



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
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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 O₂ 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.

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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

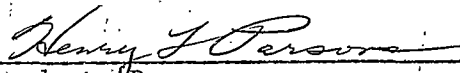
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ABSTRACT

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_2 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.

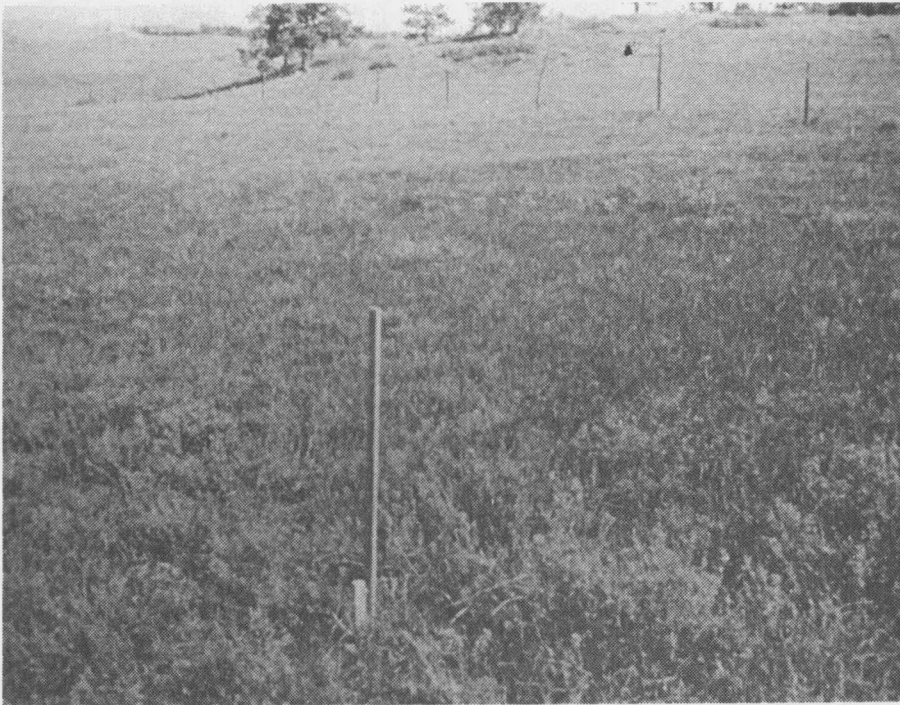


Figure 5. Photograph of Native Range Unfertilized and Native Range Fertilized native soil plots.

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 CaSO_4 - Ag_2SO_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;

KH_2PO_4 , 4 g.; Na_2HPO_4 , 6 g.; $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$, 1 mg.; MgSO_4 , 0.2 g.; CaCl_2 , 1 mg.; H_3BO_3 , 10 ug.; MnSO_4 , 10 ug.; ZnSO_4 , 70 ug.; CuSO_4 , 50 ug.; 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 steril 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 25°C at a wavelength of 260 nm on a Varian Techtron 635 UV-Vis Spectrophotometer. A Heto Ultrathermostat (London Company) was then used to raise the temperature in the spectrophotometer sample holders. The temperature was first quickly raised to 50°C and the absorbance recorded. The temperature was then raised 1°C 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 ^a	Phosphatase ^b	Pectinolyase ^c	Pectinolytic ^d 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 ^e
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 ^a	Phosphatase ^b	Pectinolyase ^c	Pectinolytic ^d 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 $\times 10^4/\text{g}$

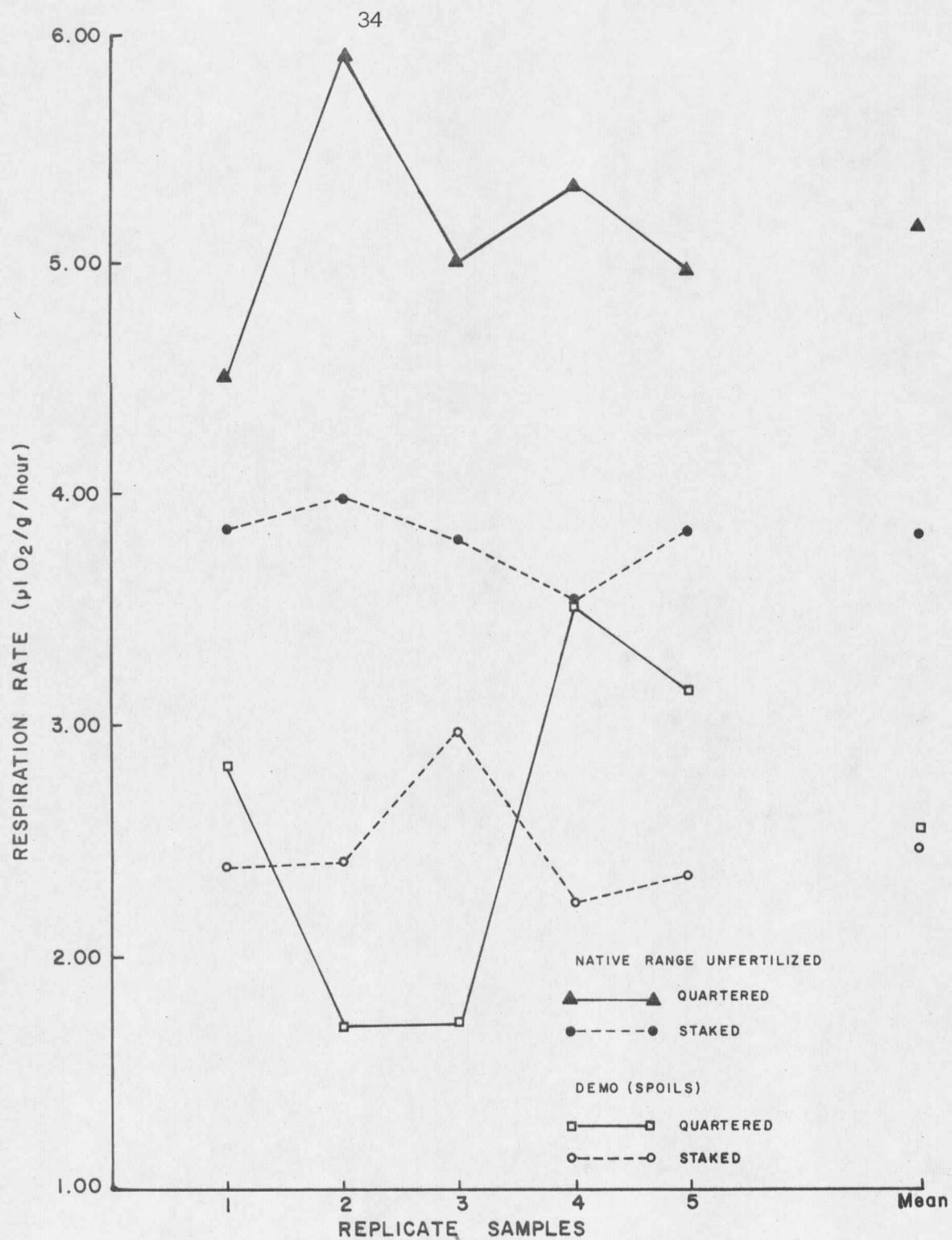


FIGURE 6. RESPIRATION RATE FOR SAMPLING VARIATION.

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-

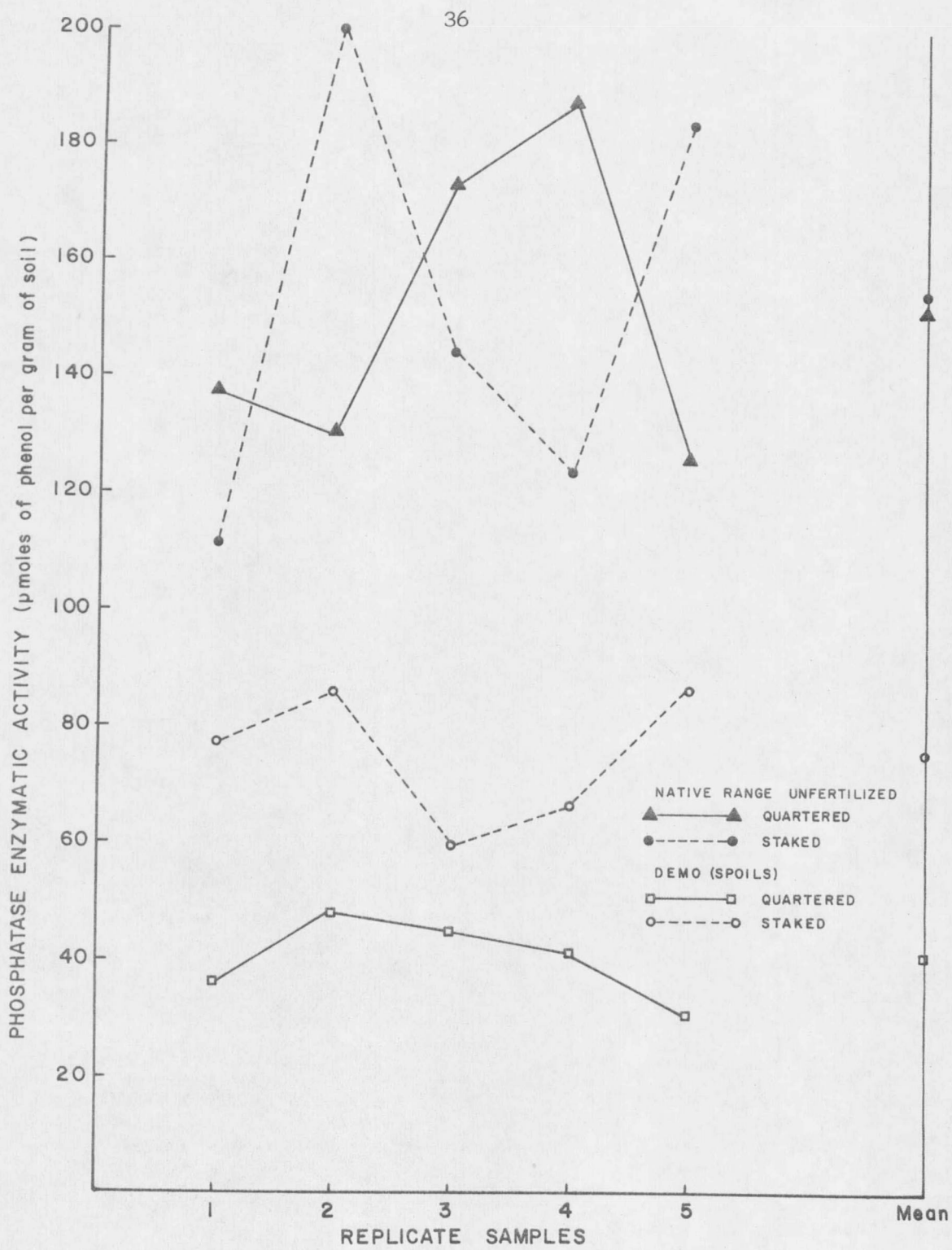


FIGURE 7. PHOSPHATASE ENZYMATIC ACTIVITY FOR SAMPLING VARIATION.

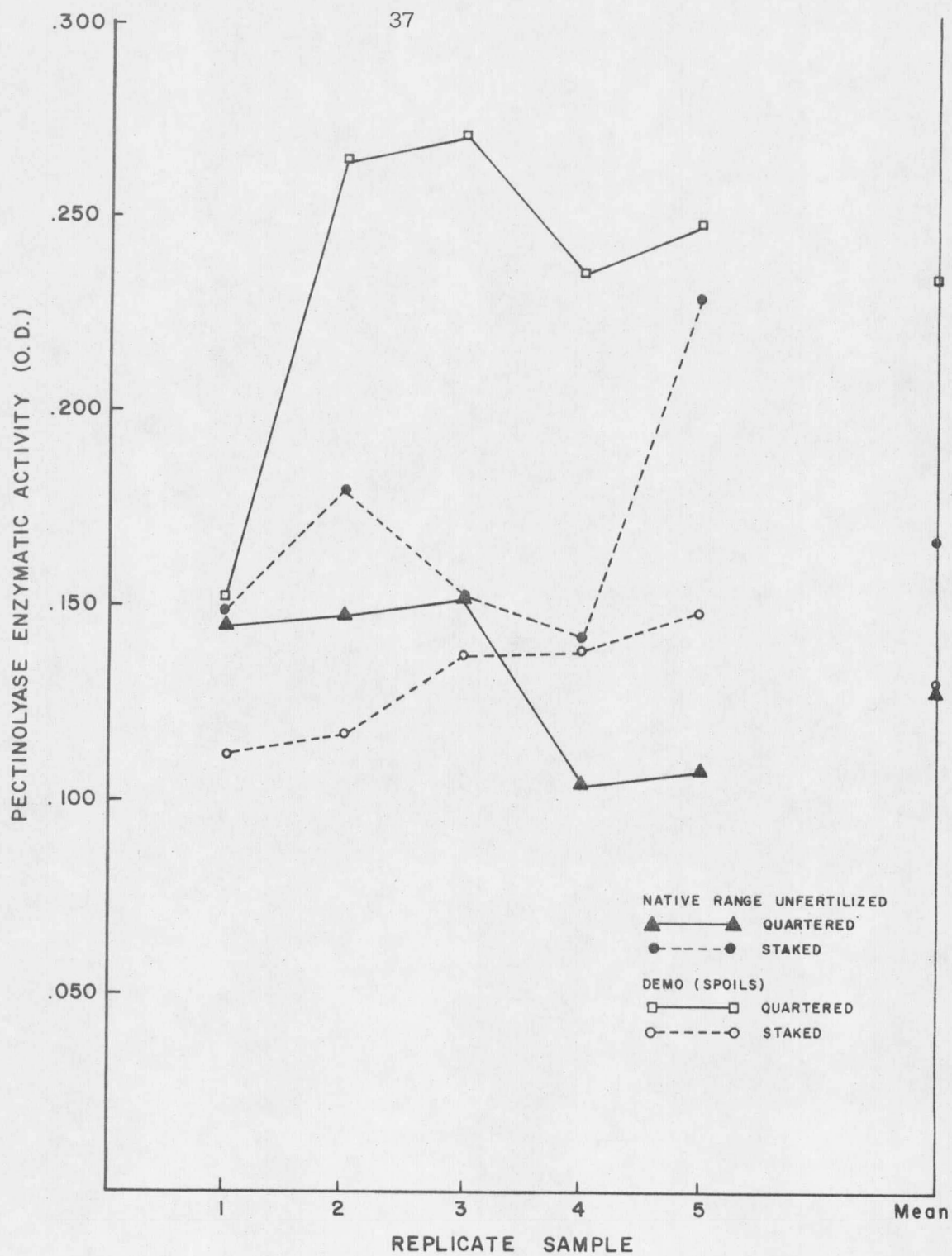


FIGURE 8. PECTINOLYASE ENZYMATIC ACTIVITY FOR SAMPLING VARIATION.

