



An investigation of microbiological parameters in the revegetation of coal mine spoils  
by Rosemary Celia Stewart

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE  
in Microbiology  
Montana State University  
© Copyright by Rosemary Celia Stewart (1975)

**Abstract:**

Soil samples were collected from six major plots at Colstrip, Montana during April, August, October and December, 1974. Three of these plots were native soil plots and the other three plots were spoils which resulted from the disturbance caused by coal strip mining.

At each plot, samples were collected from within the root region of several different plant species. Sampling experiments were conducted to determine the amount of variability which could be expected within the soil system itself and that which was due to different plant species.

Duplicate samples were collected in April 1974 and soil chemical and physical analyses were run on the duplicate samples.

The soil samples were air-dried, sieved and blended in the laboratory before performing analyses. Following this treatment, measurements were made of respiratory activity (by measuring O<sub>2</sub> uptake on a Gilson respirometer), phosphatase activity, pectinolyase activity and the number of pectinolytic bacteria for each soil sample.

Attempts were then made to correlate the measurements of each microbiological parameter with the physical and chemical properties of the soil, seasonal variation and every other microbiological parameter. The phosphatase activity of native soil was the only microbiological parameter which correlated with several of the physical and chemical properties of the soil in April, 1974. Those correlations included positive correlations with percent clay and magnesium and negative correlations with potassium, calcium, sodium and percent silt. These correlations appear to be related to the clay, which may be adsorbing the enzyme onto its surface. Respiratory activity in native soil correlated with only one soil characteristic, water-holding capacity, and that was only for native soil. This relationship is related to the pore space of the soil. Spoils did not exhibit this correlation, lack of which could be attributed to the more heterogeneous textural types.

All four microbiological parameters were observed to exhibit significant seasonal variations for both native soil and spoils, although these seasonal variations did not coincide for all four parameters. Respiratory activity and the number of pectinolytic bacteria both tended to peak in October whereas phosphatase activity tended to peak in August and pectinolyase activity was high in August and peaked in December. The possibility of seasonal variation in physical and chemical characteristics of the soil is also suggested.

The variation in the four microbiological parameters were found to be not significantly due to different plant species.

STATEMENT OF PERMISSION TO COPY

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at Montana State University, I agree that the Library shall make it freely available for inspection. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by my major professor, or, in his absence, by the Director of Libraries. It is understood that any copying or publication on this thesis for financial gain shall not be allowed without my written permission.

Signature Raymond C. Stewart  
Date August 7, 1975

AN INVESTIGATION OF MICROBIOLOGICAL PARAMETERS  
IN THE REVEGATION OF COAL MINE SPOILS

by

ROSEMARY CELIA STEWART

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

of

MASTER OF SCIENCE

in

Microbiology

Approved:



Head, Major Department



Chairman, Examining Committee



Graduate Dean

MONTANA STATE UNIVERSITY  
Bozeman, Montana

August, 1975

ACKNOWLEDGEMENTS

The author would like to thank Drs. Kenneth L. Temple, Edward J. DePuit and David G. Stuart for their guidance and assistance throughout the course of this study.

Thanks are also due to Todd GeHagen, Ruth Wicks and Jean Block for their assistance in the laboratory, and to Marie Martin for ensuring a constant supply of clean glassware.

Sincere thanks are due to Jack Williams for his assistance and guidance concerning the statistical analysis of data.

This investigation was funded by Chevron Oil Company, Denver, Colorado through the Reclamation Research Group, MAES at Bozeman.

## TABLE OF CONTENTS

	Page
VITA . . . . .	ii
ACKNOWLEDGEMENTS . . . . .	iii
TABLE OF CONTENTS . . . . .	iv
LIST OF TABLES . . . . .	vii
LIST OF FIGURES . . . . .	x
ABSTRACT . . . . .	xi
Chapter	
1. INTRODUCTION . . . . .	1
Statement of Purpose . . . . .	3
2. MATERIALS AND METHODS . . . . .	5
Study Area . . . . .	5
Sampling . . . . .	7
Soil Analyses . . . . .	18
Respiration . . . . .	19
Phosphatase . . . . .	20
Pectinolyase . . . . .	21
Pectinolytic plate counts . . . . .	22
Isolation and Identification of Soil Organisms . . . . .	25
Isolation of DNA and Determination of $T_m$ . . . . .	25
Statistics . . . . .	27

Chapter	Page
3. RESULTS . . . . .	28
Variation in a Plot at large. . . . .	28
Variation Under a Particular Plant Species. . . . .	28
Variation in Sampling Techniques. . . . .	30
Soil Horizon Comparison . . . . .	35
Soil Analyses . . . . .	35
Water-holding Capacity for Optimal Respiratory Rate . .	43
Variability in Respiratory Rate Due to Quantity of Soil	43
Interrelationship of Respiratory Activity and Soil Chemical and Physical Properties . . . . .	46
Respiratory Activity for Three Native and Three Spoils Plots. . . . .	46
Seasonal Variation of Respiratory Activity. . . . .	50
Effects of Air-drying Soil on Phosphatase Activity. . .	50
Interrelationship of Phosphatase Activity and Soil Chemical and Physical Properties. . . . .	54
Phosphatase Activity for Three Native and Three Spoils Plots. . . . .	54
Seasonal Variation of Phosphatase Activity. . . . .	57
Effects of Air-drying on Pectinolyase Activity . . . .	62
Interrelationship of Pectinolyase Activity and Soil Chemical and Physical Properties. . . . .	62
Pectinolyase Activity for Three Native and Three Spoil Plots . . . . .	64
Seasonal Variation of Pectinolyase Activity	64

Chapter	Page
Effects of Purifying Pectin on Selective Plating of Pectinolytic Bacteria. . . . .	69
Effects of Air-drying Soil on Pectinolytic Bacteria Enumeration. . . . .	72
Relative Proportion of Total Soil Bacterial Population Estimated as Pectinolytic Bacteria. . . . .	72
Interrelationship of Pectinolytic Bacterial Population and Soil Chemical and Physical Properties . . . . .	72
Pectinolytic Bacterial Population for Three Native and Three Spoils Plots. . . . .	75
Seasonal Variation of the Number of Pectinolytic Bacteria. . . . .	79
Interrelationship of Microbiological Parameters . . . . .	82
Overall Analysis of Variance for the Microbiological Parameters . . . . .	82
Isolation and Identification of Soil Organisms. . . . .	87
Isolation of DNA and Determination of $T_m$ to Identify a Soil Organism . . . . .	87
4. DISCUSSION . . . . .	89
5. SUMMARY. . . . .	131
APPENDIX . . . . .	135
LITERATURE CITED . . . . .	156

LIST OF TABLES

Table	Page
1. Sampling sites, description, dates sampled and plant species . . . . .	8
2. Six major plots and their descriptions. . . . .	15
3. Variation in a plot at large - Demo plot, August, 1974. . . . .	29
4. Variation under a particular plant species, Demo plot, August, 1974 . . . . .	31
5. Variation under a plant species at different plots, April, 1974. . . . .	32
6. Variation of plants with sampling time - Demo and Native Range Unfertilized plots . . . . .	33
7. Analysis of variance for Quartered and Staked sampling treatments . . . . .	39
8. Soil horizon comparison - Demo plot, August 1974. . . . .	40
9. Significant correlations within soil analyses for native soils and spoils, April, 1974 . . . . .	42
10. Water-holding capacity for optimal respiratory rate. . . . .	44
11. Variability in respiratory rate due to quantity of soil . . . . .	45
12. Analysis of variance due to plant and time for respiratory activity of six major plots . . . . .	48
13. Analysis of variance due to plant species for respiratory activity of six major plots . . . . .	49
14. Seasonal variation of respiratory activity of six major plots . . . . .	51
15. Significant correlations of seasonal variation for respiratory activity of six major plots . . . . .	52
16. Phosphatase activity for air-dried and field moisture treatments of soils. . . . .	53

Table	Page
17. Significant correlations between phosphatase activity and the soil analyses for native soils in April, 1974. . . . .	55
18. Analysis of variance due to plant species for phosphatase activity of six major plots . . . . .	58
19. Analysis of variance due to plant and time for phosphatase activity of six major plots . . . . .	59
20. Seasonal variation of phosphatase activity of six major plots . . . . .	60
21. Significant correlations of seasonal variation for phosphatase activity of six major plots . . . . .	61
22. Pectinolyase activity for air-dried and field moisture treatments of soils. . . . .	63
23. Analysis of variance due to plant species for pectinolyase activity of six major plots. . . . .	66
24. Analysis of variance due to plant and time for pectinolyase activity of six major plots. . . . .	67
25. Seasonal variation of pectinolyase activity for six major plots. . . . .	68
26. Significant correlations of seasonal variation for pectinolyase activity of six major plots. . . . .	70
27. Comparison of pectinolytic bacteria selective media with commercial pectin and purified pectin. . . . .	71
28. Number of pectinolytic bacteria for air-dried and field moisture treatments of soils - Manhattan silt loam . . . . .	73
29. Relative proportion of soil bacteria as pectinolytic bacteria . . . . .	74
30. Analysis of variance due to plant species for number of pectinolytic bacteria of six major plots. . . . .	77

