×10^4	5.78×10^2

$r^2 = 0.509$

* Best set of independent variables that express urease activity was determined by finding the combination of variables with the lowest mean square residual.

‡ Explanations of abbreviations of soil properties are listed in Table 4.2.

Table 4.4: Factors retained from the principal components analysis of the environmental parameters.

Factor loadings $\geq |0.30|$ shown

Test	Factors				Communiality‡
	1	2	3	4	
STEMP			.697*		.662
FC	.496		-.718*		.846
COND		.651*		.511	.695
pH	.597*	.413	.374	-.427	.849
NO_3				-.693*	.616
NH_3	-.531	.620*			.712
ORGN		.552*	-.548		.651
SOM		.541*	-.510		.680
SO_4	-.624*				.489
CN	.675*				.483
NS	.373				.199
TEH					.144
K		.628*			.629
Ca	.944*				.921
Mg	.930*				.883
Na	.758*				.662
P		.680*			.562
CEC	.904*				.886
CPS	.915*				.908
POR	.393	-.368		.471*	.523
r-value (urease vs. factor)	.374	.305	-.304	-.009	

* Highest factor loading for a given test.

‡Communiality = the proportion of the variation retained in each test by the first four factors.

5. ANALYSIS OF VARIATIONS IN DEHYDROGENASE ACTIVITY AS RELATED TO ENVIRONMENTAL PARAMETERS IN SOIL

5.1 Introduction

Dehydrogenase activity is thought to be an index of the total aerobic biological activity in the soil system and has been one of the most frequently studied soil enzymes. Stevenson (10) obtained significant correlations between oxygen uptake and dehydrogenase activity while Skujins (8) reported that dehydrogenase levels were highly correlated with soil respiration rates as well as proteolysis and nitrification. To date only one paper has discussed the relation between dehydrogenase activity and the soil environment over time (7). Pancholy and Rice examined nine surface soils through a one year period and found that dehydrogenase activity did not correlate with either organic matter content or pH and concluded that perhaps the type of vegetation determined the levels of activity. Also, they observed no seasonal fluctuations in enzymatic activity. In the work reported here, the variations in dehydrogenase activity over time were analyzed in relation to environmental parameters in six field locations in western Oregon.

5.2 Material and Methods

Site Descriptions

The six sites used in this study are located in Benton County, Oregon, in the foothills of the Coastal Mountains. Area A (sites 1-3) was a toposequence with a southwest aspect (Table 5.1). The soils were poorly to moderately well drained with a heavy clay layer in the B-horizon, well formed profiles, and abundant mottles. The area is predominately under pasture and is grazed by sheep in the fall and spring. Stie 1 (Hazelair series) is on the toeslope and site 3 (Steiwer series) is on the summit; both are under grass-clover pasture. Site 2 (Hazelair/Steiwer intergrade) is on the backslope with an old growth oak canopy and a grass-clover understory. Area B (sites 4-6) is a biosequence with an eastern aspect located on the Dixonville soil series. This soil is well drained with fractured saprolite in the C-horizon, and contains a highly developed profile with few mottles. Site 4 was on the toeslope, adjacent to a stream with a mixed fir and hardwood forest, and a substory of native grasses and small shrubs. Site 5 was on the footslope with native grasses and small shrubs, while site 6 was on the backslope with a 20 year old Douglas/Silver fir forest and a substory of native grasses (Table 5.1).

Sampling Procedure and Soil Analyses

At each site, a nine square meter plot was marked out and each plot was then divided into nine 3.0 m^2 subplots. At every sampling period, two composite samples were taken from the upper 10 cm of the A1 horizon of each site. Composite samples consisted of soil cores taken from four randomly chosen subplots so that eight of the nine subplots were sampled each period. The enzymes and certain soil characteristics were determined at each of the fourteen sampling periods over one year. Some soil characteristics were measured six times (periods 4, 8, 10, 12, 13, 14), while other, relatively constant soil characteristics (such as those in Table 5.1), were measured only once.

Established procedures were used for all analyses, and each was performed in duplicate. Ammonia-N (1, 5), nitrate-N (2, 6), soil temperature, % moisture, soil pH (using a 2:1 water to soil solution), soil electrical conductivity, and total exchangeable hydrogen (10) were determined at every sampling interval. Assays for Kjeldahl-N, exchangeable K, Ca, Na, Mg, sulfate-S, organic matter content, and available phosphorus were determined for six sampling periods, while cation exchange capacity, texture analysis (% clay, silt, and sand), and degree of porosity were measured once. Those assays performed once or for six sampling periods

were done either by or using the facilities of the Oregon State University Soil Testing Laboratory (4). The % gravimetric moisture was expressed relative to field capacity, degree of soil saturation, and air-dried soil. Organic-N was calculated as Kjeldahl-N minus NH_3-N, and for all but one observation, organic-N was equal to Kjeldahl-N within two significant figures. Total-N was assumed to be equal to the sum of the concentrations of organic-N, NH_3-N, and NO_3-N. Carbon content was estimated as equal to 58.0% of the total soil organic matter content.

Dehydrogenase activity was measured using the procedure of Casida et. al. (3). This consisted of adding 2, 3, 5-triphenyltetrazolium chloride (TTC), $CaCO_3$, and a solution of alanine (substrate) followed by incubation for 24 hours at 37°C. A control sample (without TTC) is assayed at the same time. The dehydrogenase enzymes reduce TTC to triphenyl formazan, and with the addition of methanol, a stable red color is produced. The concentration of the triphenyl formazan is determined colorimetrically and dehydrogenase activity is reported as μg formazan released/gm soil/24 hour period.

Statistical Analyses

Forward selection and backward elimination multiple regression analyses were performed using the Statistical Interactive

Programming System (SIPS) on the Oregon State University CDC-3300 computer. All other analyses were carried out on the OSU Cyber Model 73 computer. The correlation and factor analysis programs were based on those developed by Cooley and Lohnes (3). The correlation coefficients were determined using all available data; the multiple regression and the multivariate analyses included principally the data obtained from the six sampling periods for which complete soil analyses were made (72 observations).

5.3 Results and Discussion

The fluctuations in dehydrogenase activity over the sampling year at each site were markedly similar although the changes did not appear to be seasonally related (Figure 5.1). Some of the lowest levels occurred during the summer months, when drought conditions were prevalent in Oregon.

Of the environmental parameters measured, the concentration of available sodium was most highly correlated with dehydrogenase activity ($r = 0.52$, Table 5.2). Five of the seven parameters most highly correlated with dehydrogenase activity were nutrient/ion variables (Na, Ca, K, Mg, and P), and two of these were negatively correlated (P and K). Soil organic nitrogen content, pH and cation exchange capacity were also among the variables that contained a

correlation coefficient greater than 0.25. Multiple regression analysis of the data showed that 73.8% of the observed variability could be accounted for by sixteen of the twenty parameters (eliminating soil pH, % clay + silt, N:S, and C:N ratio; Table 5.3). Among the sixteen soil variables retained by the best fit equation, ten parameters were related to nutrient aspects of the soil (Na, Ca, P, K, Mg, soil organic matter, organic-N, NH_3-N, NO_3-N, and SO_4-S), two were seasonally-related parameters (soil temperature and moisture), two were relatively constant physical parameters of the soil (cation exchange capacity and porosity), and two variables were associated with the ionic equilibrium in soil (conductivity and total exchangeable hydrogen).

A principal components analysis of the environmental data was performed to aid interpreting the relationships between the parameters. Twenty factors were originally generated and four factors, representing 65.1% of the observed variability in the soil parameters, were retained for further examination (Table 5.4). Factor 1 accounted for 31.9% of the variability, and appeared to represent certain aspects of soil fertility since among the parameters which had high factor loadings were Ca, Mg, Na, and the C:N ratio. Cation exchange capacity and soil texture (% clay + silt) were also significantly expressed in the first factor, and this may be due to

the effects that these two parameters would have on the availability of cations to microorganisms which might lead to increased metabolic activity and enzyme production. The correlation coefficient for dehydrogenase activity vs. factor 1 was r = 0.337. The second factor accounted for 15.3% of the observed variation in the soil parameters, was insignificantly correlated with dehydrogenase activity (r = 0.101), and also expressed aspects of soil fertility, specifically soil organic matter content, organic-N, NH_3-N, K, and P. This suggested that perhaps the C:N ratio was more critical to the level of dehydrogenase activity than the actual amounts of C and N. Factor 3, expressing temperature and moisture, was not correlated with dehydrogenase activity (r = -0.124), as was the fourth factor (r = 0.036). Factor 3 accounted for 9.7% of the observed variation in the environmental parameters, factor 4 accounted for 8.1% and neither reflected any particular group of variables.