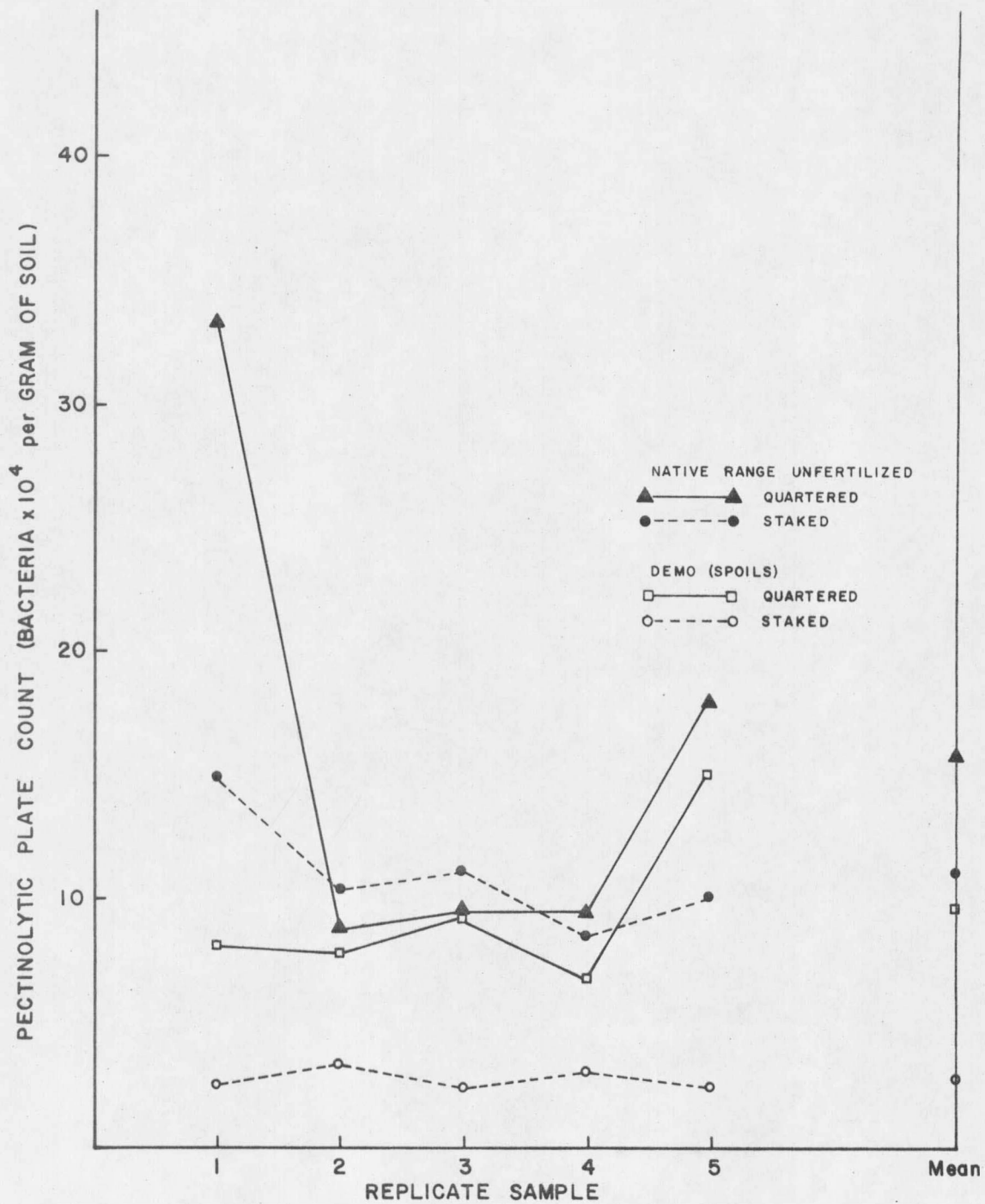


FIGURE 9. PECTINOLYTIC PLATE COUNT FOR SAMPLING VARIATION.

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

Plot and Treatment	SS ^a	df ^b SS	MS ^c	df ^d MS	F ^e	s ²	sig. ^f
<u>Native Range Unfertilized:</u>							
Staked Variation	.042	4	.01	68	.20	.05	ns ^g
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 ^a	Phosphatase ^b	Pectinolyase ^c	Pectinolytic ^d 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 = $\mu\text{mole phenol/g}$

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 ₂ 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			125%	150%	200%
			70%	50%	110%			
#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			

* 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 ^a	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 O₂/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.

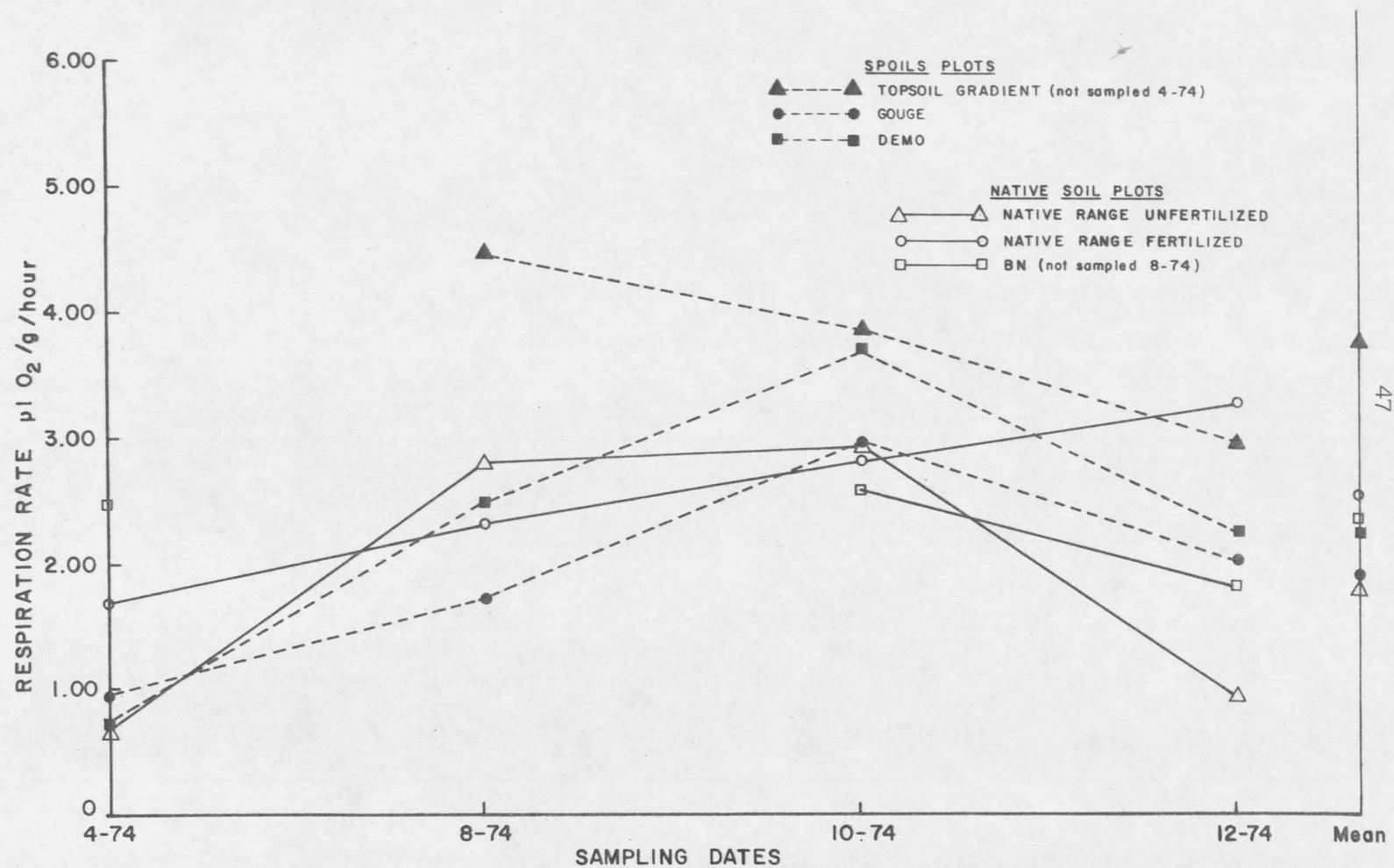


FIGURE 10. RESPIRATORY ACTIVITY FOR SIX MAJOR PLOTS.

Table 12. Analysis of variance due to plant and time for respiratory activity of six major plots

Plot	df ^a MStp	MS ^b tp	df ^c MSw	MS ^d w	F ^e	significance ^f
Native Range Unfertilized	6	4.059	42	0.379	10.70	0.5%
Native Range Fertilized	6	51.669	44	2.427	21.29	0.5%
BN	4	77.313	30	0.654	118.25	0.5%
Gouged	9	6.352	56	0.650	9.77	0.5%
Demo	6	7.425	42	0.548	13.54	0.5%
Topsoil Gradient	2	68.775	12	4.014	17.13	6.14%

a = degrees of freedom for the Mean Square of time and plants

b = Mean Square of time and plants

c = degrees of freedom for the Mean Square within

d = Mean Square within

e = F-value calculated from Mean Square_{tp}/Mean Square_w, where the null hypothesis is that the effects of time and plants are additive

f = null hypothesis rejected if significance is below 5%

Table 13. Analysis of variance due to plant species for respiratory activity of six major plots

Plots	df ^a MS _p	MS ^b p	df ^c MS _{tp}	MS ^d tp	F ^e	significance ^f
Native Range Unfertilized	2	9.166	6	4.059	2.26	ns ^g
Native Range Fertilized	2	131.398	6	51.669	2.54	ns
BN	2	9.754	4	77.313	0.13	ns
Gouge	3	13.728	9	6.352	2.16	ns
Demo	2	1.049	6	7.425	0.14	ns

a = degrees of freedom for the Mean Square of plant species

b = Mean Square of plant species

c = degrees of freedom for the Mean Square of time and plants (Variance)

d = Mean Square of time and plants

e = F-value calculated from $\text{Mean Square}_p / \text{Mean Square}_{tp}$, where the null hypothesis is that the variance due to each plant species is equal

f = null hypothesis rejected if significance level is below 5%

g = not significant

Seasonal Variation of Respiratory Activity

The effects of seasonal variation on respiratory activity are shown in Table 14. These effects are not significant at the two native soil plots, BN and Native Range Fertilized, but are significant for all three spoil plots and the other native soil plot, Native Range Unfertilized. In an attempt to determine the interrelationships of these seasonal variations, correlations were computed for respiratory activity at each plot for each sampling date against every other sampling date. Table 15 gives the significant correlations for these data. There seem to be large differences between plots in the interrelationships of sampling times on respiratory activity.

Effects of Air-drying Soil on Phosphatase Activity

To observe the effects of air-drying the soil on phosphatase activity, an experiment was conducted comparing activity of samples at field moisture with that of samples which had been air-dried. Table 16 lists the means of phosphatase activity for samples at field moisture versus air-dried and the correlation is highly significant. It was therefore concluded that the routine treatment of soil samples by air-drying did not alter the phosphatase activity.

Table 14. Seasonal variation of respiratory activity of six major plots

Plot	df_{MSt}^a	MS_t^b	$df_{s^2}^c$	$MS_{s^2}^d$	F^e	significance ^f
Native Range Unfertilized	50	462.856	3	4.802	96.39	1%
Native Range Fertilized	52	155.661	3	64.315	2.42	ns ^g
BN	36	58.540	2	41.808	1.40	ns
Gouge	68	266.208	3	9.413	28.28	1%
Demo	50	407.902	3	5.730	71.19	0.5%
Topsoil Gradient	12	68.740	2	4.019	17.10	8%

a = degrees of freedom for the Mean Square of time

b = Mean Square of time

c = degrees of freedom for s^2

d = Mean Square of s^2

e = F-value calculated from $Mean Square_t / s^2$, where the null hypothesis is that the effect of each time is the same

f = null hypothesis rejected if significance level is below 5%

g = not significant

Table 15. Significant correlations of seasonal variation for respiratory activity of six major plots

Plots	Correlated Sampling Dates	R ^a	significance
Native Range Unfertilized	April:December August:December	.829 .546	>1% >5%
Native Range Fertilized	October:December	.778	> 1%
BN	October:December	-.704	> 1%
Gouge	August:October	.501	> 5%
Demo	April:December April:October August:October October:December	-.909 -.929 .501 .681	> 1% > 1% > 5% > 1%
Topsoil Gradient ^B			

a = Correlation coefficient (R)

B = No dates were significant

Table 16. Phosphatase activity for air-dried and field moisture treatments of soils

Sample	Phosphatase Activity ^a	
	Field Moisture	Air-dried
#25	26 ^b	42
#26	130	74
#27	53	44
#28	500	290
#29	47	47
#30	455	325
#31	14	26
#32	390	345
#33	25	40
#34	1.6	0.6
#35	15	13
#36	200	250

Correlation coefficient = .930^ca = phosphatase activity measured in μ moles phenol/gram of soil

b = Mean values

c = >1% significance

Interrelationship of Phosphatase Activity and Soil Chemical and Physical Properties

Correlations were calculated for the phosphatase activity of both native soil and spoil samples from April, 1974, versus values for the soil analyses corresponding to each sample. There were no significant correlations for spoils versus soil analyses. Table 17 gives the significant correlations between phosphatase activity and soil analyses for native soils. It is interesting to note also that those soil properties which do correlate with phosphatase activity also correlate with one another. Referring back to Table 9 of significant correlations within the soil analyses, an example of the complex interrelationship exhibited is that potassium, calcium, sodium and silt, which all correlate negatively with phosphatase activity, also correlate positively with each other.

Phosphatase Activity for Three Native and Three Spoils Plots

Figure 11 gives the results of the phosphatase measurements for the six major plots, Native Range Unfertilized, Native Range Fertilized, BN, Gouge, Demo and Topsoil Gradient, on samples taken during April, August, October and December, 1974. Both Native Range sites peaked in August; this peak was not discernible in any of the other sites.