Table	Page
31. Analysis of variance due to plant and time for number of pectinolytic bacteria of six major plots . . . . .	78
32. Seasonal variation of the number of pectinolytic bacteria for six major plots. . . . .	80
33. Significant correlations of seasonal variation for numbers of pectinolytic bacteria of six major plots . . . . .	81
34. Correlations of four microbiological parameters as measured at three native soil and three spoils plots during April, August, October and December, 1974. . . . .	83
35. Chi-square for the four microbiological parameters. . . . .	85
36. Pooled variances of microbiological parameters. . . . .	86
37. Conversion of the number of pectinolytic bacteria to number of bacteria/gram of organic matter for April, 1974 . . . . .	123
38. Mean values of all four microbiological parameters for all sites and plants sampled. . . . .	136
39. Physiochemical properties of soils as determined April, 1974. . . . .	151
39b. Additional physiochemical properties of soils as determined April, 1974. . . . .	153

## LIST OF FIGURES

Figure	Page
1. Photograph of Demo spoils plot. . . . .	10
2. Photograph of Gouge spoils plot . . . . .	11
3. Photograph of Topsoil Gradient spoils plot. . . . .	12
4. Photograph of BN native soil plot . . . . .	13
5. Photograph of Native Range Unfertilized and Native Range Fertilized native soil plots . . . . .	14
6. Respiration rate for sampling variation . . . . .	34
7. Phosphatase enzymatic activity for sampling variation . . . . .	36
8. Pectinolyase enzymatic activity for sampling variation . . . . .	37
9. Pectinolytic plate count for sampling variation . . . . .	38
10. Respiratory activity for six major plots. . . . .	47
11. Phosphatase activity for six major plots. . . . .	56
12. Pectinolyase activity for six major plots . . . . .	65
13. Number of pectinolytic bacteria for six major plots . . . . .	76
14. Linear regression of pectinolyase activity against pectinolytic plate count. . . . .	84
15. Normal probability plot of $T_m$ for <u>Arthrobacter</u> . . . . .	88
16. Soil microbiology field sampling data sheet . . . . .	155

## ABSTRACT

Soil samples were collected from six major plots at Colstrip, Montana during April, August, October and December, 1974. Three of these plots were native soil plots and the other three plots were spoils which resulted from the disturbance caused by coal strip mining. At each plot, samples were collected from within the root region of several different plant species. Sampling experiments were conducted to determine the amount of variability which could be expected within the soil system itself and that which was due to different plant species.

Duplicate samples were collected in April 1974 and soil chemical and physical analyses were run on the duplicate samples.

The soil samples were air-dried, sieved and blended in the laboratory before performing analyses. Following this treatment, measurements were made of respiratory activity (by measuring O<sub>2</sub> uptake on a Gilson respirometer), phosphatase activity, pectinolyase activity and the number of pectinolytic bacteria for each soil sample.

Attempts were then made to correlate the measurements of each microbiological parameter with the physical and chemical properties of the soil, seasonal variation and every other microbiological parameter. The phosphatase activity of native soil was the only microbiological parameter which correlated with several of the physical and chemical properties of the soil in April, 1974. Those correlations included positive correlations with percent clay and magnesium and negative correlations with potassium, calcium, sodium and percent silt. These correlations appear to be related to the clay, which may be adsorbing the enzyme onto its surface. Respiratory activity in native soil correlated with only one soil characteristic, water-holding capacity, and that was only for native soil. This relationship is related to the pore space of the soil. Spoils did not exhibit this correlation, lack of which could be attributed to the more heterogeneous textural types.

All four microbiological parameters were observed to exhibit significant seasonal variations for both native soil and spoils, although these seasonal variations did not coincide for all four parameters. Respiratory activity and the number of pectinolytic bacteria both tended to peak in October whereas phosphatase activity tended to peak in August and pectinolyase activity was high in August and peaked in December. The possibility of seasonal variation in physical and chemical characteristics of the soil is also suggested.

The variation in the four microbiological parameters were found to be not significantly due to different plant species.

The statistical analyses of the microbiological parameters shows that differences exist between each plot for each of the four parameters, but the differences are not significant for spoils plots as compared to native soil plots.

The only correlation found between the four parameters was that of pectinolyase activity with the numbers of pectinolytic bacteria. This correlation is higher for native soils than for spoils, which might suggest that by following the correlation of these two measurements, an ecological trend could be observed.

It was concluded that the four microbiological parameters as applied to the ecological state of the soil were effective in showing differences. The importance of these differences is a question which requires further research.

## Chapter 1

### INTRODUCTION

The surfaces of land altered by coal strip mining are a diverse mixture of rocks and soils originating from the overburden lying over the coal. This overburden is commonly termed "spoils" following replacement after mining and is essentially a new medium for plant growth.

There are numerous publications dealing with the revegetation of spoil areas which report both successes and failures in revegetation of such areas. In the past, reclamation research has been carried out intensively on spoils from strip mining in the eastern United States. Recently, such research has been initiated to study the reclamation potential of western coal lands. These western coal lands differ most markedly from those in the eastern portions of the United States by generally low levels of pyritic materials in the coal and by occurring in a much more arid climatic zone. Therefore, the well-known problem of acidic spoils associated with eastern coal is not a problem involved in reclamation of western coal lands.

Reclamation research conducted up to this time has been focused on such factors as: surface manipulation, topsoiling, degree of slope, native and introduced plant species trials, fertilizer treatments and

time of seeding -- all of which are designed to enhance successful reclamation. However, the long-range success of these treatments is difficult to predict, for once vegetation is established on spoil its continued success depends upon the physical, chemical and biological factors that are so complexly interrelated in the soil condition known as fertility. Therefore, the reclamation research activities cited above can no longer be considered as an all inclusive approach to restoration of a desirable and productive environment in the shortest time possible, since it is necessary to observe the trends of the spoils' potential for productivity.

It has long been known that microorganisms are the active agents in the soil-forming processes and in the cycling of nutrients between soil and plant. Microbial activity can be said to prepare the environment for the plant, and it then depends on the direction and intensity of biological processes in soil whether the requirements of plants for nutritional elements will be satisfied. Therefore, the degree and type of microbial activity is probably important in determining the speed and permanency of reclamation of spoils from strip mining.

In newly manipulated spoils, it would be expected to find relatively low levels of microbial activity initially due to a few rather widely-adapted physiological groups of microorganisms. As the spoils progress to the state which supports plant life and is self-maintaining

(i.e. approaching natural conditions) the level of microbial activity can be expected to increase, and populations of specifically-adapted physiological groups of microorganisms, especially those aiding in the turnover of materials in the soil, would likewise increase. This developing ecosystem in the spoils is probably quite different from that of the pre-existing native rangeland, which would lend even more importance to the use of microbial activity as a measure of the progress of reclamation.

Traditionally, studies of soil bacteria have been concerned with taxonomic identification and populations. However, in recent years increasing attention has been given to the detection of specific enzymes and metabolic pathways in the soil in addition to the usual enumeration techniques. These parameters have been used to obtain information on the intensity of biological processes taking place in the soil. The sum of these metabolic approaches has been termed "biological activity". The main drawback of these procedures is not the use of conventional methods, but rather, the absence of standardized procedures.

#### Statement of Purpose

The long-range success of surface reclamation of strip mined spoils is related to the microbial activity of the spoils in proceeding from pioneer to climax ecosystems. The purpose of the present research

is to combine the tools of biochemistry and physiology to evolve parameters of biological activity of the soil. Specifically, parameters that measure that biological activity which is mediated by or directly related to microorganisms in the soil. These parameters would be useful in determining the ecological state of the disturbed soil as related to its natural state and in determining its potential for maintaining a steady-state population.

The specific aspects of this study involve: (1) soil sampling field experimentation in an attempt to determine the inherent variation of the system; (2) an in vitro examination of the oxygen uptake or respiratory activity of the soils, microbial extracellular phosphatase enzymatic activity, the extracellular pectinolytic enzymatic activity and a plating technique for the isolation and enumeration of pectinolytic organisms as the microbiological parameters; (3) an examination of the physical and chemical properties of soils as they interrelate to the microbiological parameters; and (4) a study of a specific biochemical taxonomic tool for the identification of soil organisms.

## Chapter 2

### MATERIALS AND METHODS

#### Study Area

The study area, as described by Sindelar, et. al. (1975), is located in southeastern Montana at Colstrip, where coal strip mining has occurred for over forty years. Western Energy Company and Peabody Coal Company have active coal mines in the immediate area. They disturb approximately 80 hectares (198 acres) of rangeland annually while producing about 8 - 10 million tons of sub-bituminous coal, and these figures are expected to increase in future years due to current energy demands.

Southeastern Montana is part of the Missouri Plateau, an unglaciated region of the Northern Great Plains in the Midland physiographic area of Montana. Geologic materials are primarily Cenozoic sedimentary rocks with some colluvium laid down in the Quaternary period. Distinctive sandstone ridges, mesas and escarpments dominate much of the landscape with broad valley lined with alluvial fans, foot slopes and stream terraces. The dendritic drainage pattern generally flows northward to the Yellowstone River.