Pancholy and Rice (7) observed that dehydrogenase activity did not fluctuate seasonally and was not correlated over time with either soil organic matter content or pH. They reported a lack of seasonal fluctuations in activity, and this was confirmed by our study; and, in addition, we found only low correlations between dehydrogenase activity and soil organic matter content (r = 0.28) and pH (r = 0.27). While the C:N ratio was included in the factor most highly correlated with dehydrogenase activity (Table 5.4), it was excluded from the

best fit multiple regression equation (Table 5.3). Although the C:N ratio had a low correlation with dehydrogenase activity in our study ($r = 0.14$), it was significantly correlated with many of the soil parameters included in the equation, such as soil organic matter content ($r = 0.54$), Ca ($r = 0.56$), Mg ($r = 0.53$), and cation exchange capacity ($r = 0.59$). Therefore, any variability seen in the C:N ratio over time was probably expressed by combinations of other soil parameters.

No single soil characteristic was highly correlated with dehydrogenase activity (Table 5.2). This was, perhaps, an indication of the complexity of the soil system, and the difficulty, when dealing with an environment containing so many variables, in setting limits for what should be considered a significant association. Since it has been suggested that dehydrogenase levels are a reflection of the respiratory activity of the soil biomass (8, 9, 10), the failure to find high correlations with soil properties might be overcome if it was possible to examine the enzymatic levels produced by selected groups of soil organisms. Dehydrogenase activity could be too non-specific an indicator and would not represent changes in the various populations comprising the soil biomass. If such were the case it would be difficult to find significant correlations between enzymatic levels and soil parameters. All of the statistical

analyses indicated that the availability of nutrients in the soil was an important factor associated with dehydrogenase activity.

5.4 Literature Cited

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Figure 5.1: Fluctuations of dehydrogenase activity over fourteen sampling periods, 1977-78.

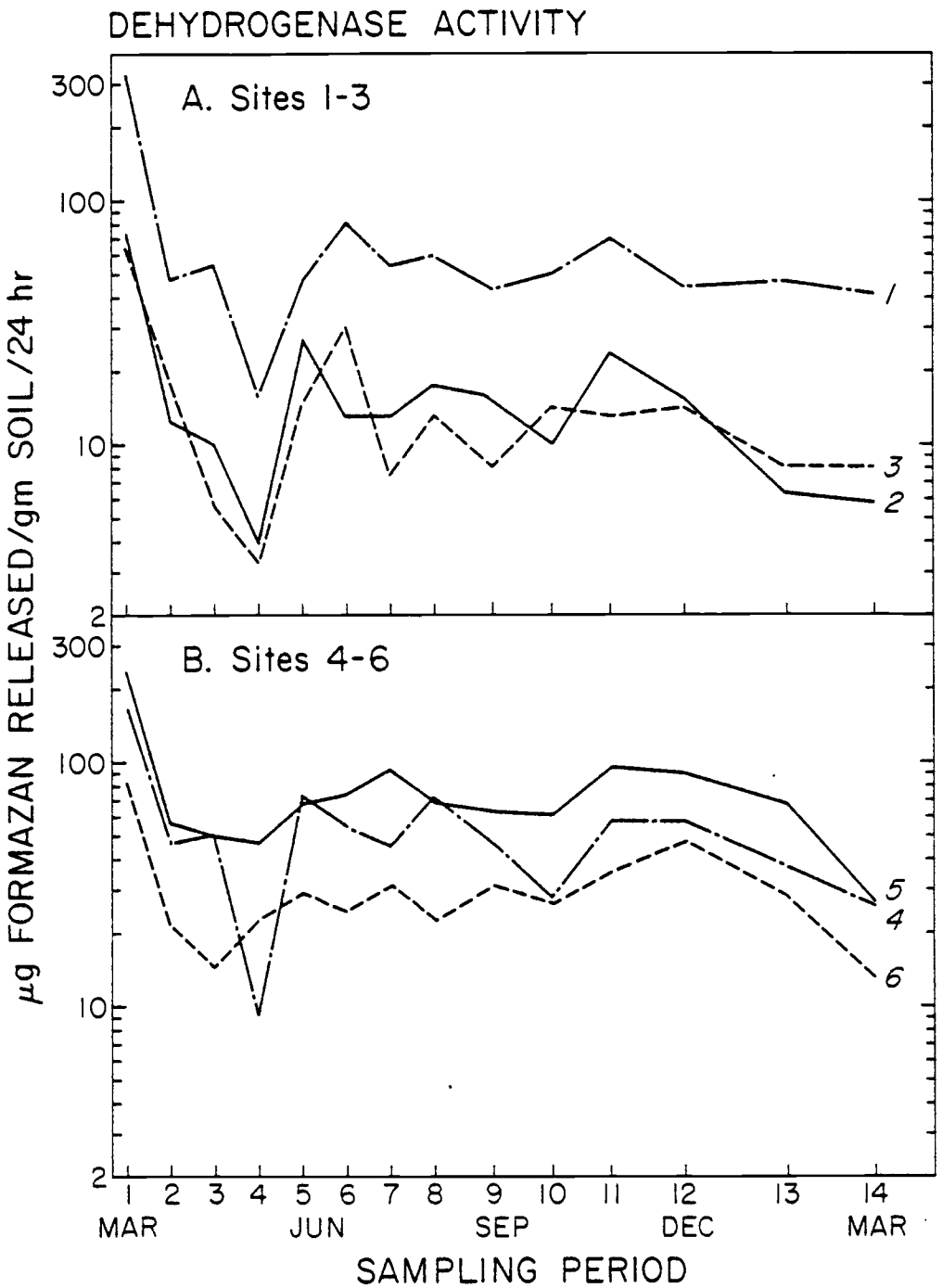
DEHYDROGENASE ACTIVITY
A. Sites 1-3
B. Sites 4-6
300
100
10
2
1
2
3
4
5
6
μg FORMAZAN RELEASED/gm SOIL/24 hr
1 2 3 4 5 6 7 8 9 10 11 12 13 14
MAR
JUN
SEP
DEC
MAR
SAMPLING PERIOD

Table 5.1: Characteristics of experimental sites.

Area	Site	Soil Series	Relief (%)	Texture (%)			Porosity (%)	Cation exchange capacity*
				Clay	Silt	Sand		
A	1	Hazelair	12	25.29	53.13	21.58	46.72	20.61
	2	Hazelair/ Steiwer Transition	19	29.87	52.93	17.20	45.62	26.39
	3	Steiwer	9	29.10	52.57	18.33	49.72	26.69
B	4	Dixonville	8	44.30	42.55	13.15	50.62	50.64
	5	Dixonville	10	40.75	47.00	12.25	45.91	47.97
	6	Dixonville	14	44.15	46.38	9.47	53.46	53.34

* CEC in meq. /100 gms soil

Table 5.2: Correlation coefficients between soil dehydrogenase activity and environmental parameters.*

Environmental parameters	Correlation coefficients (r-value)
Sodium (Na)‡	0.52
Cation exchange capacity (CEC)	0.41
Calcium (Ca)	0.39
Phosphorus (P)	-0.36
Potassium (K)	-0.30
Magnesium (Mg)	0.28
Soil organic matter (SOM)	0.28
pH	0.27
Organic nitrogen (ORGN)	0.26
Porosity (POR)	-0.21
% Clay + silt (CPS)	0.19
% Moisture relative to field capacity (FC)	0.18
Ratio of organic-N to sulfate-S (NS)	0.15
Ratio of carbon to nitrogen (CN)	0.14
Soil conductivity (COND)	0.14
Ammonia nitrogen (NH_3)	-0.13
Nitrate nitrogen (NO_3)	-0.12
Total exchangeable hydrogen (TEH)	0.12
Soil temperature (STEMP)	0.09
Sulfate sulfur (SO_4)	-0.06

*Based on periodic samplings over a one year interval.

‡ Abbreviations of soil characteristics used in computer programs.