Table 17. Significant correlations between phosphatase activity and the soil analyses for native soils in April, 1974

Correlated Properties	R ^a	significance
Potassium:Phosphatase Activity ^B	-.762	> 5%
Calcium:Phosphatase Activity	-.747	> 5%
Magnesium:Phosphatase Activity	.912	1%
Sodium:Phosphatase Activity	-.766	> 5%
Silt:Phosphatase Activity	-.957	> 1%
Clay: Phosphatase Activity	.872	> 1%

a = correlation coefficient; R

B = Phosphatase Activity in umoles phenol/gram of soil

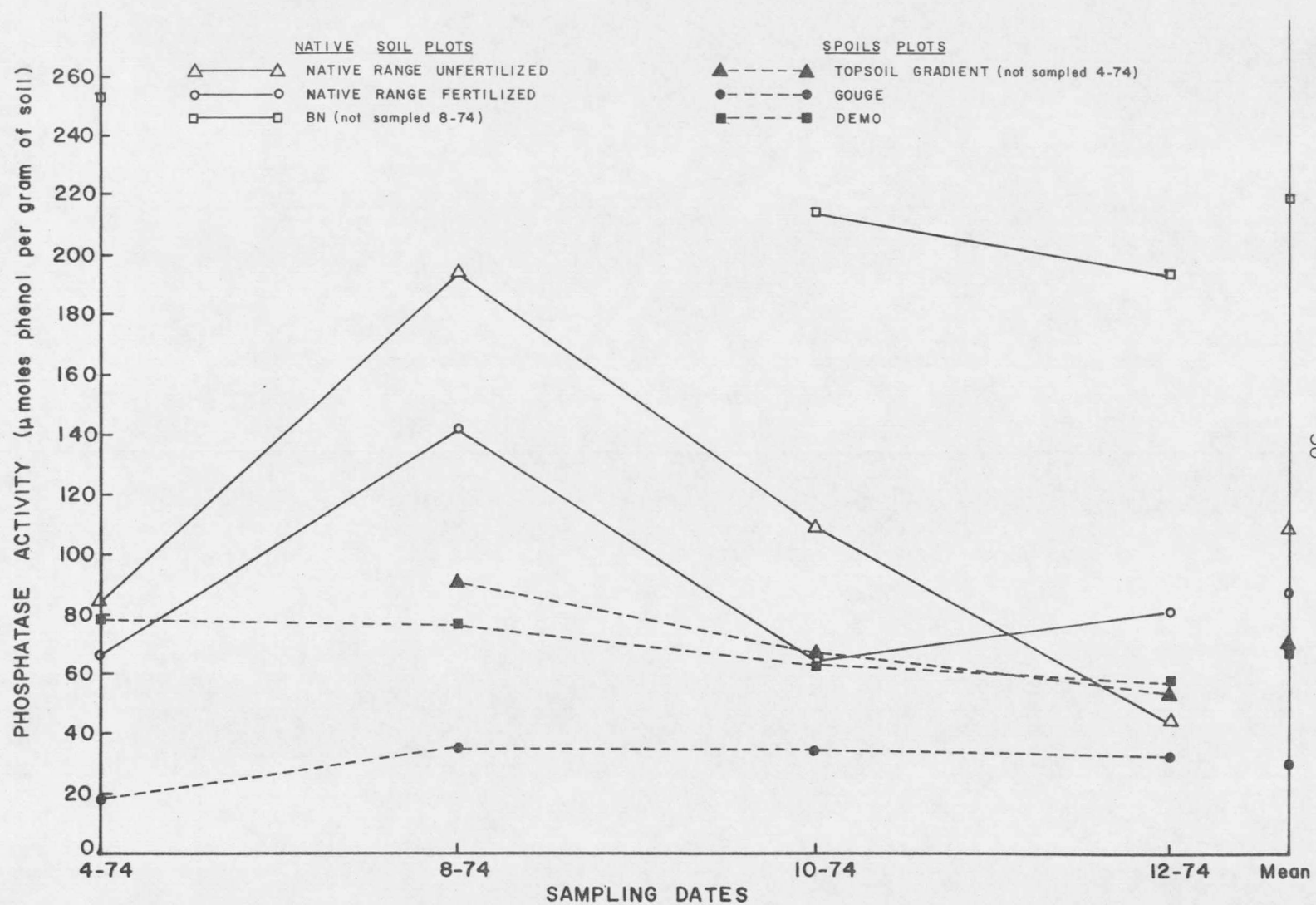


FIGURE II. PHOSPHATASE ACTIVITY FOR SIX MAJOR PLOTS.

The influence of individual plants on this activity was determined by an analysis of variance, which is summarized in Table 18. In all sites except BN the influence of individual plant species was not of great significance.

Another analysis of variance was calculated to determine the influence of the combination of individual plants and separate times on phosphatase activity. Table 19 shows that in all cases the null hypothesis that the effects of time and plants are additive is rejected, which means that the effects of time are not the same as the effects of plants.

Seasonal Variation of Phosphatase Activity

The effects of seasonal variation on phosphatase activity are shown in Table 20. These effects are significant for all plots except native soil plot BN. The level of significance is greater for spoils than for the native soil plots. The interrelationships of these seasonal variations were examined by computing correlations within each plot for phosphatase activity at each sampling date against every other sampling date. Those data which were significant, as shown in Table 21, suggest the involvement of complex interactions. For example, the correlations in the Native Range Unfertilized plot, one sampling date, April, correlated with two other sampling dates, August and October, which are in turn correlated with each other.

Table 18. Analysis of variance due to plant species for phosphatase activity of six major plots

Plots	df ^a _{MSp}	MS ^b _p	df ^c _{MStp}	MS ^d _{tp}	F ^e	significance ^f
Native Range Unfertilized	2	176.608	6	27.216	6.49	17.1%
Native Range Fertilized	2	59.136	6	31.044	1.90	ns ^g
BN	2	506.521	4	16.511	30.68	2.9%
Gouge	3	27.861	9	2.865	9.72	4.6%
Demo	2	42.134	6	4.390	9.60	10%
Topsoil Gradient	- ^h	---	-	---	---	-

a = degrees of freedom for the Mean Square of plant species

b = Mean Square of plant species

c = degrees of freedom for the Mean Square of time and plants

d = Mean Square of time and plants (variance)

e = F-value calculated from Mean Square_p/Mean Square_{tp}, where the null hypothesis is that the variance due to each plant species is equal

f = null hypothesis rejected if significance level is below 5%

g = not significant

h = not computed for TG since only one plant sampled

Table 19. Analysis of variance due to plant and time for phosphatase activity of six major plots

Plot	df ^a MStp	MS ^b tp	df ^c MSw	MS ^d w	Fe	significance ^f
Native Range Unfertilized	6	27.216	42	1.444	18.84	> 0.5%
Native Range Fertilized	6	31.044	42	0.650	47.75	> 0.5%
BN	4	16.511	29	1.206	13.69	1%
Gouge	9	2.865	56	0.795	3.61	2.5%
Demo	6	4.390	42	0.655	6.70	2%
Topsoil Gradient	2	180.600	12	2.183	82.72	2%

a = degrees of freedom for the Mean Square of time and plants

b = Mean Square of time and plants

c = degrees of freedom for the Mean Square within

d = Mean Square within

e = F-value calculated from $\text{Mean Square}_{tp} / \text{Mean Square}_w$, where the null hypothesis is that the effects of time and plants are additive

f = null hypothesis is rejected if significance is below 5%

Table 20. Seasonal variation of phosphatase activity of six major plots

Plot	df ^a _{MS_t}	MS ^b _t	df ^c _{s²}	MS ^d _{s²}	F ^e	significance ^f
Native Range Unfertilized	50	463.411	3	49.997	9.27	5%
Native Range Fertilized	50	164.394	3	32.699	5.03	11%
BN	35	72.392	2	137.392	0.53	ns ^g
Gouge	68	167.772	3	10.703	15.68	3.5%
Demo	50	142.580	3	13.196	10.80	4%
Topsoil Gradient	12	180.600	2	2.183	82.73	1.5%

a = degrees of freedom for the Mean Square of time

b = Mean Square of time

c = degrees of freedom for s²d = Mean Square of s²e = F-value calculated from Mean Square_t/s², where the null hypothesis is that the effect of each time is the same

f = null hypothesis is rejected if significance level is below 5%

g = not significant

Table 21. Significant correlations of seasonal variation for phosphatase activity of six major plots

Plots	Correlated Sampling Dates	R ^a	significance
Native Range Unfertilized	April:August	.864	> 1%
	April:October	.621	> 5%
	August:October	.673	> 1%
Native Range Fertilized	April:October	.904	> 1%
	April:December	.968	> 1%
	October:December	.902	> 1%
BN	April:October	.979	> 1%
	April:December	.955	> 1%
	October:December	.963	> 1%
Gouge	April:August	-.771	> 1%
	April:October	-.557	> 5%
Demo	August:October	.900	> 1%
	August:December	.623	> 1%
	October:December	.687	> 1%
Topsoil Gradient ^b	--	--	-

a = Correlation coefficient (R)

b = No dates were significant

April correlated significantly with October for the three native soil plots and for the spoil Gouge plot. Demo plot shared the October:December correlation with Native Range Fertilized and BN, and the August:October correlation with Native Range Unfertilized while the August:December correlation was limited to the Demo plot.

Effects of Air-drying on Pectinolyase Activity

To observe the effects of air-drying the soil on pectinolyase activity, an experiment was conducted comparing the activity of samples at field moisture with that of samples which had been air-dried. Table 22 lists the mean pectinolyase activity for samples at field moisture and after air-drying. Since the correlation between these two treatments is significant at greater than the 5% level, all further pectinolyase activity measurements were done using air-dried soil.

Interrelationship of Pectinolyase Activity and Soil Chemical and Physical Properties

Correlation coefficients were calculated for the pectinolyase activity of both native soil and spoil samples from April, 1974, versus values for the soil analyses corresponding to each sample. There were no significant correlations between pectinolyase activity and the soil analyses for either native soil or spoils.

Table 22. Pectinolyase activity for air-dried and field moisture treatments of soils

Sample	Pectinolyase Activity ^a	
	Field Moisture	Air-dried
#25	0.295 ^b	0.229
#26	0.435	0.291
#27	0.472	0.219
#28	0.475	0.174
#29	0.142	0.108
#30	0.165	0.084
#31	0.202	0.145
#32	0.130	0.100
#33	0.178	0.231
#34	0.159	0.239
#35	0.172	0.186
#36	0.142	0.201

Correlation coefficient = .487^c

a = pectinolyase activity measured as optical density at 550 nm

b = Mean values

c = >5% significance

Pectinolyase Activity for Three Native and Three Spoil Plots

Figure 12 gives the results of the pectinolyase activity measurements for the six major plots, Native Range Unfertilized, Native Range Fertilized, BN, Gouge, Demo and Topsoil Gradient, on samples taken during April, August, October and December 1974. With the exception of the BN plot, the pectinolyase activity follows the same general trend at each plot, ending with a peak for the December measurements.

To examine the question of whether individual plant species greatly influence pectinolytic activity, an analysis of variance, summarized in Table 23, was computed. The results show that the influence of individual plant species is not significant in all cases.

An analysis of variance to determine the influence of both time and plant on pectinolyase activity is summarized in Table 24. The effects of time and plant together are significant and therefore, they are not additive effects for all plots except Demo.

Seasonal Variation of Pectinolyase Activity

The effects of seasonal variation on pectinolyase activity are shown in Table 25. These effects show a high level of significance for each plot, excepting only BN. The 25% level of significance found for seasonal variation at BN is not a good level of significance, but it may nevertheless be important for a generalized idea of

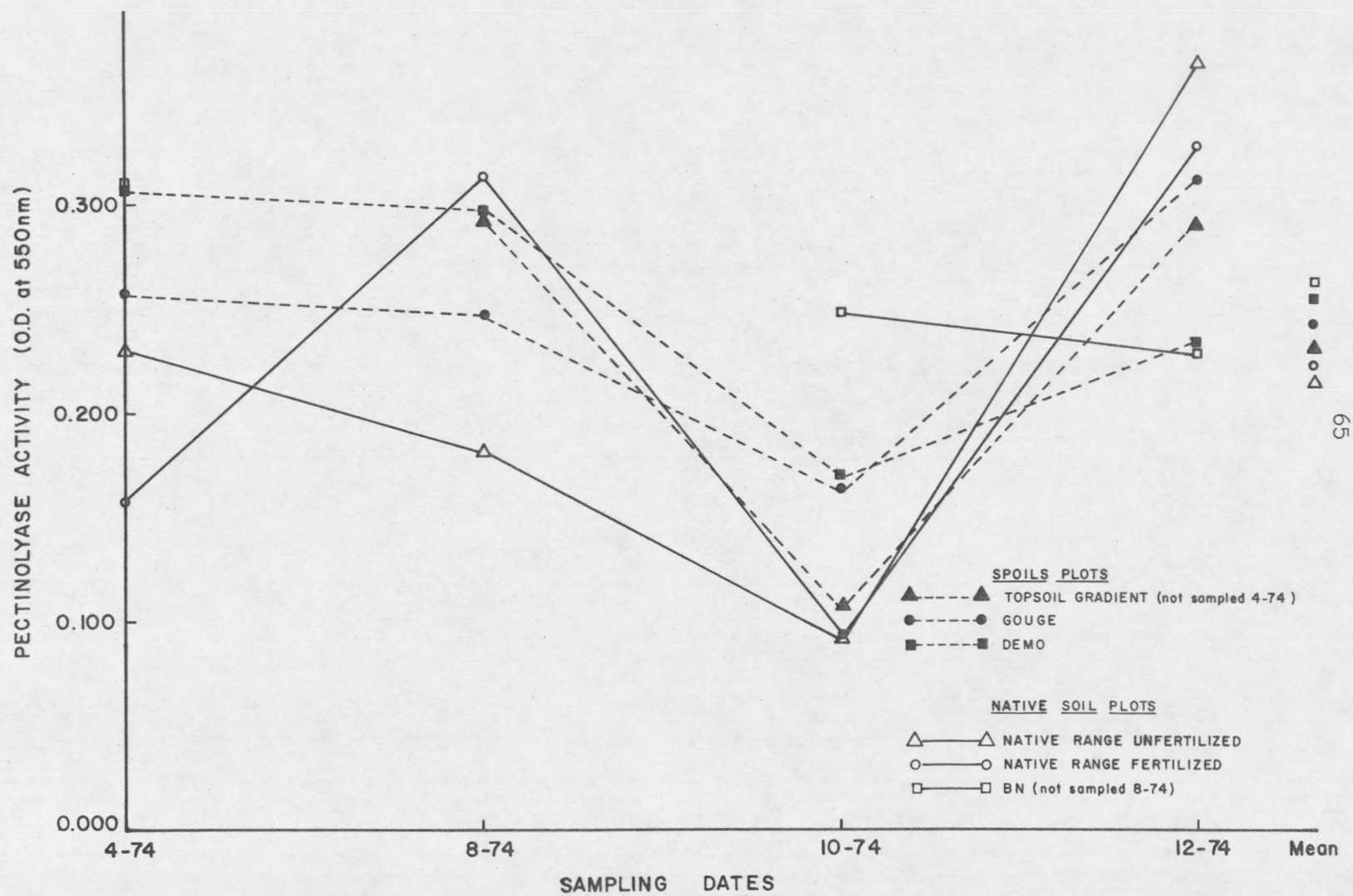


FIGURE 12. PECTINOLYASE ACTIVITY FOR SIX MAJOR PLOTS.