The elevation of Colstrip is 981 meters (3,200 feet). The continental climate is cold in winter, warm in summer and has large variations in seasonal precipitation. Average annual precipitation is

38.4 centimeters (15 inches) and maximum precipitation occurs in April through July, with about 30 percent of annual precipitation occurring as snow. The growing season extends from April through July with a frost-free period of 120-124 days. July is generally the warmest month and summers are hot, dry and windy with extreme evaporation rates.

Most soils in the study area are formed on weakly consolidated sandstone, and siltstone, stratified sands, residuum and colluvium from sandstone, clay and silt shales and siltstone. Soil development is often limited to a weak accumulation of organic matter to form the A horizons. Residuum from shale or other salty materials often is affected by the dry climate and patterns of soil drainage to produce occasionally saline and alkaline soils. These soils and the area climate restrict vegetation to adapted drought and saline-alkali tolerant species.

Vegetation of the Colstrip area is primarily mixed-prairie grassland interspersed with ponderosa pine occurring on scattered sandstone outcroppings. Livestock carrying capacity is about 1.2 - 2.4 hectare (3.0 - 6.0 acres) per animal unit month depending on range conditions.

### Sampling

Samples were collected from a total of fifteen sample plots consisting of 7 spoils sites and 8 native soil sites (Table 1) during the period of September 1973 through December 1974 on five different dates. The three dominant plants at each site were selected and samples were taken from an arbitrary location under these dominant plants at each plot. Stakes were placed at each of these sites during the April sampling and subsequent sampling in August, October and December was conducted at these stakes. Following the April sampling, three representative spoils plots (Figures 1 - 3) and three representative native soil plots (Figures 4 and 5) were selected for further study (Table 2). A data sheet was completed for each sample in the field (Appendix).

Samples were taken as close to the particular plant as possible to include the finer plant roots. The litter was scraped aside and soil was sampled to the depth of 6 inches with a spade. The samples, consisting of approximately one quart of soil, were placed in plastic lined brown-paper sampling bags supplied by the M.S.U. Soils Lab. The collected samples were immediately placed on ice and subsequently placed in a 4C refrigerator upon arrival back in the laboratory, after approximately 6 hours had elapsed.

Each sample was stored at 4C until it was air-dried for eight hours, sieved through a 1 mm mesh brass sieve, blended in a Twin shell

Table 1. Sampling sites, descriptions, dates sampled and plant species.

Site	Description, Dates Sampled and Plant Species
BN	Native soil; sampled September 1973, April, October and December 1974; <u>Agropyron smithii</u> , <u>Artemisia frigida</u> and <u>Stipa comata</u> .
Cape Oliver	Spoils; sampled September 1973 and April 1974; <u>Agropyron smithii</u> , <u>Chrysothamnus nauseosus</u> and <u>Gutierrezia sarothrae</u> .
Demo	Spoils; sampled September 1973, April, August, October and December 1974; <u>Agropyron cristatum</u> , <u>Bromus inermis</u> and <u>Melilotus officinalis</u> .
Dryland Pasture	Spoils; sampled September 1973 and April 1974; <u>Agropyron cristatum</u> , <u>Agropyron elongatum</u> and <u>Dactylis glomerata</u> .
Gouge	Spoils; sampled September 1973, April, August, October and December 1974; <u>Agropyron cristatum</u> , <u>Agropyron elongatum</u> , <u>Artemisia cana</u> and <u>Bromus inermis</u> .
McDonald	Native soil; sampled September 1973 and April 1974; <u>Koeleria cristata</u> and <u>Stipa comata</u> .
MP Lower	Native soil; sampled September 1973 and April 1974; <u>Gutierrezia sarothrae</u> and <u>Koeleria cristata</u> .

Table 1. (continued)

Site	Description, Dates Sampled and Plant Species
MP Upper	Native soil; sampled September 1973 and April 1974; <u>Bouteloua curtipendula</u> , <u>Rhus trilobata</u> and <u>Schizachyrium scoparium</u> .
Native Range Fertilized	Native soil; sampled September 1973, April, August, October and December 1974; <u>Carex</u> spp., <u>Gutierrezia sarothrae</u> and <u>Stipa comata</u> .
Native Range Unfertilized	Native soil; sampled September 1973, April, August, October and December 1974; <u>Carex</u> spp., <u>Gutierrezia sarothrae</u> and <u>Stipa comata</u> .
Raw Spoils - 1 month old	Spoils; sampled April 1974; No vegetation.
Raw Spoils - 1 year old	Spoils; sampled September 1973 and April 1974; No vegetation.
Streeter	Native soil; sampled September 1973 and April 1974; <u>Agropyron spicatum</u> , <u>Pinus ponderosa</u> and <u>Stipa comata</u> .
Old Spoils	Spoils; sampled April 1974; <u>Chrysothamnus nauseosus</u> and <u>Oryzopsis hymenoides</u> .
Topsoil Gradient	Spoils; sampled September 1973, August, October and December 1974; <u>Bromus inermis</u> .



Figure 1. Photograph of Demo spoils plot.



Figure 2. Photograph of Gouge spoils plot.



Figure 3. Photograph of Topsoil Gradient spoils plot.



Figure 4. Photograph of BN native soil plot.

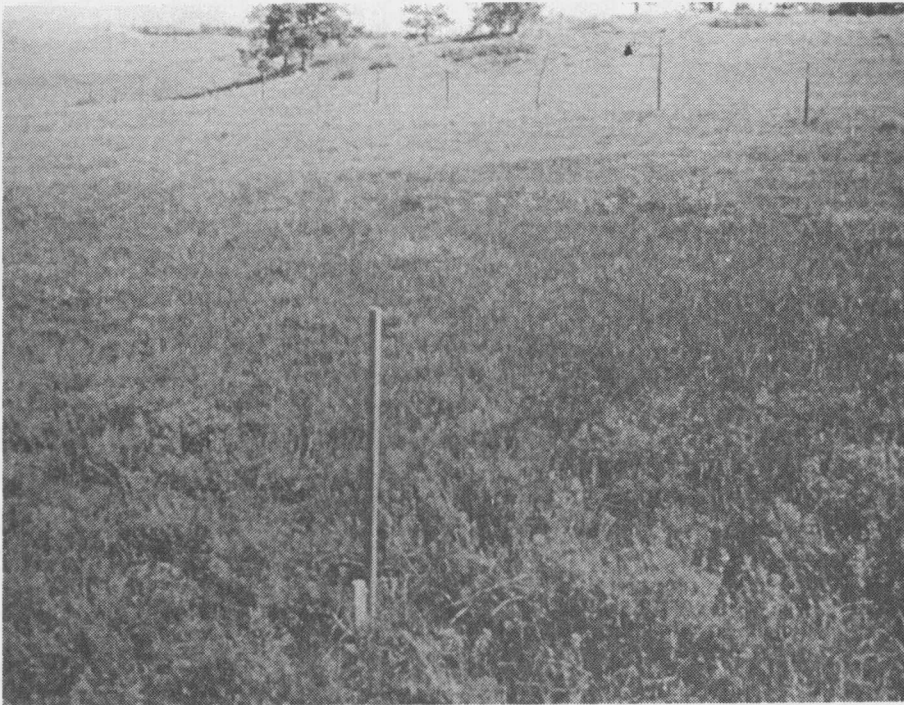


Figure 5. Photograph of Native Range Unfertilized and Native Range Fertilized native soil plots.

Table 2. Six major plots and their descriptions.

Plot	Description
BN	Native soil; mixed prairie grassland, not grazed for some time (7-8 years), range condition good to very good, dominant species are <u>Agropyron smithii</u> , <u>Stipa comata</u> ; total biomass production/year ranges between 900-1200 kg/ha; no treatment; no fertilization; no complete description of soils available as yet.
Native Range Fertilized	Native soil; mixed prairie grassland, not grazed in the past 5 years, range condition fair to good, dominant species are <u>Agropyron smithii</u> , <u>Stipa comata</u> , <u>Koeleria cristata</u> and <u>Gutierrezia sarothrae</u> ; total above ground production/year ranges between 2611-3365 kg/ha; no treatment; fertilized in spring - 80 lbs/A Nitrogen, 100 lbs/A Phosphorus; soils deep and silty, mean bulk density 1.42.
Native Range Unfertilized	Native soil; mixed prairie grassland, not grazed in the past 5 years, range condition fair to good, dominant species are <u>Agropyron smithii</u> , <u>Stipa comata</u> , <u>Koeleria cristata</u> and <u>Gutierrezia sarothrae</u> ; total above ground production/year ranges between 1449-1589 kg/ha; no treatment; no fertilization; soils deep and silty, mean bulk density 1.42.