Table 5.3: Best fit equation and analysis of variance for soil characteristics and dehydrogenase activity. *

Best fit equation

$$\text{Dehydrogenase activity} = 175.83 - 1.86\,(\text{STEMP})^{\ddagger} - 0.488\,(\text{FC}) + 0.031\,(\text{COND}) + 0.301\,(NO_3) + 2.12\,(NH_3) + 169.81\,(\text{ORGN}) - 5.71\,(\text{SOM}) - 0.723\,(SO_4) + 0.016\,(\text{TEH}) - 18.15\,(\text{K}) + 1.36\,(\text{Ca}) - 2.49\,(\text{Mg}) + 160.61\,(\text{Na}) - 0.849\,(\text{P}) + 0.285\,(\text{CEC}) - 3.20\,(\text{POR})$$

Analysis of variance

Source	Degrees of freedom	Sum of squares	Mean square
Total	71	3.97×10^4	5.58×10^2
Regression	16	2.90×10^4	1.81×10^3
Residual	55	1.07×10^4	1.94×10^2

$r^2 = 0.731$

* The "best" set of independent variables that express dehydrogenase activity was determined by finding the combination of variables with the lowest mean square residual.

‡ Explanations of abbreviations of soil properties are listed in Table 5.2.

Table 5. 4: Factors retained from the principal components analysis of the environmental parameters.

Factor loadings $\geq |0.30|$ shown

Test	Factors				Communiality‡
	1	2	3	4	
STEMP			.697*		.662
FC	.496		-.718*		.846
COND		.651*		.511	.695
pH	.597*	.413	.374	-.427	.849
NO_3				-.693*	.616
NH_3	-.531	.620*			.712
ORGN		.552*	-.548		.651
SOM		.541*	-.510		.680
SO_4	-.624*				.489
CN	.675*				.483
NS	.373				.199
TEH					.144
K		.628*			.629
Ca	.944*				.921
Mg	.930*				.883
Na	.758*				.662
P		.680*			.562
CEC	.904*				.886
CPS	.915*				.908
POR	.393	-.368		.471*	.523
r-value (dehydrogenase vs. factor)	.337	.101	-.124	.036	

* Highest factor loading for a given test.

‡ Communiality = the proportion of the variation retained in each test by the first four factors.

APPENDIX A

Data Tables

Table A1.

Sample Period	Date Sample Taken
1	March 18, 1977
2	April 7, 1977
3	April 28, 1977
4	May 19, 1977 *
5	June 9, 1977
6	June 30, 1977
7	July 21, 1977
8	August 11, 1977 *
9	September 7, 1977
10	October 6, 1977 *
11	November 1, 1977
12	December 6, 1977 *
13	January 24, 1978 *
14	March 17, 1978 *

*All determinations made.

Table A2. Subplots that comprise the composite samples for Site 1.

Sampling Period	Composite A*	Composite B*
1	7 4 6 1	8 3 5 2
2	1 6 3 2	8 4 5 9
3	2 6 1 9	3 4 8 5
4	1 7 4 9	8 6 2 3
5	9 1 7 6	5 4 8 3
6	4 1 8 6	7 5 2 3
7	5 1 6 7	3 8 4 2
8	8 7 6 3	4 1 5 2
9	4 9 2 5	1 8 6 3
10	1 7 6 5	3 8 2 4
11	2 7 6 5	9 8 4 3
12	9 6 5 4	3 2 7 1
13	6 7 9 1	3 2 4 8
14	8 6 3 1	2 7 9 5

*Soil temperature was determined for the first subplot listed for each composite.

Table A3. Subplots that comprise the composite samples for Site 2.*

Sampling Period	Composite A	Composite B
1	3 2 9 4	7 8 1 5
2	8 1 6 7	4 9 2 3
3	5 1 9 2	8 3 4 7
4	5 8 9 3	2 1 7 6
5	3 7 9 6	8 1 4 2
6	9 4 5 7	2 3 1 8
7	7 3 8 1	2 6 9 4
8	4 3 8 6	2 7 5 1
9	5 6 1 2	8 7 3 4
10	7 4 9 5	8 2 1 6
11	9 1 4 3	5 8 7 2
12	3 1 8 9	6 5 7 2
13	4 7 1 3	6 5 2 9
14	7 1 3 5	8 9 6 4

*Soil temperature was determined for the first subplot listed for each composite.

Table A4. Subplots that comprise the composite samples for Site 3.*

Sampling Period	Composite A	Composite B
1	8 4 9 1	3 2 5 7
2	2 8 3 6	9 7 5 4
3	7 9 5 6	2 8 3 4
4	4 9 7 1	3 6 2 8
5	2 4 9 6	3 7 5 1
6	8 6 7 2	3 6 4 1
7	9 6 7 5	8 3 4 1
8	4 6 9 5	1 8 7 3
9	4 6 7 1	5 2 3 9
10	4 9 2 6	7 8 5 1
11	3 5 9 6	2 7 8 4
12	2 4 9 7	5 1 3 6
13	6 5 2 9	8 1 7 4
14	7 5 8 4	2 9 3 6

*Soil temperature was determined for the first subplot listed for each composite.

Table A5. Subplots that comprise the composite samples for Site 4.*

Sampling Period	Composite A	Composite B
1	2 4 3 7	1 8 9 6
2	3 5 9 7	8 6 4 2
3	7 6 5 1	3 4 8 9
4	9 8 5 1	4 2 7 3
5	5 7 8 9	3 6 4 1
6	7 3 1 2	9 8 4 6
7	1 9 6 5	2 7 3 4
8	7 2 8 1	4 6 3 5
9	2 7 1 9	5 3 6 4
10	7 1 2 3	5 4 8 9
11	2 4 6 5	7 9 1 8
12	7 8 2 6	4 1 3 5
13	4 9 2 7	8 5 1 6
14	1 4 6 8	9 2 5 3

*Soil temperature was determined for the first subplot listed for each composite.

Table A6. Subplots that comprise the composite samples for Site 5.*

Sampling Period	Composite A	Composite B
1	7 3 6 4	1 9 2 5
2	2 7 5 9	1 8 3 4
3	4 5 8 9	2 7 3 1
4	6 3 7 2	5 8 9 1
5	4 8 3 2	7 6 5 9
6	5 9 1 8	3 4 2 6
7	8 7 2 3	9 5 1 4
8	5 6 1 3	9 4 2 8
9	1 5 4 3	2 6 9 8
10	7 1 6 8	4 5 2 9
11	5 9 2 8	3 7 1 4
12	2 7 1 9	5 3 6 4
13	5 7 3 6	4 1 2 8
14	4 7 1 5	3 9 6 8

*Soil temperature was determined for the first subplot listed for each composite.

Table A7. Subplots that comprise the composite samples for Site 6.*

Sampling Periods	Composite A	Composite B
1	8 4 6 3	9 2 7 5
2	4 3 8 7	9 2 6 5
3	7 9 4 1	3 2 6 8
4	8 4 1 6	5 9 7 2
5	4 1 3 8	2 7 6 5
6	3 4 8 2	6 7 9 5
7	9 6 8 4	5 2 7 3
8	9 2 4 6	1 3 5 7
9	8 2 6 9	3 1 5 7
10	5 2 6 1	8 7 4 9
11	9 5 7 1	8 6 2 4
12	7 1 2 6	5 9 3 4
13	9 7 5 4	1 6 2 8
14	1 4 6 5	3 8 7 9

*Soil temperature was determined for the first subplot listed for each composite.