Table 23. Analysis of variance due to plant species for pectinolyase activity of six major plots.

Plots	df ^a MS _p	MS ^b p	df ^c MS _{tp}	MS ^d tp	F ^e	significance ^f
Native Range Unfertilized	2	74.559	6	48.842	1.53	ns ^g
Native Range Fertilized	2	6.352	6	73.315	0.09	ns
BN	2	14.810	4	54.215	0.27	ns
Gouge	3	17.624	9	12.510	1.41	ns
Demo	2	9.997	6	5.616	1.78	ns
Topsoil Gradient	- ^h	--	-	--	--	-

a = degrees of freedom for the Mean Square of plant species

b = Mean Square of plant species

c = degrees of freedom for the Mean Square of time and plants

d = Mean Square of time and plants (s^2)e = F-value calculated from Mean Square_p/Mean Square_{tp}, where the null hypothesis is that the variance due to each plant species is equal

f = null hypothesis rejected if significance level is below 5%

g = not significant

h = not computed for TG since only one plant sampled.

Table 24. Analysis of variance due to plant and time for pectinolyase activity of six major plots

Plot	df ^a _{MStp}	MS ^b _{tp}	df ^c _{MSw}	MS ^d _w	F ^e	significance ^f
Native Range Unfertilized	6	48.842	42	1.922	25.42	0.5%
Native Range Fertilized	6	73.315	42	4.708	15.57	0.5%
BN	4	54.215	30	11.320	4.79	8%
Gouge	9	12.510	56	3.436	3.64	2.5%
Demo	6	5.616	42	4.823	1.16	ns ^g
Topsoil Gradient	2	1260.470	12	12.992	97.02	1%

a = degrees of freedom for the Mean Square of time and plants

b = Mean Square of time and plants

c = degrees of freedom for the Mean Square within

d = Mean Square within

e = F-value calculated from Mean Square_{tp}/Mean Square_w, where the null hypothesis is that the effects of time and plants are additive

f = null hypothesis rejected if significance is below 5%

g = not significant

Table 25. Seasonal variation of pectinolyase activity for six major plots

Plot	df ^a _{MSt}	MS ^b _t	df ^c _{s2}	MS ^d _{s2}	Fe	significance ^f
Native Range Unfertilized	50	3811.38	3	36.556	104.26	0.5%
Native Range Fertilized	50	3842.42	3	57.111	67.28	0.5%
BN	36	289.86	2	61.135	4.74	25%
Gouge	68	1349.61	3	22.035	61.25	0.5%
Demo	50	988.50	3	21.886	45.17	0.5%
Topsoil Gradient	12	1260.47	2	12.992	97.02	2%

a = degrees of freedom for the Mean Square of time

b = Mean Square of time

c = degrees of freedom for s^2

d = Mean Square of s^2

e = F-value calculated from $\text{Mean Square}_t / s^2$, where the null hypothesis is that the effect of each time is the same

f = null hypothesis is rejected if significance level is below 5%

seasonal effects on pectinolyase activity. To examine any possible interrelationships between different season effects, correlation coefficients were calculated for pectinolyase activity at each plot for each sampling date against every other sampling date. Those data showing significant correlations are found in Table 26. The correlations appeared to be quite independent for each plot. In only two instances was there any overlap in significant correlation and those were the correlation of April with December shared by the Native Range Unfertilized and Gouge plots and the correlations of October with December shared by the Native Range Fertilized and BN plots.

Effects of Purifying Pectin on Selective Plating of Pectinolytic Bacteria

It was initially found that the commercial pectin used as the sole carbohydrate source in a selective medium for pectinolytic organisms contained other carbohydrate residues. Therefore, a procedure for purifying pectin was developed and a comparison made of samples plated on commercial pectin medium and on medium with purified pectin. The results of this comparison are found in Table 27. The numbers of bacteria using purified pectin were significantly lower than the number of bacteria enumerated with commercial pectin. All further isolation and enumeration of pectinolytic bacteria was made using medium containing purified pectin.

Table 26. Significant correlations of seasonal variation for pectinolyase activity of six major plots

Plots	Correlated Sampling Dates	R ^a	significance
Native Range Unfertilized	April:December	.580	5%
Native Range Fertilized	April:August	-.895	1%
	April:October	.745	1%
	October:December	-.505	5%
BN	October:December	-.486	5%
Gouge	April:December	.718	1%
Demo	August:December	.471	5%
Topsoil Gradient ^b	--	--	--

a = Correlation coefficient (R)

b = No dates were significant

Table 27. Comparison of pectinolytic bacteria selective media with commercial pectin and purified pectin

Sample	Pectinolytic Bacteria ^A		t-value	Significance Level
	Commercial Pectin	Purified Pectin		
#78	2,383 ^B	29	13.06	99.9%
#79	1,560	13	12.70	99.9%
#83	25	3	14.31	99.9%

A = number of bacteria $\times 10^4$ per gram of soil

B = mean of five replicates

Effects of Air-drying Soil on Pectinolytic Bacteria Enumeration

The effects of air-drying the soil prior to the enumeration of pectinolytic bacteria were studied by comparing the results from enumeration of field moist and air-dried Manhattan silt loam. These results, shown in Table 28, demonstrate that rather than decreasing the number of bacteria, air-drying usually increased the numbers. This relationship is supported by the negative correlation coefficient, which is significant at greater than the 5% level.

Relative Proportion of Total Soil Bacterial Population Estimated as Pectinolytic Bacteria

To determine the relative proportion of the total soil bacterial population which the pectinolytic bacteria constitute, a comparison was made between numbers of bacteria on Soil Extract Medium for the "total" soil bacteria and numbers of bacteria on Mineral Pectin Medium with purified pectin. The results of this comparison can be seen in Table 29. The number of pectinolytic bacteria averaged 8.36% of the total soil bacterial population, most comparisons being rated highly significant.

Interrelationship of Pectinolytic Bacterial Population and Soil Chemical and Physical Properties

To examine the interrelationship between the numbers of pectinolytic bacteria and soil chemical and physical properties, correlation

Table 28. Number of pectinolytic bacteria for air-dried and field moisture treatments of soils with Manhattan silt loam

Sample	<u>Number of Pectinolytic Bacteria^a</u>	
	Field Moisture	Air-dried
#1	102.00 ^b	109.33
#2	63.33	236.00
#3	86.00	46.33
#4	83.78	130.56

Correlation coefficient = $-.678$, significance 5%

a = number of bacteria $\times 10^4$ per gram of soil

b = mean of five replicates

Table 29. Relative proportion of soil bacteria as pectinolytic bacteria

Sample	Number of Bacteria ^a		%Soil Bacteria ^d	t-value	significance
	Total ^b	Pectin ^c			
#78	360.00	19.00	5.28%	14.16	0.1%
#79	470.00	33.76	7.18%	2.71	5%
#81	873.33	4.01	0.46%	4.27	10%
#83	294.67	3.84	1.3%	3.97	0.5%
#82	145.33	27.75	19.1%	7.45	0.5%
#171	60.36	3.36	5.57%	24.48	0.1%
#173	65.22	4.87	7.47%	17.06	0.1%
#180	155.57	7.09	4.56%	8.54	0.1%
#181	390.34	86.59	22.18%	5.77	0.1%
#182	215.90	22.62	10.48%	3.96	0.5%
Mean			8.36%		

a = number of bacteria x 10⁴ per gram of soil

b = total soil bacteria from Soil Extract Medium

c = Pectinolytic bacteria from Mineral Pectin Medium

d = percent of total soil bacteria as pectinolytic bacteria

coefficients were calculated for these values at native soil and at spoil plots for April, 1974. There were no significant correlations between the number of pectinolytic bacteria and the soil analyses for either native soil or spoils.

Pectinolytic Bacterial Population for Three Native and Three Spoil Plots

Figure 13 gives the results of the pectinolytic bacteria counts for the six major plots, Native Range Unfertilized, Native Range Fertilized, BN, Demo, Gouge and Topsoil Gradient, on samples taken during April, August, October and December, 1974. The numbers of pectinolytic bacteria follow the same general trends for all plots except BN. The most striking feature of this trend is the peak for October, 1974.

To determine if the numbers of pectinolytic bacteria were directly influenced by individual plant species, an analysis of variance (Table 30) was computed. This shows that individual plant species were largely significant in determining the numbers of pectinolytic bacteria at the BN plot, much less significant at the Demo plot and not significant at all other plots.

The influence of time and plant together was also examined by an analysis of variance (Table 31). The analysis of variance shows that only for the BN plot was the influence of plant and time not significant. Thus, for all other plots the effects of plant and time

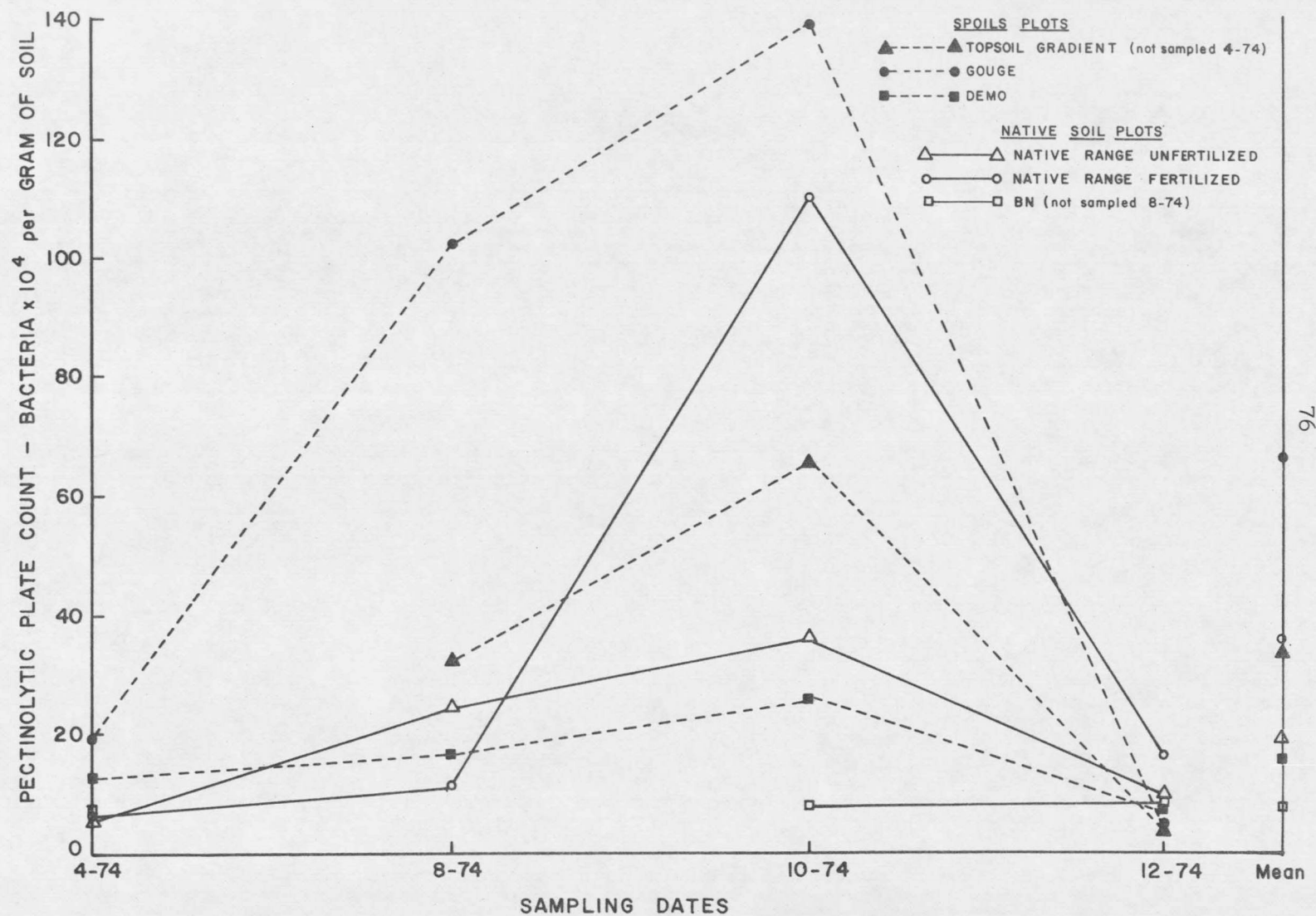


FIGURE 13. NUMBERS OF PECTINOLYTIC BACTERIA FOR SIX MAJOR PLOTS.

Table 30. Analysis of variance due to plant species for number of pectinolytic bacteria of six major plots

Plot	df ^a MSp	MS ^b p	df ^c MStp	MS ^d tp	F ^e	significance ^f
Native Range Unfertilized	2	0.252	6	4.165	0.06	ns ^g
Native Range Fertilized	2	118.795	6	98.681	1.20	ns
BN	2	2.000	4	0.011	177.09	0.8%
Gouge	3	207.261	9	116.376	1.78	ns
Demo	2	23.353	6	1.650	14.15	7.8%
Topsoil Gradient ^h	-	--	-	--	--	--

a = degrees of freedom for the Mean Square of plant species

b = Mean Square of plant species

c = degrees of freedom for the Mean Square of time and plants

d = Mean Square of time and plants (s^2)e = F-value calculated from Mean Square_p/Mean Square_{tp}, where the null hypothesis is that the variance due to each plant species is equal

f = null hypothesis rejected if significance level is below 5%

g = not significant

h = not computed for TG since only one plant sampled

Table 31. Analysis of variance due to plant and time for number of pectinolytic bacteria of six major plots

Plot	df ^a _{MStp}	MS ^b _{tp}	df ^c _{MSw}	MS ^d _w	F ^e	significance ^f
Native Range Unfertilized	6	4.165	48	0.720	5.79	2.5%
Native Range Fertilized	6	98.681	48	1.179	83.69	0.5%
BN	4	0.011	36	0.112	0.10	ns ^g
Gouge	9	116.376	64	10.443	11.14	0.5%
Demo	6	1.650	48	0.137	12.04	0.5%
Topsoil Gradient	2	47.922	12	2.805	17.09	6%

a = degrees of freedom for the Mean Square of time and plants

b = Mean Square of time and plants

c = degrees of freedom for the Mean Square within

d = Mean Square within

e = F-value calculated from Mean Square_{tp}/Mean Square_w, where the null hypothesis is that the effects of time and plants are additive

f = null hypothesis rejected if significance is below 5%

g = not significant

are not additive which means that there were interactions between plant and time.