Table 2. (continued)

Plot	Description
Demo	Spoils; seeded to introduced perennial grasses and forbes, major species are <u>Agropyron cristatum</u> , <u>Dactylis glomerata</u> , <u>Triticum aestivum</u> , <u>Melilotus officinalis</u> , <u>Onobrychis viciaefolia</u> ; total above ground production/year ranges between 1731-2200 kg/ha; shaped and topsoiled spoils; fertilized in spring - 76 lbs/A Nitrogen 50 lbs/A Phosphorus; topsoiled spoils; mean bulk density 1.61-1.78.
Gouge	Spoils; seeded to native and introduced grasses, forbes and shrubs, major species are <u>Agropyron dasystachyum</u> , <u>Agropyron elongatum</u> , <u>Agropyron sibericum</u> , <u>Agropyron smithii</u> , <u>Astragalus cicer</u> , <u>Atriplex canescens</u> , <u>Bromus inermis</u> , <u>Melilotus officinalis</u> , <u>Oryzopsis hymenoides</u> ; total above ground production/year ranges between 3524-4376; untopsoiled, shaped spoils which were mechanically gouged; fertilized in autumn - 150 lbs/A Nitrogen, 100 lbs/A Phosphorus and 50 lbs/A Potassium; untopsoiled spoils, mean bulk density 1.66.
Topsoil Gradient	Spoils; seeded to introduced grasses and forbes, major species are <u>Bromus inermis</u> , <u>Atriplex canescens</u> ; total above ground production/year ranges between 2297-2563; topsoiled spoils reshaped to slope gradients of 4:1, 3:1 and 2.5:1; fertilized in spring - 40 lbs/A Nitrogen and 20 lbs/A Phosphorus; topsoiled spoils, mean bulk density 1.45.

dry blender (Patterson-Kelley Company, Pennsylvania) and then returned to 4C until analyzed. After analysis, the samples were frozen at -20C to store for future reference.

Experiments were conducted to determine the number of replicate samples necessary to eliminate the effects of a large part of the inherent variability of the soil system itself. Three experiments were conducted at different sampling periods to determine variance within a plot and variance due to plant species.

In the first experiment, samples were taken, as previously described, from arbitrary locations throughout a revegetated spoils site (Demo plot, see Tables 1 and 2) during the August sampling. A total of four samples were taken for this purpose. Samples for the second experiment were taken, again as described, at the same site during the August sampling from 10 arbitrarily selected Bromus inermis plants.

Two methods were used for comparison in the third experiment, and these were given the titles of Staked Variation and Quartered Variation. During the August and October samplings, five samples were taken from within a one foot radius of a staked plant at the Demo and Native Range Unfertilized plots (see Tables 1 and 2). Two square feet of soil, with the same dominant plant species as at the stake, were excavated to a depth of 6 inches and the soil was mixed thoroughly by the quartering procedure. Five arbitrary samples

were taken from this "quartered" treatment. This procedure was followed for two different plant species at each of the two plots.

#### Soil Analyses

Duplicate samples were taken from each site during the April sampling and the duplicate sample was sent to the Soils Lab at Montana State University for chemical and physical analyses.

The percent of water remaining in each soil at 0.3 atmospheres was determined by a standard procedure.

The pH and conductivity were determined with a Beckman Zeromatic II pH Meter and a Serfass Model RCM 1581 Conductivity Meter on ten grams of soil to which twenty milliliters of distilled water had been added.

The available calcium, magnesium, potassium and sodium in each soil sample was determined on a Model 290-B Perkin Elmer Atomic Absorption Spectrophotometer following an ammonium acetate extraction.

The available phosphorus in each sample was determined by Brays' Method 1 as modified by Smith, Ellis and Grava (1957) and Olsen and Dean (1965).

Available nitrate was determined by a nitrophenoldisulfonic colorimetric method using a Spectronic 20 spectrophotometer following extraction with a  $\text{CaSO}_4$  -  $\text{Ag}_2\text{SO}_4$  solution.

The soil organic matter was determined by the colorimetric method of Graham (1948) as modified by Sims and Haby. (1971).

The percentage of sand, silt and clay was determined by the Bouyoucos (1926) method of mechanical analysis.

The mean bulk density was determined by the Montana Agricultural Experiment Station using the method of Sindelar, et. al. (1975).

Above Ground Production was also determined by the Montana Agricultural Experiment Station using the method of Munshower, Sindelar and Neuman (1975).

#### Respiration

The water-holding capacity for each soil was determined by the following method. A wetted filter paper disk was placed in a plastic cup with numerous holes in the bottom. The cup and filter paper were weighed on a Mettler Analytical Balance and ten grams of soil were added. The cup was then allowed to sit in a dish of distilled water until excess water was visible at the soil surface. After removal from the water, the cup was placed on an absorbant sponge to remove the visible excess water from the top surface of the soil. When no excess water could be observed, the cup was again weighed. The difference between wet weight and dry weight gave an experimental water-holding capacity value for ten grams of each particular soil sample.

Five grams of air-dried soil were placed in Warburg respirometer flasks, distilled water was added to bring the soil up

to 100% water-holding capacity and the flasks were stoppered and allowed to equilibrate over night. Two-tenths milliliters of 40% potassium hydroxide and filter paper wick were placed in the center wells. The Warburg flasks were placed on a Gilson Differential Respirometer and equilibrated at 25C for one-half hour. The respirometer was connected and a barometric pressure reading was taken with an S & M Surveying Anaeroid. Readings were taken each half hour for a total of eight hours. The respiratory rate was calculated from five replicates of each sample and these were corrected to standard conditions using the equation given in the instruction manual for the Gilson Respirometer. This procedure was based on experiments to determine the optimum water-holding capacity and the effect of sample size.

An experiment was conducted to determine the water-holding capacity which would produce the maximum respiration rate. Samples were run at 50%, 70%, 90%, 100%, 110%, 125%, 150% and 200% water-holding capacity.

To determine if less variability was possible with larger quantities of soil, special flasks were constructed with a capacity of 20 grams of soil. With these flasks, an experiment was conducted comparing respiratory rates for 5, 10, 15 and 20 gram portions of soil.

#### Phosphatase

The method used for the determination of phosphatase enzyme

activity was taken from Khazlev (1974). Five grams of air-dried soil were added to twenty milliliters of 0.3% disodium phenyl phosphate and 3-4 drops of toluene. These were stoppered and incubated in a 30C shaking water bath for one hour. Following incubation, eighty milliliters of distilled water were added and this was then centrifuged at 8,000 rpm on a Sorvall RC-2B centrifuge for ten minutes. Five milliliters of this solution were placed in a 50 ml volumetric flask to which was added 5 mls of borate buffer (pH 9.0), 3 mls of 2.5% potassium ferricyanide and 3 mls of 0.5% 4-amino anti pyrine. (Fresh solutions of  $K_3Fe(CN)_6$  and 4 amino anti pyrine were made daily.) The volume was brought to 50 mls and after 10-15 minutes a stable rose-colored complex formed with the liberated phenol and this was read on a Spectronic 20 spectrophotometer at 510 nm. The quantity of phenol was determined with a standard curve of phenol for each sample and the phosphatase activity was calculated as umoles of phenol per gram of soil.

An experiment was conducted to determine the effects of air-drying, sieving and blending on enzyme activity. Phosphatase activity was determined for samples both at the water content as sampled (without blending) and after air-drying, sieving and blending.

#### Pectinolyase

The determination of pectinolytic enzyme activity followed the method of Kaiser and DeAsconegui (1971). Two grams of air-dried soil

were mixed with 2 ml of toluene, 15 ml of Tris buffer (Tris hydroxymethylaminomethane, pH 7.5) and 5 ml of a pectin solution consisting of 0.7 g pectin, 1.1 g NaCl, 0.25 g phenol, 0.018 g  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  and 100 ml of distilled water. These were stoppered and incubated for 24 hours at 25C. Following this, the solutions were centrifuged at 8,000 rpm for 10 minutes and filtered through Whatman #4 Qualitative filter paper. The filtrate was evaporated just to dryness in porcelain evaporating dishes over a boiling water bath. The dry material was then returned to solution by the addition of 1.5 ml of distilled water. One ml of this solution was then added to 2 ml of 1 N HCl and 5 ml of 0.29% thiobarbituric acid. This mixture was boiled for one-half hour, cooled, centrifuged at 5,000 rpm and then read on a Spectronic 20 spectrophotometer at 550 nm.

A galacturonic acid standard curve was constructed, but pectinolytic activity was reported as optical density at 550 nm.

An experiment was conducted to determine the effects of air-drying on pectinolytic enzyme activity. Pectinolytic activity was determined for samples both at field water content and following air-drying.

#### Pectinolytic Plate Counts

The procedure used to determine counts of pectinolytic organisms from the soil was modified from Hankin, Zucker and Sands (1971).

Mineral pectin medium (MPM) contained, per liter:  $(\text{NH}_4)_2\text{SO}_4$ , 2 g;

$\text{KH}_2\text{PO}_4$ , 4 g.;  $\text{Na}_2\text{HPO}_4$ , 6 g.;  $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$ , 1 mg.;  $\text{MgSO}_4$ , 0.2 g.;  $\text{CaCl}_2$ , 1 mg.;  $\text{H}_3\text{BO}_3$ , 10 ug.;  $\text{MnSO}_4$ , 10 ug.;  $\text{ZnSO}_4$ , 70 ug.;  $\text{CuSO}_4$ , 50 ug.;  $\text{MoO}_3$ , 10 ug.; The pH was adjusted to 7.4 and then 5 mls of 0.4% alcoholic brom thymol blue solution, 1 g. of yeast extract, 5 g. purified pectin in 5 ml of 95% ethanol and 15 g. agar were added.