Table A8. Arylsulfatase.*

Site	Composite	Sampling Period													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	A	109.99	67.31	81.33	75.69	84.89	130.64	51.70	95.53	64.47	92.50	135.10	61.55	81.71	74.00
	B	115.31	37.51	103.32	86.32	66.45	119.99	23.34	53.32	75.10	90.40	93.20	103.20	62.82	54.66
2	A	56.79	33.98	35.40	36.09	83.46	72.49	30.43	65.75	N.D.	44.50	87.20	40.20	33.44	58.63
	B	48.48	33.26	46.03	38.23	35.25	63.60	50.28	40.91	60.21	46.10	63.70	95.00	37.29	60.95
3	A	32.18	22.98	33.27	30.42	42.33	65.39	23.41	77.09	16.32	26.50	61.30	55.00	47.09	61.07
	B	49.19	25.10	26.88	29.00	35.23	N.D.	8.16	28.31	35.11	26.80	61.30	62.00	51.64	68.08
4	A	131.27	75.81	79.36	143.74	19.63	197.66	79.36	77.45	108.45	116.00	145.60	94.40	78.57	104.09
	B	98.66	71.92	99.22	120.35	135.96	182.78	74.40	101.57	88.58	80.40	117.90	146.90	87.29	103.73
5	A	73.47	56.67	69.44	109.72	79.94	95.55	62.33	84.88	72.98	73.40	98.80	118.80	86.26	99.88
	B	86.22	58.08	94.97	100.00	79.92	138.44	63.76	67.17	85.07	86.20	88.30	199.30	77.16	99.53
6	A	44.77	47.80	62.34	56.52	45.17	82.78	51.71	67.17	53.12	24.90	80.00	94.40	61.76	81.35
	B	49.74	37.52	66.60	73.54	128.16	96.59	33.27	36.67	53.13	33.80	46.40	143.40	48.13	80.20

*Reported as µg nitrophenol/gm soil/hour of incubation.
N.D. = Not Determined.

Table A9. Urease.*

Site	Composite	Sampling Period													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	A	85.0	57.9	45.2	18.1	59.0	65.8	78.9	52.0	N.D.	49.7	81.4	41.8	17.0	76.8
	B	85.0	58.8	47.5	45.2	36.1	66.4	77.7	54.5	N.D.	46.3	58.8	22.1	32.5	88.1
2	A	108.5	68.7	41.1	40.7	125.0	77.3	106.7	71.9	45.0	36.2	20.3	39.8	38.4	74.6
	B	103.1	64.2	56.0	67.8	106.5	77.1	93.8	47.9	67.8	27.0	29.4	42.3	14.5	72.1
3	A	55.6	42.9	26.4	33.9	30.8	82.3	69.6	20.6	31.0	38.0	40.7	37.7	6.1	52.0
	B	61.0	57.0	31.6	40.7	120.6	65.3	49.9	27.1	17.4	99.4	37.3	42.3	33.2	66.4
4	A	176.3	129.3	55.4	72.3	121.4	86.6	109.4	59.9	66.0	117.5	63.3	58.5	65.5	157.3
	B	148.3	123.8	57.6	119.8	163.7	114.8	80.9	63.1	102.8	114.1	73.5	31.4	58.8	184.0
5	A	110.3	67.8	64.9	61.0	54.6	71.4	74.1	48.4	62.8	106.2	76.8	49.3	91.5	83.6
	B	97.6	62.4	73.9	54.2	75.7	89.5	79.1	42.0	39.8	64.4	62.2	78.0	57.4	98.5
6	A	108.9	60.1	47.7	36.2	31.7	52.9	53.6	46.1	27.3	26.0	53.1	30.7	54.7	87.2
	B	65.1	49.3	57.2	61.0	44.9	50.6	43.2	21.2	23.5	41.8	54.0	50.4	34.8	98.3

*Reported as µg ammonia released/gram soil/1 hour incubation.
N.D. = Not Determined.

Table A10. Dehydrogenase.*

Site	Composite	Sampling Period													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	A	311.67	56.33	39.42	10.58	47.83	75.00	42.42	63.50	37.08	41.92	80.33	47.17	61.17	43.00
	B	335.83	38.83	68.33	21.42	46.67	86.67	66.58	56.33	50.25	58.08	59.92	37.00	26.25	38.33
2	A	80.00	10.58	9.42	4.58	23.83	14.00	13.25	14.83	10.42	11.83	21.42	13.00	5.83	5.83
	B	65.00	13.58	10.67	3.33	29.83	12.17	13.00	20.83	21.58	8.17	27.50	17.33	7.00	5.83
3	A	65.00	16.58	4.27	3.33	13.00	30.33	7.92	11.25	7.42	14.17	14.75	12.50	8.17	9.42
	B	62.08	19.00	7.00	3.33	16.58	30.83	7.33	15.92	9.25	14.83	12.42	15.83	8.17	7.00
4	A	200.42	37.08	41.83	9.42	66.00	65.33	34.33	73.75	41.92	34.67	59.33	39.50	43.08	18.42
	B	125.00	56.25	58.67	9.42	80.33	45.67	56.92	67.75	50.25	21.42	56.33	74.33	32.33	22.67
5	A	245.42	56.33	46.75	44.33	70.75	70.00	109.25	67.75	74.42	67.08	99.67	77.50	51.50	25.00
	B	223.75	56.33	53.83	49.17	66.00	81.00	79.17	70.75	52.75	55.08	90.00	102.00	82.75	27.50
6	A	95.00	22.58	14.17	22.67	28.67	27.83	39.50	27.50	33.42	21.42	46.67	58.67	29.83	9.42
	B	70.83	21.42	15.33	23.83	29.83	21.83	22.42	17.83	29.92	32.33	25.08	37.00	26.25	16.58

*Reported as µg formazan released/gram soil/24 hours of incubation.

Table A11. Exchangeable potassium.*

Site	Composite	Sampling Period					
		4	8	10	12	13	14
1	A	0.37	0.59	0.67	0.27	0.35	0.36
	B	0.39	0.61	0.35	0.36	0.39	0.30
2	A	2.15	2.26	1.39	1.63	1.71	2.15
	B	1.60	1.95	1.53	1.69	1.71	1.95
3	A	0.43	0.65	0.65	0.46	0.64	0.43
	B	0.50	0.71	0.64	0.43	0.42	0.50
4	A	0.79	1.23	1.20	1.01	0.95	1.03
	B	0.99	1.20	0.99	0.93	1.00	0.99
5	A	0.55	0.94	0.88	0.55	0.62	0.64
	B	0.50	0.59	0.58	0.62	0.55	0.64
6	A	0.83	0.83	0.65	0.82	0.83	0.81
	B	0.73	0.64	0.90	0.78	0.64	0.87

*Reported in milliequivalents/100 grams soil.

Table A12. Exchangeable calcium.*

Site	Composite	Sampling Period					
		4	8	10	12	13	14
1	A	8.1	7.2	8.3	7.5	7.5	6.9
	B	7.9	7.7	7.7	7.1	6.9	6.9
2	A	14.5	15.1	14.1	13.5	12.4	12.5
	B	13.7	13.9	13.7	13.7	12.7	13.5
3	A	11.4	12.0	11.2	11.4	11.4	11.0
	B	11.8	11.4	10.6	12.2	10.8	12.0
4	A	30.0	27.0	35.0	35.0	37.0	30.0
	B	35.0	29.0	30.0	35.0	35.0	32.0
5	A	34.0	25.0	32.0	37.0	34.0	29.0
	B	32.0	24.0	32.0	35.0	35.0	29.0
6	A	32.0	25.0	30.0	35.0	35.0	27.0
	B	32.0	23.0	32.0	34.0	30.0	29.0

*Reported as milliequivalents/100 grams of soil.

Table A13. Exchangeable magnesium.*

Site	Composite	Sampling Period					
		4	8	10	12	13	14
1	A	3.3	3.4	4.1	3.8	3.3	3.4
	B	3.4	3.8	3.6	3.8	3.1	3.2
2	A	7.0	6.9	7.2	7.4	6.0	6.5
	B	6.7	6.7	7.4	6.3	7.0	7.1
3	A	5.3	6.1	6.6	6.6	5.7	5.3
	B	5.5	5.8	5.8	6.7	5.2	6.4
4	A	12.0	9.2	13.0	14.0	12.0	12.0
	B	13.0	9.2	13.0	14.0	11.0	11.0
5	A	15.0	9.9	16.0	16.0	13.0	13.0
	B	15.0	9.2	15.0	16.0	13.0	14.0
6	A	17.0	9.9	17.0	18.0	16.0	15.0
	B	17.0	9.9	17.0	18.0	14.0	17.0

*Reported as milliequivalents/100 grams of soil.