Seasonal Variation of the Number of Pectinolytic Bacteria

The effects of seasonal variation on the pectinolytic bacteria population are shown in Table 32. The seasonal variation appears to have significant effects on the pectinolytic bacterial population at all plots with the exception of BN. To determine if there were any interrelationships between the effects of one sampling period versus every other sampling period, correlation coefficients were calculated for the pectinolytic bacterial population at each plot. Those data which were significant are shown in Table 33. There were no significant correlations for the Native Range Unfertilized plot. The October:December correlation was the only correlation significant for both of the two native soil plots, Native Range Fertilized and BN, and the two spoil plots, Gouge and Demo. In comparing plots, Native Range Fertilized and Demo shared the same correlations, although Demo had three additional correlations, and BN shared correlations with Gouge, although Gouge had one additional significant correlation. There appear to be some complex interrelationships as exhibited by the significant correlations of the BN plot where April correlated with both October and December, which in turn correlated with each other.

Table 32. Seasonal variation of the number of pectinolytic bacteria for six major plots.

Plot	df ^a _{MSt}	MS ^b _t	df ^c _{s2}	MS ^d _{s2}	F ^e	significance ^f
Native Range Unfertilized	56	29.629	3	1.073	27.621	1%
Native Range Fertilized	56	366.197	3	15.827	23.14	2%
BN	42	0.090	2	0.192	0.47	ns ^g
Gouge	76	849.301	3	30.756	27.61	1%
Demo	56	8.696	3	1.128	7.71	7%
Topsoil Gradient	12	47.900	2	2.804	17.08	6%

a = degrees of freedom for the Mean Square of time

b = Mean Square of time

c = degrees of freedom for s²d = Mean Square of s²e = F-value calculated from Mean Square_t/s², where the null hypothesis is that the effect of each time is the same

f = null hypothesis is rejected if significance level is below 5%

g = not significant

Table 33. Significant correlations of seasonal variation for numbers of pectinolytic bacteria of six major plots

Plot	Correlated Sampling Dates	R ^a	significance
Native Range Unfertilized	None		
Native Range Fertilized	April:August	.553	5%
	August:October	-.512	5%
	October:December	.916	1%
BN	April:October	.549	5%
	April:December	.668	1%
	October:December	.545	5%
Gouge	April:October	.591	1%
	April:December	.602	1%
	August:December	.603	1%
	October:December	.677	1%
Demo	April:August	.884	1%
	April:October	.873	1%
	April:December	.830	1%
	August:October	.959	1%
	August:December	.911	1%
	October:December	.951	1%
Topsoil Gradient	None		

a = Correlation coefficient, R

Interrelationship of Microbiological Parameters

Correlation coefficients were calculated between each microbiological parameter and the other three microbiological parameters for the three native soil plots, the three spoil plots and for all six plots combined. These correlations are tabulated as a complete matrix in Table 34. The only significant correlation was between pectinolyase activity and the number of pectinolytic bacteria. The relationship that exists between these two is also shown by Figure 14 in a linear regression plot.

Overall Analysis of Variance for the Microbiological Parameters

The Chi-square was calculated to compare variances for each microbiological parameter on native soil plots, spoils plots and all six plots combined. The results, shown in Table 35, demonstrate that although for each microbiological parameter the intraplot variances differed from plot to plot, there were differences which were not significant between spoils and native soil. The native soil on the whole had smaller differences in intraplot variances from plot to plot for both respiratory activity and phosphatase activity than did spoils.

Table 36 gives the pooled variances of native soil and spoils. The ratio of these pooled variances is significant only for the number of pectinolytic bacteria and this is due to an extremely high variance for

Table 34. Correlations of four microbiological parameters as measured at three native soil and three spoils plots during April, August, October and December, 1974

Native Soil	Res ^a	Phos ^b	Pec ^c	PecPL ^d
Res	--	.307	-.267	.393
Phos	.307	--	.116	-.318
Pec	-.267	.116	--	-.611 ^e
PecPL	.393	-.318	-.611	--
Spoils	Res	Phos	Pec	PecPL
Res	--	.458	-.446	.171
Phos	.458	--	.144	-.296
Pec	-.446	.114	--	-.575 ^e
PecPL	.171	-.296	-.575 ^e	--
6 Plots Combined	Res	Phos	Pec	PecPL
Res	--	.118	-.318	.274
Phos	.118	--	.036	-.325
Pec	-.318	.036	--	-.523 ^e
PecPL	.274	-.325	-.523 ^e	--

a = Respiratory activity in $\mu\text{lo}_2/\text{g}/\text{hour}$

b = phosphatase activity in $\mu\text{moles phenol per gram of soil}$

c = pectinolyase activity in optical density at 550 nm

d = number of pectinolytic bacteria in bacteria $\times 10^4$ per gram of soil

e = significant at $>5\%$

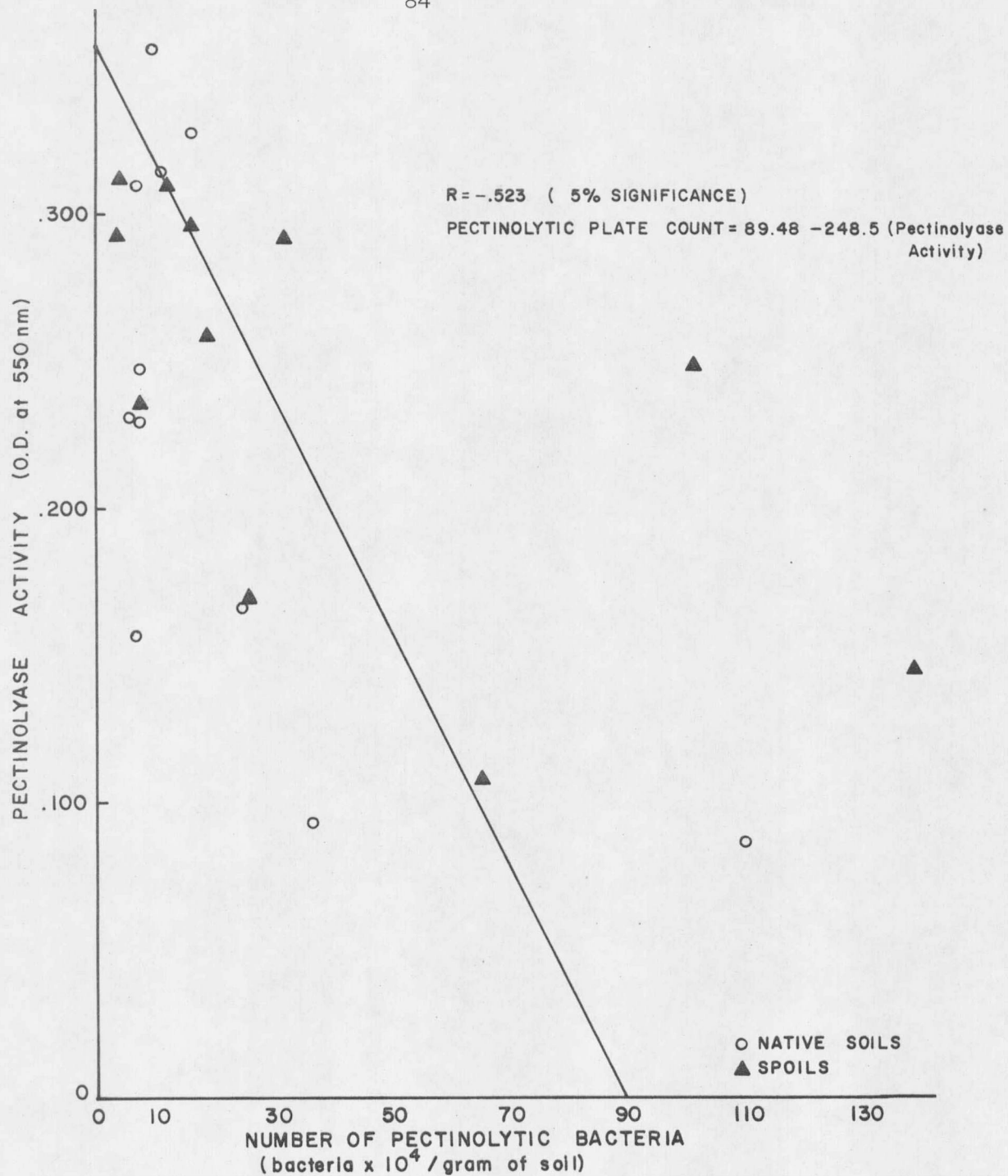


FIGURE 14. LINEAR REGRESSION OF PECTINOLYASE ACTIVITY AGAINST PECTINOLYTIC PLATE COUNT.

Table 35. Chi-square for the four microbiological parameters

Test	χ^2_T	P	df	χ^2_N	P	df	χ^2_S	P	df
Respiration	66.3	0.005	5	9.1	0.010	2	29.7	0.005	2
Phosphatase	20.1	0.005	5	6.6	0.025	2	8.6	0.010	2
Pectinolyase	33.2	0.005	5	27.1	0.005	2	11.0	0.005	2
Pectinolytic Plate Count	308.7	0.005	5	43.1	0.005	2	146.3	0.005	2

χ^2_T = Chi-square total

χ^2_N = Chi-square of native soil plots

χ^2_S = Chi-square of spoil plots

Table 36. Pooled variances of microbiological parameters

Test	<u>Pooled Variances</u>		Ratio ^c
	<u>Native</u>	<u>Spoils</u>	
Respiration	1.226	0.978	1.254 ^a
Phosphatase	1.088	0.893	1.218 ^a
Pectinolyase	5.420	5.008	1.082 ^a
Pectinolytic Plate Count	0.720	5.714	0.125 ^b

a = not significant at 10% level

b = significant at the 0.1% level

c = test for equality of pooled variances; test is especially poor when variances within are unequal

the spoils Gouge plot. The lack of significance can be interpreted as meaning that both native soil and spoils are approximately the same regarding homogeneity.

Isolation and Identification of Soil Organisms

Several colonies commonly occurring on the Mineral Pectin Medium were isolated and biochemical tests were performed for identification. By this means, several organisms were identified as Bacillus spp. An additional organism showed pleomorphic morphology and was not identified with certainty. This organisms, a suspected Arthrobacter, was used for the DNA T_m determination.

Isolation of DNA, and Determination of T_m to Identify a Soil Organism

DNA was isolated from the unidentified soil organism; the final concentration of DNA was determined to be 280 ug/ml and the thermal melting point was determined twice for a solution of the DNA containing 56 ug/ml. The T_m was determined as 86.6C and from this the percent G:C equals 81.78. The normal probability plot of the T_m is shown in Figure 15.

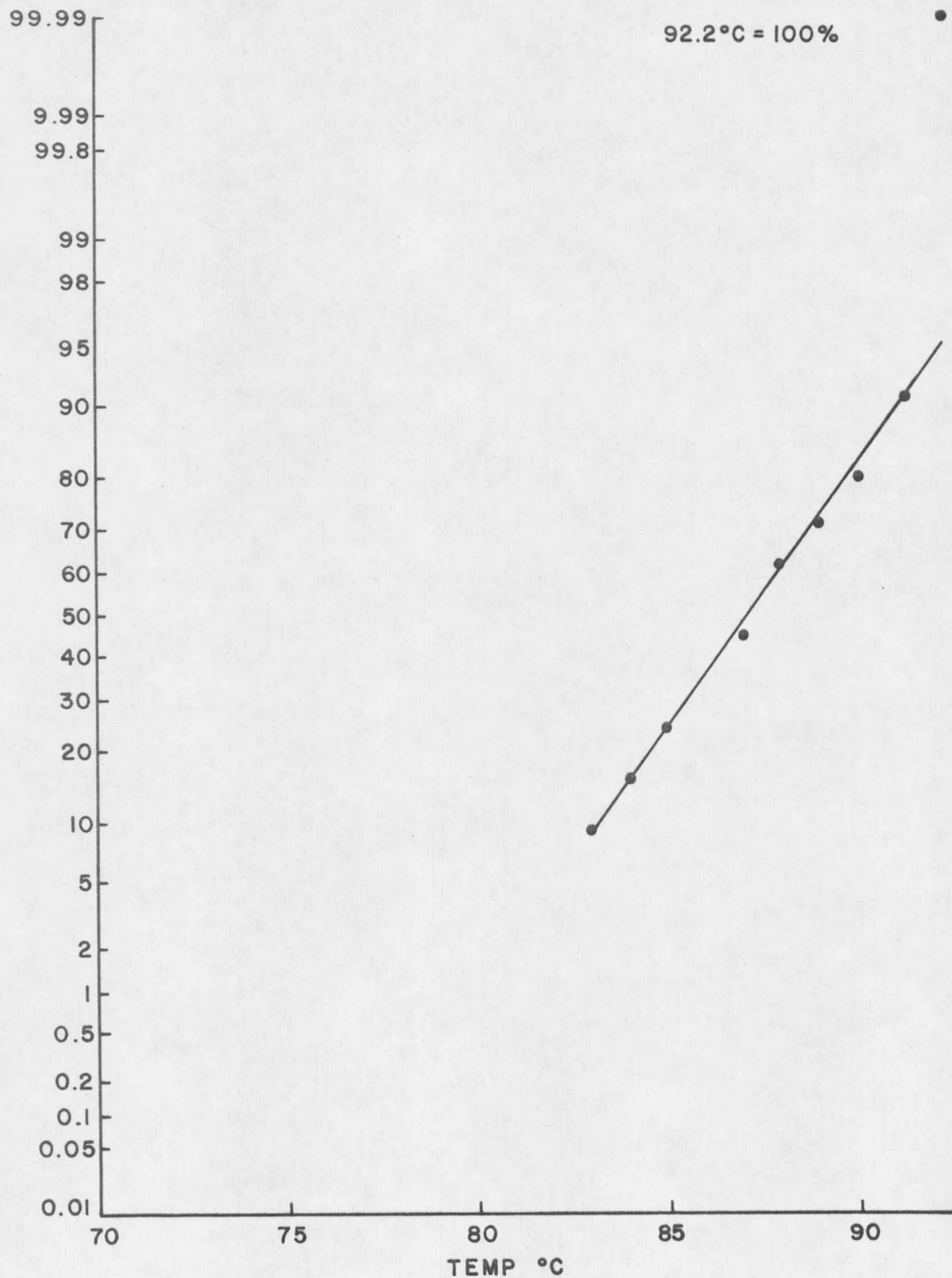


FIGURE 15. NORMAL PROBABILITY PLOT OF T_m FOR ARTHROBACTER
($T_m = 87.2^\circ\text{C}$)

Chapter 4

DISCUSSION

Many attempts to improve coal strip mine reclamation have been made, but the majority of these attempts have been focused on revegetation trials (Struthers and Vimmerstadt, 1965a, 1965b; Roger, 1951; Jacoby, 1969; and Everett and Henry, 1974). While the importance of soil formation as a fundamental objective of strip mine reclamation has often been recognized, this objective has been overlooked to a large extent. As research continues on strip mine reclamation, however, revegetation and other types of field trials will no longer prove to be the best means of approaching the problems since it is necessary to recognize that better success in revegetation depends on improving the growing conditions directly.