The purified pectin was prepared by combining 250 grams of commercial pectin with 500 ml of 70% ethanol, shaking for 2 hours, filtering and repeating the above procedure once. Following the second filtration, the pectin was placed in a 37C incubator and allowed to dry for 24 hours prior to use.

The medium was sterilized by autoclaving at 121C for 15 minutes, poured into sterile petri dishes and allowed to dry for 24 hours prior to use.

Five grams of soil were added to 495 mls of sterile double-distilled water in a sterile Waring blender head and blended at high speed for one minute. One ml was removed with a sterile pipette, while the blender was in motion, and this was added to a sterile 9 ml distilled water dilution blank. The dilution blank with the added soil was shaken on a Vari-Whirl (VWR Scientific) and 1 ml was removed and added to a second 9 ml distilled water dilution blank. A second 1 ml was removed, of which 0.2 ml was added to three separate MPM plates. The same procedure was followed

for the second and third 9 ml dilution blanks resulting in three MPM plates each of a  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  dilution. A bent glass rod was dipped in alcohol, flamed and used to spread the liquid on the surface of the medium. The plates were then inverted and incubated for 48 hours at 30C. Following incubation, the number of colonies was counted on a New Brunswick Scientific Colony Counter. The number of bacteria per gram of soil was then calculated from five replications of the above procedure for each sample.

Plates were flooded with 1% hexadecyltrimethylammoniumbromide (aqueous) and zones of pectinolysis were taken as clear zones surrounding any colony after fifteen minutes to one-half hour.

An experiment was conducted to compare this modified Mineral Pectin Medium (MPM) with the original Mineral Pectin Medium containing unpurified pectin and also with Soil Extract Medium. The Mineral Pectin Medium with unpurified pectin was the same as MPM except that commercial pectin was used with no further purification and the Soil Extract Medium used was taken from Bunt and Rovira (1955). Samples were plated on MPM and either Soil Extract Medium or the original Mineral Pectin Medium, then incubated and counted as above.

Pure cultures of isolates from the soil and stock cultures (from the Montana State University Microbiology Department) of Bacillus subtilis, Bacillus macerans and Erwinia carotovora, all of which were known to be pectinolytic, were also streaked on MPM to confirm the

effectiveness of the medium for determining pectinolytic organisms.

An additional control check was run on the MPM by replicate plating from MPM onto the same medium without pectin, without yeast extract and without both pectin and yeast extract.

#### Isolation and Identification of Soil Organisms

Several characteristic colonies were isolated and standard biochemical tests were performed for identification according to the Seventh Edition of Bergey's Manual of Determinative Bacteriology.

One isolate was also used to determine the possibility of identification by means of the thermal melting point of the organisms' DNA.

#### Isolation of DNA and Determination of Thermal Melting Point

The method employed for determining the Thermal melting ( $T_m$ ) value is based on the observations of Thomas (1953) and Marmur and Doty (1962), which state that when a DNA sample is slowly heated a sharp increase in the extinction coefficient occurs at the temperature at which the double-stranded DNA separates into a single-stranded random coil. The midpoint of the rise was called the  $T_m$  value and it was found that the value depended on the G+C content of the DNA sample.

A large volume (12 liters) of sterile TSY broth with 3% yeast extract was inoculated with a 24-hour culture of the organisms desired. This was incubated for 48 hours at 30C. Cells were collected by continuous-flow centrifugation on a Sorvall RC-2B centrifuge at

15,000 rpm. The cells were washed in a saline-EDTA solution (0.15 M - 0.1 M) at pH 8.0, recentrifuged and then resuspended in 300 ml of the saline-EDTA solution. The DNA was isolated from these cells by following the method of Marmur (1961). The isolated DNA was dissolved in 10 ml of saline-sodium citrate solution (0.15 M - 0.015 M) at pH 7.0 and 1 ml of this final solution of extracted DNA was used to determine the concentration of DNA present in the sample according to the diphenylamine method of Burton (1956). The extracted DNA was then adjusted to an approximate concentration of 50 ug/ml in saline-sodium citrate.

Five-tenths ml of the DNA suspension containing 50 ug/ml was placed in a quartz microcuvette and 0.5 ml of saline-sodium citrate solution was added to a second quartz microcuvette as the blank. The absorbance was read at 25C at a wavelength of 260 nm on a Varian Techtron 635 UV-Vis Spectrophotometer. A Heto Ultrathermastat (London Company) was then used to raise the temperature in the spectrophotometer sample holders. The temperature was first quickly raised to 50C and the absorbance recorded. The temperature was then raised 1C every 10 minutes until no increase in absorbance was noted for consecutive temperature increases.

The  $T_m$  was calculated as that temperature corresponding to 50% of the increase in absorbance using the Normal Probability Paper method of Knittel, et. al. (1968). The % G-C was calculated according to

the method of Bowie, et. al. (1972), using the equation:  $T_m = 53.07 + 0.41 \%G-C$ .

For comparison of results, several determinations were made for the soil isolate, an E. coli culture from the MSU Microbiology Department and for E. coli Type VIII DNA from Sigma Chemical Company.

#### Statistics

Analysis of variance was computed by using a computer program titled "One-way and multi factor analysis of variance" by R.E. Lund, Statistical Laboratory, MSU. F-tests were calculated from the computer program according to the method of Snedecor and Cochran (1967), section 11.2. Correlation coefficients were computed on a Victor model 3600 statistical calculator. Chi-square was calculated from Bartlett's Test of Homogeneity of Variance as found in Snedecor and Cochran (1967).

## Chapter 3

### RESULTS

#### Variation in a Plot at Large

In an experiment to determine the amount of variation to be expected at any one plot, four sites were arbitrarily selected on the Demo plot August, 1974. Following the routine procedure of air-drying, sieving and blending, the three parameters of respiratory activity, phosphatase enzymatic activity and pectinolytic enzymatic activity were measured in 5 replications for each of these four samples. The results of these three tests are presented in Table 3 with their respective statistical analyses. Both the phosphatase enzymatic activity and pectinolytic enzymatic activity showed large variations between plots, while the variation exhibited by respiratory activity was much less. It appears from this that there is a great deal of inherent variation due to the soil system itself which must be taken into consideration when analyzing results of this or any other soil study.

#### Variation Under a Particular Plant Species

The variation in results that could be expected from measurements for one particular plant species was determined by taking samples beneath ten individual Bromus inermis plants (Lincoln Smooth Brome)

Table 3. Variation in a plot at large - Demo plot, August 1974

Sample #	Respiration ( $\mu\text{l O}_2/\text{g/hr}$ )	Phosphatase ( $\mu\text{moles phenol/g}$ )	Pectinolyase (O.D.)
102	1.7363	35.20	0.284
103	1.7683	34.00	0.282
111	1.2656	12.68	0.455
112	2.0573	33.20	0.262
Mean	1.7063	28.77	0.321
Standard deviation	0.4123	11.259	0.117
Chi-square	2.00 (n=20)	88.12 (n=20)	0.86 (n=20)

on the Demo plot (Table 2) August, 1974. Measurements of respiratory activity, phosphatase enzymatic activity and pectinolytic enzymatic activity were made on 5 replicates of each of these samples. Table 4 presents the results of these measurements. From these data it appears that any particular plant at one plot also has a large variation associated with it at arbitrary locations.

The differences between the same plant species on different plots at the same time can be seen from Table 5. In addition to the variation found for one plant species at a particular time, Table 6 presents the data for plants sampled at different times. These data indicate that a variation can again be expected for a particular plant on a specific plot at different times.