Table A14. Exchangeable sodium.*

Site	Composite	Sampling Period					
		4	8	10	12	13	14
1	A	0.19	0.17	0.23	0.19	0.17	0.13
	B	0.19	0.17	0.21	0.21	0.15	0.13
2	A	0.26	0.23	0.23	0.26	0.13	0.10
	B	0.17	0.21	0.26	0.21	0.13	0.13
3	A	0.13	0.13	0.19	0.15	0.10	0.07
	B	0.13	0.13	0.17	0.13	0.09	0.07
4	A	0.30	0.30	0.43	0.30	0.28	0.21
	B	0.33	0.33	0.33	0.35	0.28	0.21
5	A	0.30	0.26	0.50	0.35	0.26	0.21
	B	0.30	0.28	0.37	0.30	0.26	0.21
6	A	0.30	0.23	0.35	0.33	0.23	0.21
	B	0.28	0.23	0.28	0.30	0.23	0.21

*Reported as milliequivalents/100 grams of soil.

Table A15. Available phosphorus (ppm).

Site	Composite	Sampling Period					
		4	8	10	12	13	14
1	A	4.8	9.0	8.0	7.0	11.0	9.0
	B	4.5	17.0	8.0	7.0	11.0	7.0
2	A	22.2	40.0	32.0	17.0	50.0	23.0
	B	20.0	38.0	23.0	23.0	24.0	40.0
3	A	17.1	19.0	10.0	10.0	22.0	15.0
	B	10.4	13.0	10.0	14.0	19.0	16.0
4	A	10.2	20.0	18.0	27.0	22.0	21.0
	B	38.2	20.0	18.0	14.0	18.0	19.0
5	A	2.1	9.0	11.0	8.0	15.0	13.0
	B	0	8.0	7.0	7.0	14.0	10.0
6	A	3.1	6.0	5.0	8.0	14.0	11.0
	B	7.0	5.0	7.0	7.0	14.0	10.0

Table A16. Sulfate Sulfur (ppm).

Site	Composite	Sampling Period					
		4	8	10	12	13	14
1	A	10.32	14.51	21.50	6.64	10.52	8.78
	B	10.85	11.90	11.37	11.54	10.32	8.68
2	A	7.48	26.78	7.66	3.55	5.98	1.42
	B	10.22	27.71	8.51	2.07	2.93	5.98
3	A	4.70	7.76	6.38	10.55	6.81	6.64
	B	5.20	1.28	10.35	10.62	5.29	1.48
4	A	4.54	3.22	3.85	3.75	6.94	2.67
	B	5.26	4.87	5.13	4.57	5.43	3.00
5	A	6.51	1.68	2.24	3.55	5.23	1.52
	B	2.63	1.28	0.73	4.37	7.43	2.27
6	A	2.63	3.68	5.29	3.85	7.46	2.99
	B	2.34	4.80	1.94	5.06	7.96	3.16

Table A17. % Total Carbon*.

Site	Composite	Sampling Period 4	8	10	12	13	14
1	A	4.42	4.58	3.79	3.31	4.26	3.47
	B	4.26	3.94	3.79	3.63	3.63	3.63
2	A	3.31	3.79	4.10	3.31	3.94	3.63
	B	3.31	3.63	3.31	3.47	3.63	3.63
3	A	2.84	3.94	2.84	2.37	3.63	3.31
	B	3.31	3.00	2.84	2.68	3.63	3.31
4	A	5.21	4.42	6.78	3.63	5.36	6.78
	B	5.21	5.68	3.31	5.05	4.73	6.78
5	A	5.21	4.26	4.58	3.94	5.05	4.10
	B	3.94	3.79	3.79	4.73	4.10	4.26
6	A	3.31	3.94	2.52	2.68	3.47	4.26
	B	3.47	3.63	2.68	3.16	3.47	3.79

*Determined as % organic matter x 0.58.

Table A18. % Organic Nitrogen*.

Site	Composite	Sampling Period 4	8	10	12	13	14
1	A	0.31	0.31	0.28	0.26	0.33	0.30
	B	0.28	0.31	0.28	0.28	0.27	0.29
2	A	0.25	0.31	0.27	0.24	0.29	0.24
	B	0.23	0.29	0.27	0.25	0.25	0.26
3	A	0.25	0.28	0.23	0.21	0.28	0.25
	B	0.23	0.24	0.23	0.24	0.26	0.26
4	A	0.24	0.33	0.38	0.31	0.32	0.33
	B	0.29	0.36	0.24	0.33	0.30	0.38
5	A	0.29	0.30	0.17	0.27	0.29	0.28
	B	0.22	0.27	0.23	0.29	0.27	0.30
6	A	0.15	0.22	0.29	0.18	0.20	0.22
	B	0.18	0.18	0.20	0.20	0.22	0.22

*Organic nitrogen = total nitrogen - ammonium nitrogen.
Total nitrogen was determined by the Kjeldahl method.

Table A19. Ratio of total carbon to total nitrogen*.

Site	Composite	Sampling Period					
		4	8	10	12	13	14
1	A	14.56	14.72	13.38	12.71	12.89	11.54
	B	15.19	12.68	13.42	12.94	13.42	12.50
2	A	13.22	12.17	15.07	13.76	13.55	15.07
	B	14.37	12.47	12.43	13.85	14.47	13.87
3	A	11.35	14.04	12.18	11.25	12.95	13.22
	B	14.37	12.48	12.13	11.15	13.94	12.69
4	A	21.68	13.38	17.82	11.70	16.74	20.52
	B	17.95	15.77	13.77	15.29	15.75	17.81
5	A	17.95	14.18	26.88	14.58	17.40	14.63
	B	17.89	14.02	16.33	16.30	15.70	14.18
6	A	22.03	17.89	8.68	14.87	17.33	19.35
	B	19.24	20.13	13.38	15.78	15.76	17.21

*Total nitrogen = organic nitrogen + ammonium + nitrate.

Table A20. Ratio of organic nitrogen to sulfate sulfur.

Site	Composite	Sampling Period					
		4	8	10	12	13	14
1	A	300.05	213.02	130.13	391.54	313.35	341.28
	B	257.85	259.96	246.05	242.29	261.31	333.68
2	A	334.89	115.34	352.03	675.30	484.23	1687.68
	B	224.95	104.33	304.52	1206.33	852.06	433.23
3	A	531.70	360.41	360.34	198.96	410.94	376.27
	B	442.00	1872.89	222.12	225.88	448.85	1753.58
4	A	528.22	1024.25	986.64	826.40	460.95	1234.63
	B	551.14	738.92	467.49	721.88	552.30	1265.00
5	A	445.32	1785.13	758.19	760.25	554.30	1840.99
	B	835.97	2108.60	3147.26	663.39	363.26	1320.26
6	A	569.97	597.56	548.00	467.28	267.96	735.45
	B	768.40	374.79	1029.95	395.06	276.26	695.89

Table A21. Air Temperature (^{o}C).

Site	Sampling Period													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	9.0	20.0	21.0	17.5	17.0	25.0	28.0	34.0	24.0	11.0	14.0	11.5	4.0	12.0
2	8.0	19.0	18.0	17.5	16.0	21.0	21.0	26.0	19.0	10.0	13.0	12.0	4.0	11.0
3	8.0	20.0	20.0	16.5	19.0	26.0	29.0	30.0	23.0	12.0	13.0	11.5	4.0	12.0
4	7.0	21.0	17.0	17.0	20.0	24.0	27.0	27.0	18.0	11.0	13.0	10.0	2.5	11.0
5	7.0	20.0	18.0	24.0	21.0	28.0	34.0	30.0	27.0	10.0	13.0	10.5	3.5	11.0
6	6.0	11.0	19.0	17.0	21.0	23.0	24.0	29.0	19.5	10.0	12.0	10.0	3.0	10.0

Table A22. Soil Temperature (^{o}C).