A feasible approach to studying the actual soil-forming processes directly is through the study of the microorganisms in the soil. Microorganisms play numerous roles in soil genesis and their importance for plant nutrition is accepted. Soil fertility is in great measure controlled by the diverse biochemical activities of the microflora, which are collectively called biological activity.

From the time of Koch and Pasteur, microorganisms have been studied by incubation on nutrient media. Originally, the activity of soil microorganisms was determined through classical techniques by counting

bacteria, actinomycetes and fungi. Winogradsky and Beijerinck, in the late nineteenth century, devised the methods using selective media based upon physiological or nutritional requirements and this concept has since been enlarged upon by many. Lochhead and his colleagues (Lochhead and Chase, 1943) succeeded in grouping soil microorganisms into seven nutritional categories. The greatest problems involved in these methods, as illustrated by the findings of Starkey (1958) and others, are that less than 10% of the bacterial population of the soil was recovered with plating procedures.

In recent years, Soviet and European scientists have attached great importance to the evaluation of the biological state of the soil with biochemical and physiological methods (Kuprevich, 1951; Mashtakov, Kulakovskaya and Gol'dina, 1954). These methods aim not only to detect particular physiologically or metabolically functional groups of microorganisms, but to also determine the approximate numbers of bacteria in each group. This activity depends on the number of bacteria within a given group, in addition to the level of their metabolism and growth rate.

Respiration is one of the earliest and still most frequently used indices of microbial activity in soil (Fred and Waksman, 1928). Recently, the recognition of enzymes in soil and development of methods for their measurement have led to their use for studying biological activity in soil (Hofmann, 1962). The respiratory intensity of soil

makes it possible to characterize the general biological activity of soil, but the study of the enzymatic activity of soil makes it possible to devise methods to stimulate the transformation of individual organic compounds important for plant nutrition and enzymatic activity reflects the dynamics of readily assimilable substances in soil (Galstyan and Ashkhababyan, 1960). Some authors (Katsnel'son and Yershov, 1958; Mashtakov, et. al., 1954) have found a direct relationship between the activity of enzymes, the numbers of microorganisms and the amount of oxygen uptake (or respiration) and consider that these indices can give a picture of soil fertility. However, many others discount this direct correlation and the direct correlation occurs so infrequently that it seems to be the exception rather than the rule. However, with combinations of these various techniques an overall evaluation of soil biological activities can be obtained.

As research continued on the biological activity of the soil, it was recognized that the microbial populations and their activities were a reflection of the interplay of the many physical, chemical and biological factors in a specific environment. Attempts were made to determine the influence that various factors, such as seasonal variation, temperature, pH, oxygen, soil minerals, soil structure and clay content, had on biological activity. In 1966, Mishustin recognized that the dynamic state of the soil microflora was determined by several factors influencing the condition of microorganisms, including soil humidity,

soil temperature, penetration of organic compounds into soil and others. Mishustin also found that the variation of the microbial population in the soil did not obliterate the specific features that characterized the composition of the soil population in the individual soil types (Mishustin, 1966).

It was shown in several investigations (Krasilnikov and Nikitina, 1945) that different microbial populations are responsible for individual stages in the decomposition of organic substances, and almost any kind of disturbance promotes microbial activity (Dobbs and Hinson, 1960). Therefore, the sharp changes in the course of the soil processes brought about by strip mining should open the way for entirely different stages of associations of soil microorganisms. It has been previously observed that an area disturbed by strip mining seldom reaches the same ecological equilibrium that existed prior to the disturbance (National Academy of Science, 1974). This possibility of different microbial ecosystems existing in close proximity and even within the same soil series has been previously suggested (Ekert, 1956). There is limited information concerning the succession of soil microorganisms accompanying plant communities, but on developing soils close associations have been found between the development of soil microflora and the progress of vegetational succession (Stout, 1961; Ross, 1965; Clark and Paul, 1970; Remacle and DeLeval, 1975). In addition to these observations, enzyme activity has been observed to change as

soils were developing (Galstyan, Sarkisyan and Bakhalbashyan, 1962).

While very few studies of strip mine reclamation have included considerations of soil microorganisms, and these have been mainly patterned after the classical techniques of enumeration and isolation of microorganisms present (Grandt and Lang, 1958; Wilson, et. al., 1965; Richardson and Dicker, 1975), it may prove to be a valuable means of assessing the biological factors and interactions involved in establishment and maintenance of the climax necessary for successful reclamation.

In determinations of the biological activity of soil, the exceedingly heterogeneous nature of soil must be recognized. Many have noted that the heterogeneous character remained even following sieving and blending of the soil taken from a discrete soil horizon (Stotzky, 1965; Pochon and Tardieux, 1968; Parkinson, Gray and Williams, 1971). The results of the microbiological parameters on air-dried, sieved and blended soils in sampling experiments performed at the Demo plot (Table 3) confirmed this persistent heterogeneity of the soil. It may be that the variations shown in Table 3 simply represent the heterogeneity of soils studied. Sampling from the same plant species throughout the same plot failed to decrease the standard deviation of these microbiological parameters (Table 4). Therefore, the variance found for one plant species at different plots (Table 5) would be expected to be greater than that for a given plant at one plot. The differences of results obtained at different sampling times for a particular plant species

at a specified plot (Table 6) would have been due to the heterogeneous nature of the soil itself, and to the different stages in the plant growth cycle exhibited. Attempts to decrease the sampling variability had no significant effect, as shown from the analysis of variance for the two sampling techniques used (Table 7), staked and quartered variations. The F-test for variances was not significant in any case and the variance for each of the two treatments was not significantly different from that for the other sampling treatment. The accuracy of the microbiological parameters in representing the soil processes depended on the variability within the soil unit (Parkinson, et. al., 1971). Thus, these determinations of the variability within the soil unit were considered necessary and quite useful for all further study.

In addition to the determinations of the general variability throughout a plot, the variation with depth was determined. The statistical analysis for the microbiological parameters, as measured at two depths, (Table 8) showed no significant differences. The variability in samples taken from arbitrary locations of one plot were not present in this analysis of samples taken from two different depths in the same plot. This implied that the variability within the soil unit was not due to variability of depth, within the top 12 inches of soil.

The biological activity of soil has been found to be closely inter-related with the complex physiochemical properties of the soil (Collins-George, 1962; Russell, 1961; Stanford, Kelly and Pierce, 1942; Chapman,

1965; Stotzky, 1968). Although some chemical properties of soils have been found to be fairly constant over the course of a year, as determined at monthly intervals, various other chemical properties of soil, including potassium and phosphorus, varied significantly (Weaver, 1975 - personal communication). However, very little work has been done to determine the possible correlations between variations in soil properties and variations in the activity of soil microorganisms (Hagedorn and Holt, 1975). The variations in the physiochemical properties of strip mine spoils, although expected to be quite large, have themselves been infrequently studied (Sandoval, et. al., 1973). In terms of vegetation alone, these properties of spoils assume a significant role which becomes even more important in determining microbial activity.

During soil formation, a homogenizing effect transpires. Soils in the same climatic-vegetational zones but formed from mineralogically different parent material (different C horizon) are more similar than the parent rock. So spoils should be more heterogeneous than native range in the Colstrip area. This heterogeneity should decrease with time due to the effects of weathering, plant growth and microbial activity.

To determine any obvious effects of the soil physiochemical properties in the study area in this initial study, soil chemical and physical analyses were conducted on samples taken during April 1974 (Appendix).

There was a difference in the mean of these properties for spoils as compared to native soil in all but the soil property of conductivity. In addition to these differences between native soil and spoils, the spoils tended to have a greater range of most properties. In general, the mean spoils values were lower than the native soil values for all but phosphorus, pH, sodium and percent sand.

The mean spoils pH of 8.6 as compared to the mean pH of native soil of 8.22 has been found to often indicate problems in Great Plains soil, e.g. undesirable soluble carbonate content, low solubility of calcium salts, nutritional imbalance and others (Sandoval, et. al, 1973). The mean concentration of available phosphorus in spoils was approximately two times as high as that in native soil; possibly this was due to the increased availability of mineral or sorbed phosphates in sandy soil (Swaby and Sperber, 1958) or the decreased percentage of clay in spoils which would decrease adsorption sites for phosphorus. The potassium (ppm) in spoils was twice as high as that found in native soils; one possible explanation for this was the finding that biological activity increased potassium availability (Chapman, 1965). The nitrate concentration in native soil was almost four times as great as that in spoils, and the respective values were 5.714 ppm and 1.37 ppm; this difference was possibly due to the difference in length of time during which nitrogen fertilizer was applied and the greater time period of organic matter decomposition, since organic matter is a primary source of nitrogen in

soil. The nitrate concentration in spoils was below the value of 3 ppm which is considered to be the concentration below which only nitrogen-fixing plants grow well (Sandoval, et. al., 1973). The mean conductivity for both spoils and native soil was the same at 0.6 millimhos and a conductivity of less than 2 millimhos signifies that salinity has little effect in these soils (Sandoval, et. al., 1973). The organic matter of spoils was lower than that of native soil and this was expected since spoils were merely newly established soils. The milliequivalents of calcium in native soil and spoil were quite similar values, which could possibly be attributed to similar calcium composition of topsoils (native) and overburden (Spoils) soils. On the whole, spoils contained less magnesium than native soils, which might be attributed to the original mineral composition of parent material. The sodium concentration of spoils was higher than that for native soil, possibly due to imperfect drainage conditions and high surface evaporation rates. There was a large difference between the available water-holding capacity percent at 0.3 atmospheres of spoils in comparison with native soils, with the spoils having the lower value. Evidence has been presented which showed that the available water-holding capacity was directly related to the textural composition of the soil, and high clay and silt percentages have a higher water-holding capacity in comparison to soils consisting primarily of sand (Buckman and Brady, 1969). These results followed these findings since spoils consisted primarily of sand

and had a much lower water-holding capacity. In a comparison of soil textures, spoils generally had a much high percentage of sand and were classified on the basis of this composition (Buckman and Brady, 1969) as sandy loams. Native soils, on the other hand, were composed primarily of clay and silt which led to the classification of native soils as clay loam and loam (Buckman and Brady, 1969). The mean bulk density of spoils was slightly higher than that of native soils. This property is also related to the textural composition of soils since less dense soils are loose, porous and composed of small particles whereas soils with higher density have less pore space, are more compact and are composed of larger particles (Buckman and Brady, 1969).

According to Southard (1973), the soils in the Colstrip area are of the Bainville-Flasher-Badlands soil series and have a dominant surface texture of silt loam, which also dominates the subsoil. The underlying material consists of soft siltstone and sandstone and this soil type is subject to wind and water erosion and is used almost exclusively for grazing. In the 1938 Great Soil Group classification these soils were classed as Lithosols. More recently, in the 1968 soil classification, they were classed as Torriorthent. However, during 1974 and again in 1975, the soils at Colstrip appear to have been moist for a longer period than that described for Torriorthents, which was three consecutive months.

Of the significant correlations within soil analyses (Table 9)

for native soils, several correlations between certain soil properties are easily explained. The positive correlations for native soil between potassium and conductivity and also sodium and conductivity (both significant at the 5% level) are to be expected since both sodium and potassium are the two most motile cations and are not substantially fixed in the soil so they have the largest influence on conductivity. Both nitrate and phosphorus positively correlated with fertilization and above ground production and this indicated that the fertilized plots still had nitrate and phosphorus available for plant growth. In addition to these correlations for native soils, the correlation between magnesium and clay also represents a phenomenon which was expected. The predominant type of clay at Colstrip has been determined to be montmorillonite and it is known that magnesium occupies some of the sites within the lattice of this clay. Therefore, magnesium would be expected to vary in proportion with the amount of clay.

The significant correlations within soil analyses for spoils are much less complex than those for native soils. The correlation between percent sand and mean bulk density is an obvious one since the greater the percent sand present, the higher the mean bulk density will be. Organic matter correlates with water-holding capacity since organic matter is a major factor in the water-holding capacity of a soil. The remaining significant correlations are not so easily explained and

may be due to a number of things including interactions with other physical and chemical properties not measured in this analysis.

Although significant correlations were obtained within the soil analyses, there is no basis for the assumption that cause and effect relationships exist. It is possible that the increase in one soil physiochemical property is the direct result of an increase in some other soil property, but there is no basis for making such an assumption. Further studies would be necessary to determine a proper interpretation of these correlations, but the fact that significant correlations were observed is interesting in itself.

One of the earliest indices of soil microbial activity was the respiration of microorganisms in soil and it is still one of the most frequently used (Stotzky, 1965). Originally, Boussingault and Lewy (1853), Moller (1879) and Wollny (1881) used the production of CO_2 as an in situ measurement of the microbial activity of soils. Probably the first to use this measurement in the laboratory were Deherain and Demoussey (1896). Measurements of respiration have been extensively used by Russell (1905), Neller (1922), Waksma and Starkey (1924) and subsequently many others in soil microbiological studies. The measurement of respiration by means of O_2 uptake has been found to have wider applications since CO_2 was found to have evolved from anaerobic as well as aerobic environments and some CO_2 evolved from soil may have non-biological origins (McLaren and Skujins, 1971). The quantities of O_2

consumed have been found to depend on the type of substrate, the environmental conditions and the microorganisms involved (Stotzky, 1965). Measurements of respiration have been found to be well correlated with other parameters of microbial activity such as organic matter content, nitrogen or phosphorus transformations, metabolic intermediates, pH, average microbial numbers and changes in soil weight (Hausenbulla, 1950; Stotzky and Norman, 1961; Stotzky, 1965; Ross, 1965).

In the past, a 60-70% of water-holding capacity has been assumed to be optimum for aerobic microorganisms. Therefore, the measurement of O₂ uptake in vitro has usually been determined with soil which was brought to 60% of water-holding capacity (Stotzky, 1965; Ross and Roberts, 1970). However, since the O₂ uptake of soil was found to be dependent on the degree of water saturation (Buckman and Brady, 1969), the possibility that optimum O₂ uptake in vitro occurred at a water-holding capacity different than 60% was investigated in this study. A preliminary study by Ross and Boyd (1970) found that optimum O₂ uptake in vitro did not necessarily occur at 60% of water-holding capacity. Among various percentages of water-holding capacity which were investigated in this research, optimal O₂ uptake was found to occur at 100% of the water-holding capacity (Table 10). Consequently, all respiratory measurements were made with the soil at 100% of water-holding capacity.