#### Variation in Sampling Techniques

Two different methods were employed in sampling for this experiment. In the first method, 5 samples were taken within a one-foot radius about a staked plant; this method was termed "Staked Variation". The second method involved field mixing the top six inches of soil within a two-square foot area dominated by the same plant species as that at the stake. Five samples were then taken from this "Quartered Variation" site arbitrarily. The data for respiratory rate of the 5 replicates for both Quartered and Staked Variation can be seen in Figure 6 for Stipa comata at the Native

Table 4. Variation under a particular plant species - Demo plot, August 1974

Sample #	Respiration ( $\mu\text{l O}_2/\text{g/hr}$ )	Phosphatase ( $\mu\text{moles phenol/g}$ )	Pectinolyase (O.D.)
90	1.5084	32.2	0.264
91	1.0675	27.5	0.232
92	2.3383	38.8	0.205
93	2.3780	31.0	0.218
94	2.1927	29.2	0.480
95	1.9350	23.4	0.440
96	2.4610	40.6	0.333
97	2.0897	41.8	0.349
100	2.3626	43.6	0.391
101	2.5019	64.0	0.361
Mean	2.0835	37.2	0.327
standard deviation	0.501	13.618	0.097

Table 5. Variation under a plant species at different plots April 1974

Plant and Plot	Respiration <sup>a</sup>	Phosphatase <sup>b</sup>	Pectinolyase <sup>c</sup>	Pectinolytic <sup>d</sup> Plate Counts
<u>Bromus inermis:</u>				
Gouge	1.1414	15.20	0.225	35.373
Demo	0.3478	56.30	0.293	6.513
standard deviation	0.570	20.55	0.034	14.430
<u>Stipa comata:</u>				
McDonald	1.7643	60.00	0.364	nd <sup>e</sup>
BN	1.8758	120.00	0.207	2.256
Native Range Fertilized	1.7386	75.00	0.118	5.567
Native Range Unfertilized	0.3572	103.70	0.296	8.183
Streeter	0.4017	253.30	0.396	nd
standard deviation	0.6940	68.75	0.102	2.425
<u>Gutierrezia sarothrae:</u>				
Cape Oliver	1.4339	36.70	0.123	nd
MP-Lower	2.3101	80.30	0.293	nd
Native Range Fertilized	1.7008	81.00	0.137	9.427
Native Range Unfertilized	0.2577	33.30	0.275	1.833
standard deviation	0.7450	22.86	0.078	3.797

a =  $\mu\text{l O}_2/\text{g}/\text{hour}$

b =  $\mu\text{moles phenol/g}$

c = optical density at 550 nm

d = number of bacteria  $\times 10^4/\text{g}$

e = not determined

Table 6. Variation of plants with sampling time - Demo and Native Range Unfertilized

Plant and Time	Respiration <sup>a</sup>	Phosphatase <sup>b</sup>	Pectinolyase <sup>c</sup>	Pectinolytic <sup>d</sup> Plate Counts
<u>Bromus inermis</u> (Demo):				
April 1974	0.3478	56.30	0.293	6.513
August 1974	2.4671	52.30	0.268	6.084
October 1974	3.7490	54.60	0.163	8.087
December 1974	2.7140	45.40	0.222	1.280
Mean	2.5386	51.70	0.230	5.491
Standard deviation	1.1453	7.47	0.057	2.874
<u>Stipa comata</u> (Native Range Unfertilized):				
April 1974	0.3572	103.67	0.296	8.183
August 1974	3.0962	206.00	0.147	33.763
October 1974	3.1460	109.60	0.088	22.800
December 1974	0.8760	11.30	0.419	12.133
Mean	1.8688	107.64	0.238	19.195
Standard deviation	13.7390	68.88	0.129	9.954

a =  $\mu\text{l O}_2/\text{g}/\text{hour}$

b =  $\mu\text{mole phenol/g}$

c = optical density at 550 nm

d = bacteria  $\times 10^4/\text{g}$

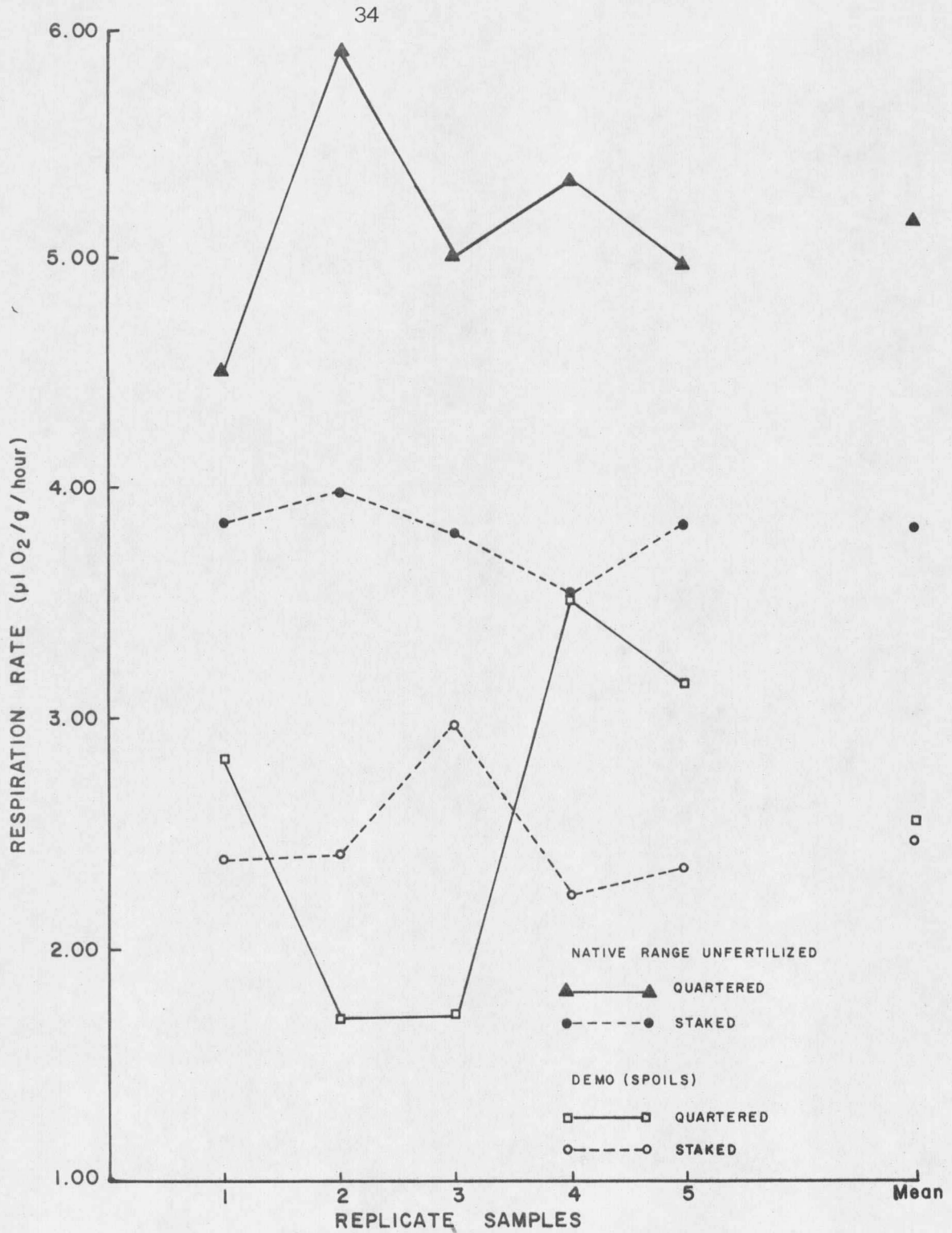


FIGURE 6. RESPIRATION RATE FOR SAMPLING VARIATION.

Range Unfertilized plot and Bromus inermis at the Demo plot in October, 1974. Figures 7, 8 and 9 present the data for phosphatase enzymatic activity, pectinolytic enzymatic activity and pectinolytic plate count, respectively, for these two plots with their associated plant species. The analysis of variance for this composite data, as presented in Table 7, shows that the comparison of variances between these two sampling treatments is not significant.

#### Soil Horizon Comparison

During the August 1974 sampling, the soil at Demo plot was sampled at two different depths to determine if there was a difference in levels of activity corresponding to the soil horizons in spoils. The upper six inches were taken for the Upper Soil Horizon and soil from below 6 inches was sampled as the Lower Soil Horizon. The four proposed microbial activity parameters were then measured on 5 replicates from each of these samples and the results are presented in Table 8. The difference in levels of activity, as measured with these four tests, is not obvious or significant.