Site	Composite	Sampling Period													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	A	7.5	14.0	14.0	15.0	20.0	23.0	25.0	25.0	19.0	13.0	12.0	9.0	1.0	10.5
	B	8.0	15.0	15.0	16.0	19.0	21.0	24.0	23.0	19.0	13.0	12.0	9.0	1.0	10.5
2	A	7.0	13.0	13.0	12.0	15.0	19.0	19.0	25.0	19.0	13.0	11.0	9.0	5.0	10.0
	B	7.5	13.0	14.0	13.0	17.0	20.0	19.0	23.0	18.0	12.0	11.0	9.5	6.0	9.5
3	A	8.5	15.0	14.0	15.0	18.0	23.0	24.0	26.0	22.0	12.0	11.0	9.0	6.0	10.0
	B	8.0	16.0	14.0	15.0	18.0	23.0	23.0	26.0	20.0	12.0	11.0	9.0	5.0	10.0
4	A	6.0	13.0	13.0	11.5	14.0	15.0	15.0	17.0	15.0	11.0	10.5	9.0	5.5	9.0
	B	7.0	11.0	14.0	11.0	15.0	14.0	16.0	18.0	15.0	11.0	11.0	9.0	5.5	8.5
5	A	8.5	13.0	16.0	15.0	19.0	23.0	27.0	25.0	20.0	12.0	11.0	9.0	5.0	10.0
	B	7.0	14.0	16.0	18.0	17.0	19.0	27.0	24.0	18.0	13.0	11.5	9.0	6.0	9.5
6	A	6.5	9.5	14.0	12.0	13.0	16.0	16.0	19.0	15.0	11.0	10.5	8.5	5.0	9.0
	B	5.5	10.0	11.0	11.0	13.0	17.0	17.0	20.0	15.0	11.0	10.5	8.5	5.0	8.0

Table A23. % Moisture (absolute).

Site	Composite	Sampling Period													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	A	29.0	27.0	21.0	24.0	21.0	7.0	12.0	4.0	16.0	25.0	31.0	28.0	38.0	33.0
	B	26.0	27.0	21.0	23.0	19.0	11.0	7.0	4.0	20.0	24.0	29.0	31.0	36.0	33.0
2	A	26.0	25.0	14.0	20.0	18.0	9.0	1.0	3.0	14.0	23.0	30.0	26.0	29.0	28.0
	B	24.0	25.0	15.0	19.0	14.0	8.0	5.0	3.0	15.0	24.0	27.0	27.0	27.0	28.0
3	A	21.0	20.0	15.0	12.0	15.0	6.0	3.0	2.0	12.0	17.0	23.0	21.0	25.0	25.0
	B	22.0	6.0	11.0	16.0	13.0	6.0	1.0	4.0	15.0	20.0	22.0	25.0	23.0	27.0
4	A	33.0	40.0	18.0	27.0	26.0	24.0	14.0	11.0	21.0	33.0	32.0	32.0	33.0	32.0
	B	29.0	28.0	22.0	23.0	30.0	23.0	15.0	16.0	21.0	26.0	25.0	32.0	33.0	35.0
5	A	27.0	23.0	18.0	28.0	23.0	13.0	15.0	9.0	26.0	31.0	28.0	31.0	32.0	30.0
	B	29.0	27.6	19.0	27.0	22.0	17.0	17.0	9.0	23.0	31.0	28.0	33.0	33.0	30.0
6	A	28.0	28.0	20.0	25.0	24.0	18.0	12.0	12.0	21.0	23.0	25.0	28.0	30.0	30.0
	B	15.0	29.0	19.0	24.0	25.0	19.0	14.0	11.0	22.0	26.0	24.0	30.0	30.0	30.0

Table A24. % Moisture relative to field capacity.

Site	Composite	Sampling Period													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	A	81.7	76.1	59.2	67.6	59.2	19.7	33.8	11.3	45.1	70.4	87.3	78.9	107.0	93.0
	B	73.2	76.1	59.2	64.8	53.5	31.0	19.7	11.3	56.3	67.6	81.7	87.3	101.4	93.0
2	A	75.4	72.5	40.6	58.0	52.2	26.1	2.9	8.7	40.6	66.7	87.0	75.4	84.1	81.2
	B	69.6	72.5	43.5	55.1	40.6	23.2	14.5	8.7	43.5	69.6	78.3	78.3	78.3	81.2
3	A	56.3	53.6	40.2	32.2	40.2	16.1	8.0	5.4	32.2	45.6	61.7	56.3	67.0	67.0
	B	59.0	16.1	29.5	42.9	34.9	16.1	2.7	10.7	40.2	53.6	59.0	67.0	61.7	72.4
4	A	102.2	123.8	55.7	83.6	80.5	74.3	43.3	34.1	65.0	102.2	99.1	99.1	102.2	99.1
	B	89.8	86.7	68.1	71.2	92.9	71.2	46.4	49.5	65.0	80.5	77.4	99.1	102.2	108.4
5	A	84.4	71.9	56.3	87.5	71.9	40.6	46.9	28.1	81.3	96.9	87.5	96.9	100.0	93.8
	B	90.6	86.3	59.4	84.4	68.8	53.1	53.1	28.1	71.9	96.9	87.5	103.1	103.1	93.8
6	A	82.4	82.4	58.8	73.5	70.6	52.9	35.3	35.3	61.8	67.6	73.5	82.4	88.2	88.2
	B	44.1	85.3	55.9	70.6	73.5	55.9	41.2	32.4	64.7	76.5	70.6	88.2	88.2	88.2

Table A25. pH.

Site	Composite	Sampling Period												
		1	2	3	4	5	6	7	8	10	11	12	13	14
1	A	5.06	5.38	5.31	5.26	5.22	5.09	5.07	5.20	5.20	5.25	5.43	5.15	4.78
	B	5.15	5.43	5.20	5.26	5.15	5.24	5.12	5.23	5.46	5.28	5.40	5.12	4.95
2	A	5.97	6.09	5.87	6.07	6.22	5.73	5.86	5.94	6.12	5.97	5.99	5.84	5.67
	B	5.99	6.05	5.07	5.93	6.11	5.79	5.86	5.80	6.02	5.93	6.01	5.85	5.78
3	A	5.24	5.49	5.31	5.33	5.37	5.31	5.25	5.33	5.43	5.32	5.45	5.15	4.91
	B	5.27	5.40	5.40	5.35	5.39	5.11	5.24	5.34	5.41	5.23	5.43	5.12	4.93
4	A	6.03	5.84	5.85	5.70	5.81	6.13	5.62	5.89	5.80	5.81	5.70	5.62	5.58
	B	5.60	5.84	5.80	5.70	5.93	5.66	5.96	5.80	5.65	5.66	5.51	5.74	5.60
5	A	6.29	6.23	6.22	6.32	6.07	6.14	5.99	6.22	6.40	6.24	6.01	5.87	6.10
	B	6.39	6.08	6.07	6.29	6.95	6.00	6.11	6.13	6.29	6.28	6.05	6.13	5.90
6	A	6.05	5.60	5.79	5.85	5.60	5.90	5.62	6.03	5.51	5.98	5.84	5.50	5.57
	B	5.92	5.86	5.84	5.83	6.07	6.05	5.37	5.50	5.90	5.61	5.66	5.54	5.43

Table A26. Conductivity (μmhos).

Site	Composite	Sampling Period												
		1	2	3	4	5	6	7	8	10	11	12	13	14
1	A	450.0	360.0	270.0	360.0	239.0	392.4	639.0	630.0	72.0	81.0	459.0	486.0	324.0
	B	486.0	306.0	360.0	540.0	327.0	356.4	477.0	648.0	91.8	95.4	423.0	396.0	297.0
2	A	792.0	288.0	414.0	342.0	720.0	680.4	918.0	972.0	60.3	43.2	396.0	630.0	468.0
	B	342.0	360.0	360.0	342.0	450.0	648.0	792.0	1044.0	57.6	70.2	405.0	306.0	792.0
3	A	306.0	234.0	144.0	180.0	174.6	313.2	396.0	378.0	147.6	167.4	320.4	288.0	243.0
	B	288.0	288.0	171.0	234.0	270.0	291.6	360.0	396.0	149.4	122.4	378.0	234.0	360.0
4	A	630.0	324.0	378.0	288.0	388.4	390.6	549.0	468.0	70.2	46.8	606.6	594.0	612.0
	B	288.0	270.0	396.0	414.0	390.6	531.0	369.0	450.0	64.8	50.4	561.6	414.0	540.0
5	A	486.0	360.0	450.0	225.0	189.0	379.8	311.4	396.0	120.6	63.9	561.6	594.0	630.0
	B	378.0	288.0	360.0	306.0	359.2	376.2	414.0	504.0	86.4	63.0	612.0	522.0	594.0
6	A	342.0	324.0	486.0	396.0	306.0	354.6	459.0	432.0	93.6	57.6	414.0	450.0	702.0
	B	252.0	306.0	450.0	306.0	288.0	291.6	378.0	360.0	93.6	50.4	486.0	396.0	504.0

Table A27. Total exchangeable hydrogen*.