Using the Gilson respirometer in determining O₂ uptake, it was necessary to run 5 replicates of each soil sample to ensure a statistically

acceptable value. An attempt was made to determine the possibility of reducing the variation between replicates by increasing the quantity of soil used for respiratory measurements. The results from this experiment (Table 11) showed that by increasing the quantity of soil used in the determination of respiration, the standard deviation between replicates was increased and the respiration rate per gram of soil was decreased. This was in contrast to findings of Stotzky (1960). A possible explanation is that the specially constructed larger vessels provided suboptimum conditions. In particular there was a possibility that the decreased O_2 uptake rate in larger vessels resulted from a less efficient gas exchange and a poorer O_2 -soil particle contact per soil volume. with increased quantities of soil. Based on these findings, subsequent measurements were made with standard vessels and smaller quantities (5 grams) of soil.

The interrelationship of respiratory activity and the physical and chemical properties of soils has not been investigated in great detail. In the past, measurements of respiration have been found to be correlated with organic matter content, pH, cation exchange capacity and surface area of clays (Hausenbuiller, 1950; Stotzky, 1960; Stotzky and Norman, 1961). However, the respiratory activity in the present investigation was found to have no highly significant interrelationships with the physiochemical properties of the soil. The only correlation found between respiration and soil properties was that obtained

with water-holding capacity in native soil. This interrelationship could be attributed to soil pore space, texture and density since soil with higher water-holding capacity in all likelihood is more porous which would result in higher respiratory activity. Usually this interrelationship is also related to organic matter content, but no such interrelationship was observed in this case. The lack of significant correlations between respiration and physiochemical properties of the soils investigated may be due to the heterogeneous nature of the soils involved or to interrelationships which are not readily apparent at this time. There exists the possibility that if the soil properties were to be examined in conjunction with respiration at various different times, interrelationships would become apparent.

There was no overall pattern in respiration for the six major plots (Figure 10). However, the respiratory activity for each plot changed with time and there appeared to be a peak in October for the respiratory activity of spoils. The analysis of variance due to the combination of plants and times (Table 12) shows that the effects of times and plants are not additive for all plots, while the analysis of variance due to plant species alone (Table 13) shows that the variance due to one plant species is essentially the same as that due to any other plant species. The variation in respiratory activity exhibited by these 6 plots thus seems to be a function of time rather than plant species. The analysis of variance for seasonal variation (Table 14) confirms the effect of time

in determining variation which was significant for all but two plots, those of Native Range Fertilized and BN. Both of the plots for which time effects were not significant were native soils. These results lead to the speculations that in these two cases the influence of time was insignificant for the reason that native soils exist in a relatively stable state in comparison to spoils. The variation exhibited by these two native soils (seen in Figure 10) may be due to some other factor such as physiochemical properties since it cannot be attributed to either plant species or to times. The correlations of the respiratory activity at different times (Table 15) do not reveal a pattern among plots. Such a pattern for sampling times at different plots would be expected if the seasonal effects were the same for each plot. This lack of pattern suggests that factors other than time are also important in determining respiratory activity and that the plots are generally different in regard to respiration.

These findings of seasonal variations have various implications for ecological studies utilizing respiratory activity measurements. Often, the measurement of respiratory activity is made only once during ecological studies based on the assumption that soil respiratory activity is relatively constant with time. One example of this is a study to determine the effects on soil respiration of weather modification (Weaver and Klarich, 1973). The assumption of stable soil respiratory activity with time is invalidated by the results of the present study.

The total living constituency of any single soil, although closely associated with the origin of the living soil, has never been successfully isolated and identified. Very little is known even now about the make-up of the species of soil inhabitants. Therefore, the most useful approach to a study of soil microorganisms is through the transformation of substrates taking place in the soil, mediated by enzymes of microbial origin. At present, the number of enzymes whose activity has been detected in soil approaches 50 (McLaren and Skujins, 1971). However, not more than 10 of these have been studied in detail.

Most phosphorus in soil exists as organic phosphate. The assimilation of phosphates from organic compounds by plants and microorganisms is preceded by their hydrolysis catalyzed by extracellular phosphatases (McLaren and Skujins, 1971). Therefore, the phosphatase activity in soil has been increasingly linked with the activity of microorganisms living in the soil. Fifteen to twenty separate enzymes have been characterized as phosphatases and are involved in the metabolism of carbohydrates, nucleotides and phospholipids. These include phytases, glycerophosphatase, fructosediphosphatase, lecithinase and nucleases among others. The phytase (myo-inositol polyphosphatase) activity of Aspergillus niger was known for a long time (Dox and Golden, 1911); Rotini (1931) was one of the first investigators to describe soil phosphatases and MacFadyen (1934) found phosphatases in a number of soil

microorganisms. The inorganic phosphate released from substrates was assayed and correlated with enzymatic activity by Roger (1942) and he suggested that phosphatases were excreted from plant roots. Other examined the relationship between phosphatase activity and the clay content of soil (Mortland and Gieseking, 1952). A variety of substrates was used in assaying for soil phosphatase activity including phenylphosphate (Kroll and Kramer, 1955), phenolphthalein phosphate (Krasilnikov and Kotelev, 1957) and glycerolphosphate (Skujins, Braal and McLaren, 1962). The most commonly used substrate was phenyl phosphate because its use involved a simple procedure and phenyl phosphate was not absorbed by soil constituents. This substrate was used by Kramer and Yerdei (1959) in their studies of the correlation between phosphatase activity and soil fertility.

The literature on phosphatase activity in soil is numerous, but contradictory in observations and interpretations. Various subjects such as the pH of maximal phosphatase activity (Keilling, et. al., 1960; Drobnikova, 1961;), inhibition by inorganic phosphorus (Kuo and Blumenthal, 1961), absorption of the enzyme by clay minerals (Kroll and Kramer, 1967; Ross, 1965), influence of physiochemical properties of soil (Halstead, 1964; Paw and Hughes, 1974), adverse effect of air-drying soil (Jansson, 1967, Ross, 1965), correlations with microorganisms and others have been studied.

Contradictions resulted from the majority of these investigations.

However, Krasilnikov and Kotelev (1957) have demonstrated that phosphatase is produced by a large number of soil bacteria and other investigators have shown that it accumulates as a result of microbial activity (Geller and Dobrotvorskaya, 1961; Jacquet, Villette and Richou, 1956). In general, it can be concluded that there exists in soil a wide range of microorganisms capable of dephosphorylating all known organic phosphates (McLaren and Peterson, 1967), and the enzymatic activity in soil appears to be correlated with the concurrent or immediately past activity of intact cells (Kuprevich and Shcherbakova, 1971).

The preservation of the initial biological activity present in a soil sample at the time of sampling is still an unsolved problem because it is impossible to store a soil sample for more than a few hours without changes occurring within the microbial population (James and Sutherland, 1939; Grossbard and Hall, 1954). However, according to G. Hoffman (1959), the enzyme activity of a soil did not change during the course of a year following proper air-drying and storage. Contrasting results have been reported by many (Jansson, 1967), but Rosee (1965) concluded from studies of invertase activity that the storage of soil samples at 4°C does not cause substantial changes in enzyme activity and Hofmann (1962) suggested that air-drying stabilized the enzymes activity.

The effects of air-drying the soil on phosphatase activity in this study were determined (Table 16). The change in phosphatase activity mediated by air-drying was not significant. Therefore, this

procedure was adopted to facilitate the handling of a larger number of samples.

The effects on phosphatase activity of various physiochemical properties of the soil have been discussed by many authors (Drobnikova, 1961; Kroll and Kramer, 1955; Kiss, 1958; Janossy, 1963). In this present study, although soil analyses were performed at only one sampling period, an attempt to examine the interrelationship of phosphatase activity with individual soil properties was made. The phosphatase activity of native soil (during April 1974) significantly correlates with several soil properties (Table 17). Potassium, calcium, and sodium all negatively correlate with phosphatase activity at the 5% level of significance and percent silt also correlates negatively, but at the 1% level of significance. However, both magnesium and percent clay correlate positively with phosphatase activity at the 1% level of significance. This pattern of correlation suggests that phosphatase enzymes are adsorbed by clays. This suggestion is strengthened by re-examining the correlations within soil properties (Table 9). Potassium, sodium, calcium and percent silt all appear to be interrelated in that they share positive correlations with each other and also share negative correlations with magnesium and percent clay. Together, these correlations suggest that the phosphatase enzyme competes with potassium, sodium and calcium for adsorption sites on the clay and since the type of clay is largely montmorillonite, magnesium occupies sites within the clay lattice and this contributes to the high cation-exchange capa-

city of the clay. However, it must be remembered that these results, along with their possible implications, only hold true for the April 1974 sampling time since soil analyses were not conducted during any other time. Therefore, this apparent pattern might be affected by seasonal changes and, in fact, may only appear during certain time periods. In addition, it must be emphasized that the appearance of such an apparent pattern does not allow any definite conclusions to be drawn regarding cause and effect relationships without further detailed research into the problem. In contrast to native soils, the spoils exhibited no significant correlations between phosphatase activity and the soil properties. These results lead to the speculation that: (1) in terms of phosphatase activity, spoils and native soils are dissimilar and (2) any significant correlation between phosphatase activity and soil properties was masked by some other property or the heterogeneous physiochemical nature of spoils.

In the present study, both Native Range Fertilized and Native Range Unfertilized plots had a peak in phosphatase activity in August 1974 (Figure 11) which was not discernible for any of the other plots at that time. The phosphatase activity did not follow any general pattern for the various plots at the four other sampling times. The analysis of variance of phosphatase activity due to different plant species (Table 18) shows that, although the level of significance was actually only acceptable for the BN plot, there may be some minor

effects on phosphatase activity caused by different plant species at all plots except Native Range Fertilized. No real conclusion can be drawn from these statistically rather weak significance levels, but they do suggest that the effects of individual plant species on phosphatase activity cannot be completely disregarded. In contrast to the analysis of variance due to plants, the analysis of variance due to the combination of plants and times (Table 19) was statistically significant for all plots. Thus, the effects of time and plant are not additive. Since the effects of plants alone were not highly significant, the effects of different times on phosphatase activity may be more important. The analysis of variance for the effects of different times on phosphatase activity (Table 20) confirms this idea since the time effects were significant for all plots with the exception of the BN plot. The only other plot for which time effects were not highly significant was Native Range Fertilized, which, in addition, showed no significance for the effects of plants. Apparently, some other phenomenon is largely responsible for the variation of phosphatase activity at this plot, although further investigations are necessary to confirm this result. Although the effects of plants are significant for the phosphatase activity of the BN plot, the time effects are not significant and thus, the effects of time and plant are not additive. From this it can be assumed that the phosphatase activity is different for at least the Native Range Fertilized plot, where the

variation was due to time and possibly some other factor, and the BN plot, where the variation was due to the effects of plants. By contrast, the other four plots the variation was dominantly due to times, but was also affected by plants to some extent. Another interesting result from these analyses of variance is the fact that the spoils plots were more highly significant for both the variance due to plants and the variance due to time than were the native soil plots, but the native soil plots were more significant for the variance due to the combination of plants and times. This might suggest that the variation of phosphatase activity for native soil is affected by something in addition to time and plant, which might also be related to the pattern of correlations between phosphatase activity and the soil physiochemical properties of the native soil. The correlations of phosphatase activity at different sampling times (Table 21) show several common characteristics, but no actual overall pattern. From the results of the previous analyses of variance, the plots of Native Range Unfertilized, Gouge, Demo and possibly Topsoil Gradient appear to be the most closely related in terms of causes of variation in phosphatase activity. Therefore, if the variations were due only to plants and/or times and these effects were the same for each plot, these four plots would be expected to exhibit the same patterns in terms of seasonal correlations. This assumption appears to have some basis between Native Range Unfertilized and Gouge plots, but not for

Demo and Topsoil Gradient. (Since only one plant was sampled at the Topsoil Gradient plot, seasonal correlations could not be calculated.) However, the two dissimilar plots, Native Range Fertilized and BN, also share an identical pattern in seasonal correlations. In addition to this, the correlation of April: October was shared by all but two plots, Demo and Topsoil Gradient. Therefore, although interactions are present, and most likely other factors effect the variation in addition to time and plant, it would be impossible to interpret these data until further investigations are conducted.

These various findings concerning phosphatase activity might have various implications on other ecological studies utilizing phosphatase activity. The possibilities of: interaction between phosphatase enzymes and the physiochemical properties of the soil; variation in activity caused by different plant species; variation in activity with season; and variation in activity due to other factors, all must be taken into consideration before any conclusions can be drawn concerning phosphatase activity.

Essentially, the ecological methods which can be used in soil microbiology are based on the determination of functional or physiological groups since the dissemination of microorganisms in soils proves to be a most sensitive indicator of the substrate's properties (Mishustin, 1966). These are defined as populations of bacteria which

have a common physiological, biochemical or metabolic property, enabling them to act together (Casida, 1968). The methods involve first detecting the activity of a particular functional group and then counting the approximate numbers of bacteria belonging to that group. The functional group on which the present research is focused consists of the population of soil bacteria responsible for the degradation of plant pectin.

Pectic substances, polysaccharides principally composed of α -1,4 linked galacturonic acid, are found in the tissues of higher plants where they have an important structural role. They are deposited in the primary cell wall during the early stages of growth and in the middle lamella where they act as intracellular cement. The enzymatic breakdown of pectic substances in the middle lamella leads to plant tissue maceration (MacMillan and Sheiman, 1974). Therefore, the enzymes that degrade pectin are essential in the rapid decay of dead plant material and assist in recycling carbon compounds in the soil.