#### Soil Analyses

Soil analyses, including pH, phosphorus, potassium, nitrate-

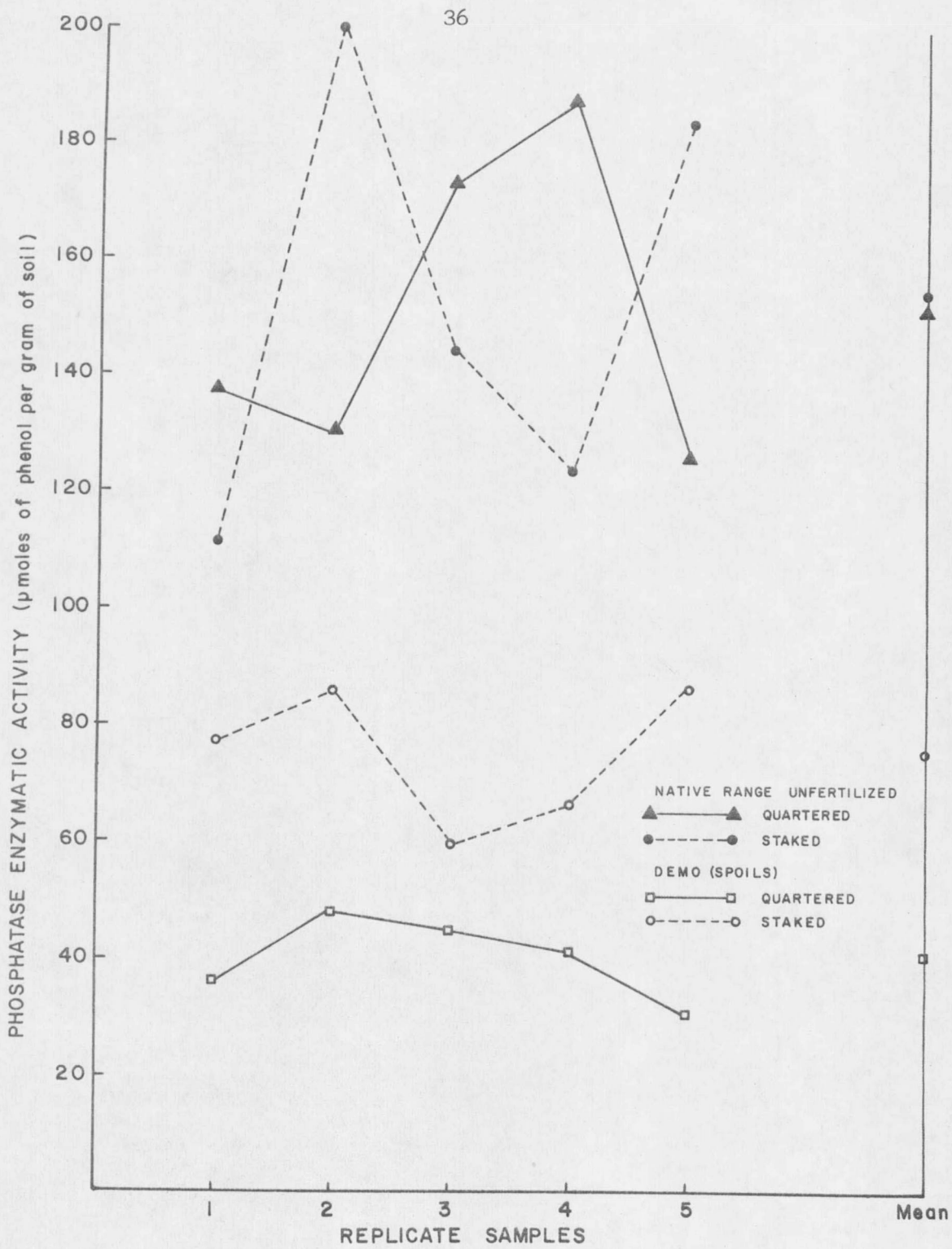


FIGURE 7. PHOSPHATASE ENZYMATIC ACTIVITY FOR SAMPLING VARIATION.

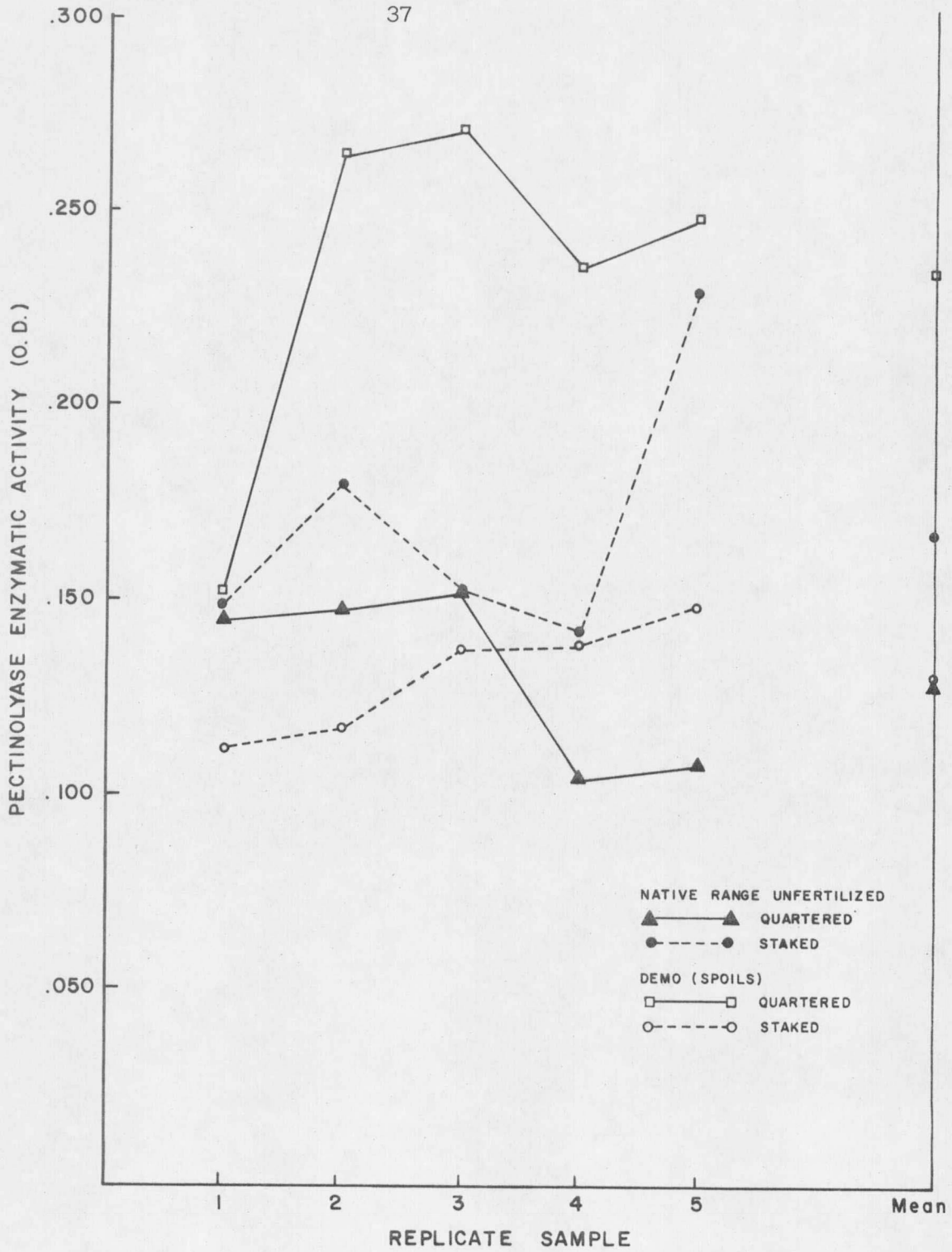


FIGURE 8. PECTINOLYASE ENZYMATIC ACTIVITY FOR SAMPLING VARIATION.

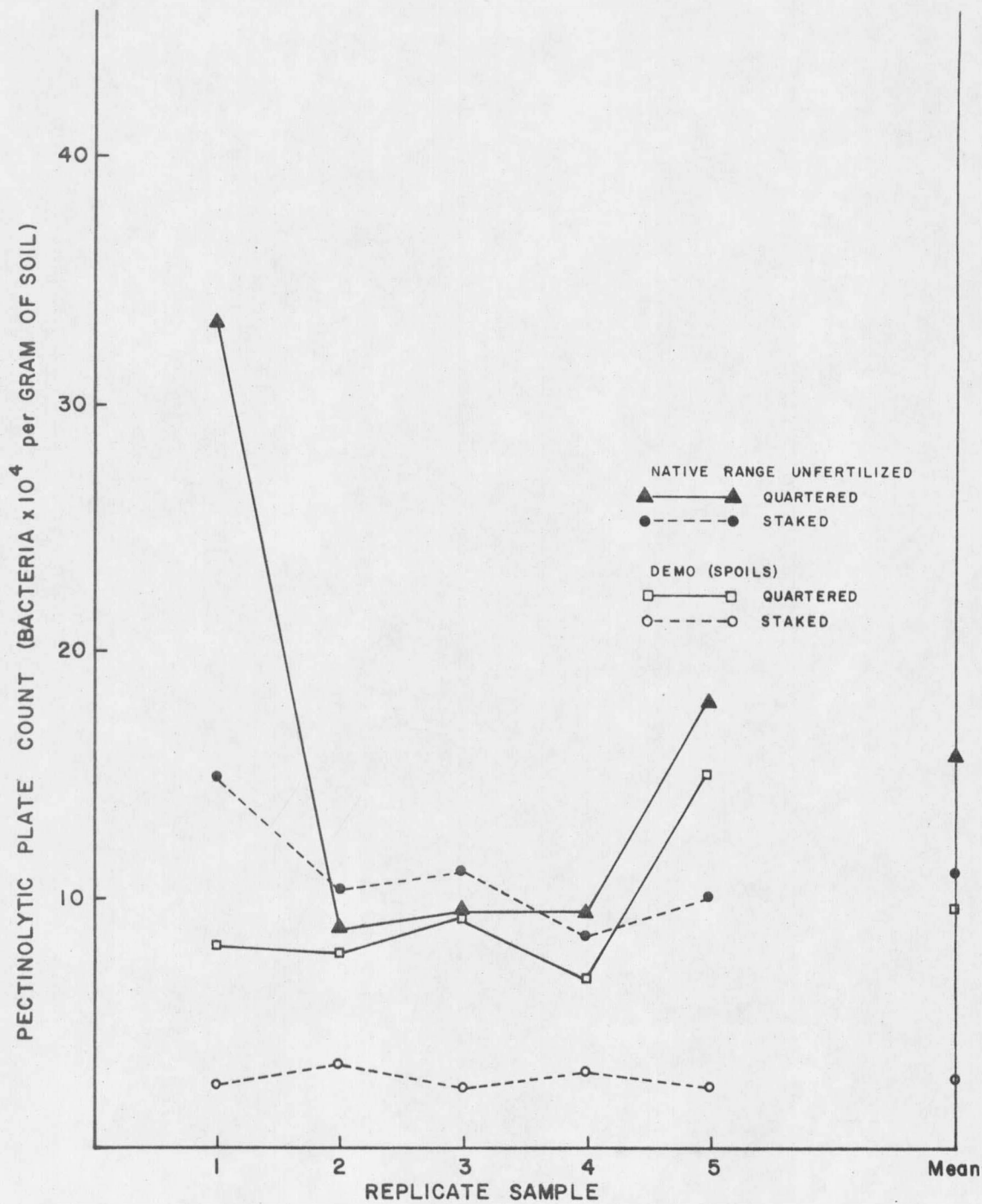


FIGURE 9. PECTINOLYTIC PLATE COUNT FOR SAMPLING VARIATION.