Site	Composite	Sampling Period												
		1	2	3	4	5	6	7	8	10	11	12	13	14
1	A	8.83	8.58	9.97	7.83	6.80	12.57	13.93	15.62	15.90	13.06	11.98	13.21	12.78
	B	8.41	8.93	10.21	8.55	5.62	12.65	14.31	15.62	15.22	12.23	12.46	13.05	12.73
2	A	5.46	4.95	7.60	4.53	3.45	9.00	10.44	11.28	11.45	10.09	8.79	10.20	9.18
	B	5.34	5.52	6.46	4.86	3.49	9.18	10.72	10.91	11.70	9.22	8.98	9.01	8.92
3	A	7.07	7.00	9.06	6.41	5.12	11.01	13.32	14.76	13.75	10.31	10.08	11.31	11.23
	B	6.99	7.32	7.73	6.85	5.12	11.25	13.15	12.85	13.64	11.56	9.88	11.31	11.81
4	A	9.37	9.42	10.69	11.12	7.50	13.03	18.05	15.29	19.36	16.48	13.66	15.47	15.41
	B	9.37	11.04	11.07	10.73	7.37	15.88	15.26	15.51	19.72	18.08	14.41	14.30	14.95
5	A	6.31	7.75	8.31	7.27	6.61	11.88	13.94	14.43	13.13	15.00	11.29	12.55	10.46
	B	6.48	8.36	10.00	7.20	4.94	12.97	12.42	13.49	14.15	12.39	10.77	10.93	11.91
6	A	8.01	10.35	11.78	8.74	7.67	13.04	16.10	13.77	18.17	15.20	10.79	14.49	13.67
	B	8.01	9.60	11.24	9.01	5.46	11.57	17.20	17.84	15.75	14.38	13.08	14.92	14.78

*Reported as milliequivalents of exchangeable hydrogen/100 grams of soil.

Table A28. Nitrate nitrogen (ppm).

Site	Composite	Sampling Period												
		1	2	3	4	5	6	7	8	10	11	12	13	14
1	A	2.50	4.50	4.62	1.94	2.50	1.52	1.96	1.91	30.4	1.51	1.98	2.45	2.85
	B	2.55	3.70	4.15	1.96	2.80	1.48	1.36	1.96	21.9	1.70	2.03	2.05	1.07
2	A	3.50	9.40	4.20	1.79	2.70	90.0	23.0	2.00	17.4	3.80	3.66	3.55	4.90
	B	1.76	10.00	4.50	1.79	2.70	1.58	1.43	2.80	55.4	8.50	1.67	5.35	7.60
3	A	2.25	4.45	3.81	1.66	2.40	1.64	2.80	3.40	30.4	9.40	5.15	2.65	2.15
	B	2.53	4.29	3.39	1.55	1.99	1.77	1.95	1.57	40.0	5.00	2.07	1.98	3.40
4	A	2.30	2.35	2.82	1.42	2.10	1.26	1.58	1.23	1.91	1.36	1.53	1.65	0.83
	B	1.25	3.10	2.72	1.52	1.92	1.94	2.45	1.30	1.90	1.13	1.41	1.63	0.89
5	A	3.25	2.10	2.96	1.26	1.20	1.18	1.81	3.10	2.50	1.61	1.38	1.73	1.04
	B	3.10	5.40	3.14	1.16	1.06	1.22	1.96	1.43	18.20	1.74	1.22	1.46	1.14
6	A	1.41	2.98	3.54	1.34	1.04	1.22	2.30	1.42	2.13	1.48	1.39	1.50	1.01
	B	2.02	2.90	2.80	1.15	1.23	1.34	2.60	2.60	1.66	1.51	1.24	1.42	1.10

Table A29. Ammonium nitrogen (ppm).

Site	Composite	Sampling Periods*										
		3	4	5	6	7	8	10	11	12	13	14
1	A	4.20	3.70	5.95	1.61	1.40	9.10	2.10	5.10	1.50	3.55	3.60
	B	3.20	2.40	3.90	1.11	1.20	6.50	2.40	7.60	3.95	3.25	3.65
2	A	4.10	1.80	9.10	3.65	3.40	11.20	3.45	6.30	2.70	4.30	3.50
	B	10.0	<1.0+	2.85	1.34	2.10	9.10	1.40	5.00	2.90	3.45	9.30
3	A	1.45	<1.0	2.45	3.20	1.45	3.20	1.05	1.75	<1.00	1.50	1.60
	B	3.10	1.60	2.90	1.48	1.20	2.70	1.05	<1.00	1.20	1.15	4.70
4	A	3.25	2.00	2.30	<1.0	2.00	1.90	1.83	75.0	<1.00	<1.00	3.55
	B	4.20	<1.0	3.20	4.63	1.45	1.45	1.80	65.5	<1.00	<1.00	5.00
5	A	9.10	<1.0	2.25	<1.0	<1.0	<1.0	1.65	76.0	1.10	<1.00	1.70
	B	2.05	1.10	1.55	1.30	<1.0	<1.0	2.50	80.0	<1.00	<1.00	2.20
6	A	1.30	<1.0	1.70	1.14	1.05	<1.0	1.10	<1.00	<1.00	<1.00	<1.00
	B	1.15	1.90	1.60	1.00	2.50	<1.0	1.90	<1.00	<1.00	<1.00	<1.00

*No readings for periods 1, 2 and 9.

+Readings of less than 1.0 ppm were entered into the computer as .99 ppm.

APPENDIX B

Statistical Tables

Table B1: Environmental parameters and enzyme activities assayed.

Soil temperature (STEMP)

% Moisture relative to field capacity (FC)

Electrical conductivity of the soil (COND)

pH

Nitrate nitrogen (NO_3)

Ammonia nitrogen (NH_3)

Organic nitrogen (ORGN)

Soil organic matter (SOM)

Sulfate sulfur (SO_4)

Carbon to nitrogen ratio (C:N)

Organic nitrogen to sulfate sulfur ratio (N:S)

Total exchangeable hydrogen (TEH)

Potassium (K)

Calcium (Ca)

Magnesium (Mg)

Sodium (Na)

Phosphorus (P)

Cation exchange capacity (CEC)

% Clay + silt (CPS)

Porosity (POR)

Urease activity (UREASE)

Arylsulfatase activity (ARYL)

Dehydrogenase activity (DEHYD)

Table B2: Correlation matrix (r-values)*

	STEMP	FC	COND	pH	NO_3	NH_3	ORGN	SOM	SO_4	C:N	N:S	TEN	K	Ca	Mg	Na	P	CEC	CPS	TOR	UREASE	ARYL	DEHYD
STEMP	1.00																						
FC	-.04	1.00																					
COND	.11	-.10	1.00																				
pH	.13	.11	.06	1.00																			
NO_3	.02	-.08	-.43	.01	1.00																		
NH_3	.33	-.33	.50	-.15	-.05	1.00																	
ORGN	.06	.06	.27	-.14	-.07	.31	1.00																
SOM	-.02	.31	.23	.18	-.21	.08	.65	1.00															
SO_4	.23	-.36	.27	-.28	.22	.56	.21	-.12	1.00														
C:N	-.06	.30	.01	.39	-.22	-.21	-.27	.54	-.35	1.00													
N:S	.14	.09	-.03	.35	-.09	-.10	.04	.16	-.61	.13	1.00												
TEN	-.07	.14	.00	-.07	-.16	-.09	-.30	-.19	-.09	.11	.07	1.00											
K	.09	-.13	.29	.45	.07	.40	.07	.07	.13	.00	.04	-.09	1.00										
Ca	-.18	.44	.09	.57	-.28	-.42	-.05	.41	-.46	.56	.29	.01	.02	1.00									
Mg	-.19	.41	.02	.55	-.22	-.42	-.29	.17	-.47	.53	.27	.10	.01	.93	1.00								
Na	.03	.29	-.14	.64	-.04	-.31	-.01	.35	-.22	.49	.17	-.01	.08	.76	.71	1.00							
P	-.04	-.13	.37	.11	.03	.48	.31	.13	.25	-.17	-.09	-.20	.73	-.12	-.21	-.18	1.00						
CEC	-.11	.40	.16	.58	-.29	-.29	-.03	.43	-.40	.59	.27	.03	.11	.90	.85	.76	-.14	1.00					
CPS	-.09	.25	.06	.58	-.25	-.43	-.30	.17	0.51	.54	.31	.05	.09	.91	.93	.62	-.14	.87	1.00				
TOR	-.09	-.01	-.15	-.26	-.17	-.35	-.30	-.06	-.27	.22	-.07	.02	-.20	.37	.44	.14	-.21	.33	.51	1.00			
UREASE	-.13	.37	.13	.15	-.08	.04	.26	.58	-.18	.40	.24	.00	.11	.41	.32	.25	.14	.38	.28	.06	1.00		
ARYL	-.11	.43	.23	.19	-.23	-.11	.32	.58	-.14	.37	.14	.12	-.17	.50	.40	.45	-.19	.47	.26	-.01	.35	1.00	
DEHYD	.09	.18	.14	.27	-.12	-.13	.26	.28	-.06	.14	.15	.12	-.30	.39	.28	.52	-.36	.41	.19	-.21	.02	.54	1.00

* Positive signs excluded; explanations of abbreviations in Table B1

Table B3: Multiple regression equation and analysis of variance for arylsulfatase activity.