Table 7. Analysis of variance for quartered and staked sampling treatments

Plot and Treatment	SS <sup>a</sup>	df <sup>b</sup> <sub>SS</sub>	MS <sup>c</sup>	df <sup>d</sup> <sub>MS</sub>	F <sup>e</sup>	s <sup>2</sup>	sig. <sup>f</sup>
<u>Native Range Unfertilized:</u>							
Staked Variation	.042	4	.01	68	.20	.05	ns <sup>g</sup>
Quartered Variation	.062	4	.02	72	.34	.05	ns
<u>Demo:</u>							
Staked Variation	.004	4	.00	70	.01	.11	ns
Quartered Variation	.058	4	.02	70	.19	.08	ns

a = Sum of Squares

b = degrees of freedom for sum of squares

c = Mean Square

d = degrees of freedom for mean square

e = F-test, variance ratio

f = significance

g = not significant

Table 8. Soil horizon comparison - Demo plot August 1974

Soil Horizon	Respiration <sup>a</sup>	Phosphatase <sup>b</sup>	Pectinolyase <sup>c</sup>	Pectinolytic <sup>d</sup> Plate Counts
Upper	1.3880	29.5	0.340	2.167
Lower	1.0554	20.6	0.245	2.200
Mean	1.2217	25.0	0.292	2.184
Standard deviation	0.166	4.45	0.048	0.023

a =  $\mu\text{l O}_2/\text{g}/\text{hour}$

b =  $\mu\text{mole phenol}/\text{g}$

c = optical density at 550 nm

d = bacteria  $\times 10^4/\text{g}$

nitrogen, conductivity, organic matter, calcium, magnesium, sodium, percent available water at 0.3 atmospheres and percent sand, silt and clay were completed only for the samples taken during April, 1974. These values, along with the values of experimental water-holding capacity, mean bulk density, fertilizer treatment and above ground production (Appendix) were correlated with each other to determine any interrelationships. Table 9 presents the significant correlations within these soil analyses for both the native soil and spoils. The chemical and physical properties of spoils show very few significant interrelationships in comparison with the same properties of native soil. This fact appears to support the theory that spoils are very heterogeneous. The large differences may, in turn, represent differences between the developing ecosystem of spoils as compared to that of the native soils.

For the native soils, the properties of pH, organic matter, experimental water-holding capacity and mean bulk density appeared to be independent of all other physical and chemical properties. Both nitrate and phosphorus correlate with fertilizer treatment and above ground production. Among the other properties a complex interrelationship appears to be involved as illustrated by the positive correlation between potassium and %silt, and the negative correlation between potassium and %clay, while there also exists a negative correlation between %silt and %clay.

Table 9. Significant correlations within soil analyses for native soils and spoils April 1974

Soil Type	Correlated Properties		Significance Level
	Positive	Negative	
Native	Phosphorus:Above Ground Production Potassium:Conductivity Potassium:Calcium Nitrate:Fertilizer Nitrate:Above Ground Production Conductivity:Sodium Conductivity:Fertilizer Conductivity:Above Ground Production Sodium:%Sand	Magnesium:Sodium Magnesium:%Sand	5%
	Phosphorus:Fertilizer Potassium:Sodium Potassium:%Silt Conductivity:% H <sub>2</sub> O at 0.3 atm Calcium:Sodium Magnesium:%Clay	Potassium:Magnesium Potassium:%Clay Calcium:%Clay Magnesium:%Silt Sodium:%Clay %Sand:%Clay %Silt:%Clay	1%
Spoils	Phosphorus:Sodium %Sand:Mean Bulk Density Water-holding Capacity:Organic Matter	Organic Matter:%Clay %Sand:Above Ground Production	5%
		pH:Potassium %Sand:%Silt	1%

Spoils, however, show much less complex interrelationships between soil physical and chemical properties. Although for spoils there is only a total of two more properties than for native soils that are independent, only two of the interrelated properties are correlated with more than one other property. Thus, there are only seven significant correlations for spoils as compared to twenty-four for native soil.

#### Water-holding Capacity for Optimal Respiratory Rate

The respiratory rate of samples at water-holding capacities of 50%, 70%, 90%, 100%, 110%, 125%, 150% and 200% were measured to determine the water-holding capacity which would result in optimal respiratory activity. In each case, the respiratory activity was greatest at 100% water-holding capacity, as seen in Table 10.

#### Variability in Respiratory Rate Due to Quantity of Soil

In attempt to decrease the variation between replicates of respiratory activity, different quantities of soil were used for determining respiratory activity. Five grams, 10, 15 and 20 grams of soil were used in these measurements of respiratory rates.

Table 11 shows the results of these experiments. In each case, increasing the quantity of soil tended to increase the variance and decrease the respiratory rate per gram of soil.

Table 10. Water-holding Capacity for Optimal Respiratory Rate

Sample	100%	90%	Percent Water-holding Capacity					
			70%	50%	110%	125%	150%	200%
#1	10.7369	nd	7.7876	7.6852	nd	8.8529	8.6379	7.5953
	11.8421	nd	8.9793	8.1725	nd	7.3998	8.9726	7.7242
Mean	11.2895		8.3834	7.9288		8.1264	8.8052	7.6598
T-value*			6.194	9.644		6.002	7.452	11.300
P			P .01	P .01		P=.01	P .01	P .01
#2	15.6444	10.0069	nd	nd	11.3070	nd	nd	nd
	12.9915	11.0856	nd	nd	10.3027	nd	nd	nd
	14.8074	10.8350	nd	nd	11.8290	nd	nd	nd
Mean	14.4811	10.8350			11.1462			
T-value		4.526			3.697			
P		P .025			P .050			
#3	14.673	10.0708	nd	nd	11.7515	nd	nd	nd
	14.2927	12.4709	nd	nd	12.6969	nd	nd	nd
	10.4993	13.2145	nd	nd	13.8442	nd	nd	nd
Mean	13.1550	11.9187			12.7642			
T-value		0.756			0.778			
P		P .500			P .500			

44

\* T-value calculated comparing 100% Water-holding capacity with the given Water-holding capacity.

Table 11. Variability in respiratory rate due to quantity of soil

Run Number	Quantity of Soil(grams)			
	5	10	15	20
#1	13.9838 <sup>a</sup>	nd	nd	43.1415
	14.8078	nd	nd	54.2022
	15.2390	nd	nd	39.8979
	13.0580	nd	nd	64.9799
	12.6223	nd	nd	64.6265
	11.9813	nd	nd	54.9255
	<u>14.7202</u>	nd	nd	<u>51.9139</u>
Mean	13.7732			53.3839
Mean/gram	2.7546			2.6692
Standard deviation	1.147			8.887
#2	nd	27.5451	37.1718	45.9272
	nd	26.9220	41.2732	50.3269
	nd	26.7316	39.1134	38.4718
	nd	31.4732	34.6079	46.9614
	nd	<u>25.7867</u>	<u>30.8716</u>	<u>nd</u>
	Mean	27.6917	36.6076	45.4218
Mean/gram	2.7692	2.4415	2.2711	
Standard deviation	1.973	3.613	4.330	

a = respiration rate in uliters O<sub>2</sub>/hour

### Interrelationship of Respiratory Activity and Soil Chemical and Physical Properties

Correlations were calculated from the respiratory activity of both native soil and spoil samples from April, 1974, versus the corresponding values of the soil analyses. There were no significant correlations for spoils. The only significant correlation for native soil was for water-holding capacity which was significant at the 5% level.

### Respiratory Activity for Three Native and Three Spoils Plots

Figure 10 illustrates the results of the respiratory activity measurements for the six major plots, Native Range Unfertilized, Native Range Fertilized, BN, Gouge, Topsoil Gradient and Demo (Table 2), on samples taken April, August, October and December, 1974. An analysis of variance to determine the effects of different plants and different times for each plot is given in Table 12. In all cases, the effects of time and plants are not additive since the F-test was significant. The lower level of significance for the Topsoil Gradient plot can be easily accounted for by the smaller number of samples collected at that plot. For the effects of plant species alone, the analysis of variance given in Table 13 shows that individual plant species had no significant effect on respiratory activity. This value could not be calculated for the Topsoil Gradient plot since only one plant was sampled.



















































































































































































































