Regression equation

Arylsulfatase activity = 598.84 + 1.37 (STEMP) + 0.294 (FC)
+ 0.054 (COND) + 4.35 (pH) + 0.128(NO_3)
- 1.90 (NH_3) - 441.59 (ORGN)
+ 40.54 (SOM) - 0.258 (SO_4) - 7.24 (C:N)
- 0.002 (N:S) - 0.015 (TEH)
- 7.80 (K) + 0.445 (Ca) + 4.93 (Mg)
+ 51.72 (Na) - 0.307 (P) + 0.255 (CEC)
- 7.55 (CPS) + 0.658 (POR)

Analysis of variance

Source	Degrees of freedom	Sum of squares	Mean square
Total	71	7.69×10^4	1.08×10^3
Regression	20	5.16×10^4	2.58×10^3
Residual	51	2.53×10^4	4.96×10^2

$r^2 = 0.671$

Table B4: Multiple regression equation and analysis of variance for urease activity.

Regression equation

Urease activity = 164.82 + 2.64 (STEMP) + 0.699 (FC)
- 0.006 (COND) - 33.52 (pH) + 0.320 (NO_3)
+ 1.05 (NH_3) - 710.74 (ORGN) + 51.87 (SOM)
+ 0.339 (SO_4) - 8.37 (C:N) + 0.006 (N:S)
+ 0.002 (TEH) + 2.12 (K) + 0.011 (Ca)
+ 1.56 (Mg) - 9.70 (Na) + 0.880 (P)
- 0.043 (CEC) + 1.58 (CPS) - 1.22 (POR)

Analysis of variance

Source	Degrees of freedom	Sum of squares	Mean squares
Total	71	7.42×10^4	1.04×10^3
Regression	20	3.93×10^4	1.97×10^3
Residual	51	3.49×10^4	6.84×10^2

$r^2 + 0.530$

Table B5: Multiple regression equation and analysis of variance for dehydrogenase activity.

Regression equation

Dehydrogenase activity = 307.04 - 1.94 (STEMP) - 0.586 (FC)
+ 0.029 (COND) - 0.584 (pH)
+ 0.360 (NO_3) + 1.94 (NH_3)
+ 314.63 (ORGN) - 14.76 (SOM)
- 0.971 (SO_4) + 2.31 (C:N) - 0.002 (N:S)
+ 0.016 (TEH) - 15.19 (K) + 1.79 (Ca)
- 1.77 (Mg) + 124.99 (Na) - 0.918 (P)
+ 0.445 (CEC) - 2.03 (CPS)
- 3.15 (POR)

Analysis of variance

Source	Degrees of freedom	Sum of squares	Mean square
Total	71	3.97×10^4	5.58×10^2
Regression	20	2.93×10^4	1.46×10^3
Residual	51	1.04×10^4	2.03×10^2

$r^2 = 0.738$

Table B6: Principal components analysis of the environmental parameters (factor loadings)

Tests	Factor 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
STEMP	-.226	.276	.697	.224	-.542	.046	.082	-.029	.079	-.052	.032	.007	.052	.033	.001	-.086	-.110	.010	-.021	-.002
FC	.496	-.134	-.718	-.258	.266	-.114	.140	.058	-.018	.128	-.104	-.040	.002	-.001	.012	-.036	-.123	.013	-.037	-.002
COND	-.056	.651	-.077	.511	.208	-.166	.045	.217	-.149	.213	.288	-.059	-.126	.119	.035	.010	-.006	.003	-.004	-.002
pH	.597	.413	.374	-.427	.011	-.103	.179	.105	-.202	-.109	.063	-.014	.059	-.031	.022	.181	-.049	-.002	.009	.009
NO_3	-.267	-.183	.174	-.693	-.024	.326	.041	-.087	.258	.377	.231	-.019	.018	.074	-.005	-.001	.007	-.005	.001	.001
NH_3	-.531	.620	.066	.210	.073	-.068	.129	-.059	.043	.329	-.344	.020	.145	.073	-.004	.051	.021	-.018	-.007	-.001
ORGN	-.212	.552	-.548	-.031	-.430	.091	-.138	.195	.268	-.123	.046	-.043	.058	.004	.014	.022	.002	.033	-.014	.044
SOM	.352	.541	-.510	.055	-.419	.120	.066	-.317	.082	-.028	.092	-.016	.034	-.051	.049	.029	.002	-.010	.020	-.049
SO_4	-.624	.283	.054	.128	.157	.490	.354	.173	.067	.092	-.026	.061	-.108	-.249	.001	.007	-.013	.006	.001	.002
C:N	.675	.103	-.026	.126	-.064	.067	.302	-.602	-.199	.086	.033	.042	-.052	-.009	-.012	-.012	.018	.018	-.010	.039
N:S	.373	.091	.138	-.179	-.401	-.674	-.239	.027	.128	.249	-.081	.051	-.159	-.122	-.005	.005	.005	.003	.005	.002
TEH	.098	-.272	.058	.239	.260	-.462	.548	-.056	.496	-.121	.094	.018	.034	.008	-.002	.028	.006	-.003	.001	.000
K	-.022	.682	.286	-.287	.443	-.088	-.135	-.182	.116	-.128	-.063	-.253	-.032	-.052	.086	-.077	.013	-.008	.000	.003
Ca	.944	.159	-.019	.057	.013	.114	-.052	.143	.059	.010	.046	.123	.045	-.024	.057	-.055	.001	-.115	-.005	.016
Mg	.930	.005	.118	.065	.155	.078	-.019	.156	.039	.110	-.035	.104	.095	.000	.124	-.061	.003	.071	.057	.002
Na	.758	.184	.116	-.200	-.145	.299	.248	.138	.125	-.128	-.212	.039	-.201	.161	.014	.008	.033	.010	-.020	-.012
P	-.263	.680	-.027	-.172	.430	-.036	-.316	-.169	.119	-.134	.050	.277	-.026	.035	-.092	-.006	-.031	.051	.002	-.004
CEC	.904	.243	.002	.094	.003	.115	.044	.114	.063	.052	-.018	-.149	.023	-.011	-.230	-.032	-.009	-.004	.042	.001
CPS	.915	.054	.231	.122	.130	.058	-.158	.066	.024	.062	.085	.002	.102	-.086	-.035	.009	.058	.035	-.081	-.018
POR	.393	-.368	.107	.471	.139	.327	-.456	-.189	.270	.059	-.058	-.066	-.067	.008	.035	.115	-.056	-.004	.004	.008

Table B7: Principal components analysis of the environmental parameters.

Factor	Eigenvalue	Percent of variation	Cumulative percent
1	6.3824	31.9	31.9
2	3.0622	15.3	47.2
3	1.9435	9.7	56.9
4	1.6111	8.1	65.0
5	1.4949	7.5	72.5
6	1.3399	6.5	79.2
7	1.0939	5.5	84.6
8	0.7859	3.9	88.6
9	0.6486	3.2	91.8
10	0.5077	2.5	94.4
11	0.3640	1.8	96.2
12	0.2117	1.1	97.2
13	0.1571	0.8	98.0
14	0.1450	0.7	98.7
15	0.0948	0.5	99.2
16	0.0739	0.4	99.6
17	0.0400	0.2	99.8
18	0.0220	0.1	99.9
19	0.0148	0.1	100.0
20	0.0068	0.0	100.0