Historically, the subject of pectic enzymes has been one of much confusion. Originally, only two major types of enzymes were thought to be involved in pectin degradation. The first, pectinesterase, hydrolyzed the ester linkage and the glycosidic linkage was then hydrolyzed by polygalacturonase. As research continued, more than one type of depolymerizing enzyme was found to exist. Specificity

for pectin as the substrate rather than polygalacturonic acid, differences in the action pattern and exo- and endo- splitting enzymes were among the characteristics determined (Roboz, Barrat and Tatum, 1952). Deuel and Stutz (1958) clarified much of the confusion by classifying the depolymerases based on substrate attacked, mode of attack and optimal pH for enzyme activity, but they assumed the only mechanism for cleavage of the glycolytic bond was hydrolysis. A pectic enzyme which split the α -1,4 bonds of pectin by a transelimination mechanism rather than by hydrolysis was soon found (Albersheim, Neukom and Deuel, 1960; Edstrom and Phaff, 1964). Although the first transeliminases (pectin lyases) were specific for pectin, enzymes capable of degrading polygalacturonate with both endo- and exo-action patterns were soon discovered (Nagel and Vaughn, 1961; MacMillan and Phaff, 1966). A new classification scheme was proposed by Bateman and Miller (1966) which incorporated these new enzymes. More recently, another group of pectic enzymes has been described which degrade oligogalacturonides and which join the other nine previously described pectic enzymes. Due to the confusion concerning pectic enzymes, only a few general studies have actually been conducted in which pectinolytic activity was assayed (Kaiser and DeAscongeni, 1971; Hankin, Sands and Hill, 1974).

The decomposition of organic residues in soil presents problems of very great biochemical complexity. In the breakdown of pectin, extracellular enzymes released by microorganisms diffuse to the substrate

and cause a relatively simple hydrolytic cleavage of the insoluble molecule, giving rise to water soluble fragments which can then diffuse away from the site of decomposition and become absorbed by microorganisms in the vicinity (Burgess, 1965). However, studies of the incidence of different enzymes which occur in close association with each other and which come from the same microorganisms have shown that a complex control mechanisms, i.e. catabolite repression, is responsible for the control of pectic enzyme activity (Voznyakovskaya, Avrova and Andronikashvili, 1974). Before such an assay can be generally accepted, the confusion surrounding types of pectic enzymes must be further clarified.

In the present study, the effects of air-drying upon pectinolyase activity were determined (Table 22). Although the correlation between pectinolyase activity of field moist soil and air-dried soil was low, it was still significant at more than the 5% level. Since the treatments of the soil samples for each test were kept as uniform as possible, air-dried soil samples were used.

Many enzymes in soil have been found to be related to or affected by the physiochemical properties of the soil. However, no significant correlations between pectinolyase activity and the analyses were obtained in the present study.

The pectinolyase activity for the six major plots versus sampling time (Figure 12) shows several peaks. The Native Range Fertilized plot

exhibits a peak at the August sampling and again in December. All but the BN plot exhibited a low in pectinolyase activity during October and a peak again in December. This suggests a similarity between plots. From the analysis of variance due to plant species (Table 23) it can be concluded that the differences in pectinolyase activity due to individual plant species were not significant. However, the analysis of variance due to a combination of plants and times (Table 24) shows that the effects of plants and times are not additive for any plots except Demo. Since the effects of individual plant species were not significant, this suggests that the major variation in pectinolyase activity is due to time. The analysis of variance due to time (Table 25) confirms that the variation in pectinolyase activity is due to time for all but the BN plot. The low levels of significance for variation due to time and times in combination with plants suggest that the variance in pectinolyase activity at this site is caused by some other factor or factors. To determine if the apparent relationship between plots, as suggested by the analyses of variance, exists in any type of seasonal pattern, correlations of pectinolyase activity between one sampling date and each other sampling date were calculated (Table 26). No real pattern was apparent, although there were some interrelationships. In terms of pectinolyase activity, these analyses of variance suggest that each plot is independent of all other plots, and most important of all,

individual plant species do not have separate effects, which is ideal for the measurement of pectinolyase activity.

In general, the pectinolyase activity varied with time and was also possibly affected by some other undetermined factor or factors.

The determination of the number of pectinolytic bacteria present in conjunction with the pectinolyase activity is a vital part of the study of functional groups in soil.

A primary assumption in the classical soil dilution plate method (Waksman, 1927; Johnson, et. al., 1959) is that each viable micro-organism present in soil develops into a visible colony following inoculation of a soil suspension into the agar medium and incubation. However, in comparison with direct staining methods used in observing microorganisms from soil, plating gives counts of only 1-10% of those obtained directly. This failure to grow can be due to: incorrect substrate, improper incubation, senile or injured bacterial cells, dormant spores, antagonisms between organisms (Wieringa, 1939), adherence of the bacterial cells to soil particles (Pochon and Tardieux, 1968) and the cohesion of cells in colonies (Jensen, 1968). It has been generally accepted that most soil bacteria have a low metabolic rate and that their nutritional requirements are poorly understood (Gibson, 1957). Many of these bacteria grow poorly on the rich media used for other bacteriological studies. However, it can be assumed that the limitations apply to soil generally and therefore, for any

given group of soil samples, empirical relative microbial populations can be enumerated.

Again, the confusion surrounding the classification of pectic enzymes must be taken into consideration. In addition, the problem of substrate specificity cannot be completely overlooked. However, since the number of bacteria present and their activity in nature is dependent upon the environmental conditions as well as their physiological abilities and the plant material containing pectin is the actual substrate within the soil system, the problem of substrate specificity can be circumvented by using pectin as the substrate. This recognizes that such a "pectinolytic" count does not define a physiologically precise group of bacteria.

With soil, it is especially important that the medium for plate counts be low in readily available energy materials such as sugars so that a few fast-growing microorganisms do not dominate growth on the plates and inhibit the development of a larger number of slow-growing species (Clark, 1965). In the selective plating of pectinolytic organisms, this precaution assumes even more importance since the enzymes involved are subject to complex controls such as catabolite repression (MacMillan and Sheiman, 1974; Voznyakovskaya, Avrova and Andronikashvili, 1974).

Reports of attempts to develop a selective medium for the enumeration of pectinolytic microorganisms are numerous in the literature.

The type of medium most commonly used relies on finding depressions or soft areas in a calcium-polypectate gel medium (Wieringa, 1949; Vaugh, et. al., 1957). Kerr (1953) employed sterile vegetable tissue to isolate pectinolytic organisms. These media may be useful with different conditions, but a solid medium offers distinct advantages in preparation as well as in detection and enumeration of organisms. Jayasankar and Graham (1970) described a solid medium containing pectin and yeast extract on which pectinolytic activity was determined following growth by flooding the plates with cetyltrimethylammoniumbromide, which precipitated intact polysaccharides. Hankin, Zucker and Sands (1971) revised this medium to contain a mineral base, which had a stimulatory effect on pectinolytic enzyme synthesis and determined pectinolytic organisms on spread plates with hexadecyltrimethylammoniumbromide. However, it has often been observed that commercial pectin preparations contain contaminating residues of sugars (Kertesz, 1951; Elrod, 1942; Luh and Phaff, 1951; Jermyn and Tompkins, 1951; Dehority, 1969). This contamination would reduce the selectivity of a pectin medium. Therefore, in the present research, the solid medium of Hankin, Zucker and Sands (1971) was modified by purifying the pectin following a slightly modified method of Jermyn and Tompkins (1951). This modified Mineral Pectin Medium was then used in the present research to enumerate pectinolytic bacteria. The indicator used by Hankin, Zucker and Sands (1971) for determining

pectolytic activity did not give good results, so its use was dispensed with and the number of colonies per plate of MPM were taken as the number of pectinolytic bacteria.

A comparison of enumeration of pectinolytic bacteria on both the original Mineral Pectin Medium with unpurified pectin and on the modified MPM with purified pectin was made (Table 27). In all cases, the number of pectinolytic bacteria, as determined on MPM with purified pectin was significantly lower. These results were interpreted as follows: the higher numbers of bacteria on Mineral Pectin Medium with unpurified pectin were probably due to the presence of other carbohydrates in pectin and by eliminating these contaminating residues, the effectiveness of this medium in selecting pectinolytic bacteria was increased. This justifies the pectin purification step used in these studies.

In previous work, it was found that air-drying the soil reduced the microbial counts (Clark, 1965). In the present research, air-drying resulted in increased microbial counts (Table 28). This significant increase over soil which is at field moisture suggests that by air-drying the soil prior to plating, a burst of organisms was stimulated. This pattern has been discussed in the literature (Dobbs and Hinson, 1960; Griffiths and Birch, 1961; McLaren and Skujins, 1968) and is a well-known phenomenon attributed to the drying process rendering organic matter more soluble and to the disruption of aggregates.

Today, a widely accepted medium for total counts of soil bacteria is Soil Extract Agar (Casida, 1968). This medium was therefore used in the present research to compare the number of pectinolytic bacteria with the "total population" of soil bacteria (Table 29). In all cases, the number of pectinolytic bacteria was significantly different than the number of total soil bacteria. The number of pectinolytic bacteria as a percent of the total soil bacteria ranged from 0.46% to 22.18%, with a mean of 8.36%. It is of interest to note that the highest percentage was for the Gouge plot, which is a spoils plot. In addition, the relative percentage values for the Native Range Unfertilized plot were quite similar for the two different sampling times. These results show that the pectinolytic bacteria comprise a small proportion of the total soil bacteria in numbers, although they mediate a very important process in the soil.

The number of pectinolytic bacteria from April 1974 did not correlate with any of the physiochemical properties of the soils which were also determined in April. This lack of correlation is not known to hold true for any other sampling period, because the soil analyses were only performed in April. Any speculation about interrelationships at other times is impossible to make at this time. However, this lack of correlation in April suggests that at this time the number of pectinolytic bacteria were not related to the soil characteristics determined. In addition, Mishustin (1966) has

suggested that the results of counts of soil microorganisms be reported as the number of microorganisms per gram of organic matter rather than the number of microorganisms per gram of soil, since the activities of microorganisms are not connected with the entire soil mass, but only with the organic fraction of the soil. The data for pectinolytic Plate counts of the April samples were converted to the number of bacter/gram of organic matter (Table 37). From these results, Chi-square values were calculated as an index of dispersion and these values for the number of bacteria/g organic matter were much lower than the respective values for the number of bacteria/g of soil. These results suggest that there is a realistic association between microorganisms and organic matter.

The numbers of pectinolytic bacteria follow the same general trends with sampling times for all plots except BN (Figure 13). The peak for October 1974 is the most striking feature of this trend. This trend is opposite that of pectinolyase activity (Figure 12). The variation due to plant species (Table 30) on the numbers of pectinolytic bacteria was highly significant for only the BN plot, although the Demo plot exhibited a weakly significant value. In contrast, the analysis of variance due to the combination of plants and times (Table 31) showed that these two factors caused different effects for all plots with the exception of BN. The analysis of variance due to time (Table 32) showed that the variation in numbers

Table 37. Conversion of the number of pectinolytic bacteria to number of bacteria/gram of organic matter for April, 1974

Plot	PecPL ^a	X ² _{PPL} ^b	%ORM ^c	PBORM ^d	X ² _{PBORM} ^e
Native Range Unfertilized	5.904 ^f	4.23	2.173	0.137	0.363
Native Range Fertilized	6.980	1.30	2.293	0.142	0.042
BN	6.269	5.622	2.693	0.159	0.148
Gouge	19.040	24.300	0.850	0.236	1.566
Demo	12.538	7.44	1.38	0.149	0.025

a = pectinolytic plate count in number of bacteria per gram of soil

b = Chi-square of pectinolytic plate count

c = percent organic matter

d = pectinolytic bacteria per gram of organic matter

e = Chi-square of pectinolytic bacteria per gram of organic matter

f = mean of fifteen replicates

of pectinolytic bacteria was significantly affected by time for all plots with the exception of BN. The behavior at the BN plot suggests that the variance in the numbers of pectinolytic bacteria at that plot is due to the nature of the vegetation and/or some other factor(s) rather than times. These results were similar to those for pectinolyase activity at the native plots, Native Range Unfertilized and Native Range Fertilized, and the spoils plots, Gouge and Topsoil Gradient. The pectinolyase activity analyses of variance were opposite the results of the analyses of variance in numbers of pectinolytic bacteria at the BN plot. These differences are impossible to explain on the basis of the results of this study. The significant correlations for the numbers of pectinolytic bacteria at the different sampling times (Table 33) do not exhibit any outstanding pattern. The only correlation between dates that was consistently significant for the plots Native Range Fertilized, BN, Gouge and Demo was October:December. These correlations for each plot shared at least one correlation with those found for the pectinolyase activity of the corresponding plot. It is impossible to draw any conclusions from these relationships at this time.

Determination of the numbers of pectinolytic bacteria in a soil may be important for the degradative capacity of a soil in terms of land used predictions (Hankin, Sands and Hill, 1974). Few studies have been conducted relation the changes in a given population of soil

microorganisms to changes in the properties of the soil (Hagedorn and Holt, 1974), but the results of this study show that interrelationships occur which might be useful in determining the stage of soil development. The effects of plant species and seasons on the number of pectinolytic bacteria in the soil must be taken into consideration when determining this population in the soil. In addition, calculation of the number of pectinolytic bacteria per gram of organic matter may be an especially useful index since these organisms are directly involved in the cycling of carbon in the soil.

A survey of the results in the literature shows that positive correlations between the enzymatic activity, respiratory activity and numbers of microorganisms in soil is the exception rather than the rule (Gel'ster, 1930; Stotzky, 1965; Khrenova and Kulai, 1966; Ramirez-Martinez and McLaren, 1966; Kuprevich and Shcherbakova, 1971).

In terms of respiratory activity, the quantity of O₂ consumed depends on the type of substrate, environmental conditions and microorganisms involved. Stotzky (1965) found respiratory activity well correlated with the enzymatic activity of soil, but maximum respiratory rates preceded the maximum number of microorganisms isolated. This suggested that respiration reflects metabolic activities rather than density of soil microorganisms.

A number of microorganisms in soil release phosphatases (Jacquet, et. al., 1956) and Hofmann and co-workers (1962) believe that micro-

organisms in the soil are the exclusive suppliers of free enzymes, but it has been difficult to establish a correlation between numbers of microorganisms and phosphatase activity (Ramirez-Martinez and McLaren, 1966; Balicka and Trzeblinski, 1956).

The role of pectinolyase activity in soil has been clearly established as important for the decomposition of plant residues and recycling of carbon, but little or no work has been done in attempting to correlate this enzymatic activity with other indices of biological activity in the soil.

Enzymatic activities of soil likewise are dependent on the presence of minerals, availability of substrate, environmental conditions and microorganisms involved. Since the enzyme content of individual cells as they are found in soil cannot be measured and in addition to the extracellular enzymes of viable cells, both the enzymes present in non-viable cells and those liberated by lysed cells may be active, a poor correlation may exist between the number of microorganisms capable of carrying out a specific transformation and the actual intensity of the transformation (Krasilnikov, 1962).

These techniques all possess a similar fault in that respiratory and enzymatic activities are general characteristics of the flora and fauna, both micro- and macro-, of the soil. Hence the sum of these activities is measured in the above determinations. Thus, it must be considered that the information gained is an overall evaluation of

soil biological activities and not necessarily limited to living microorganisms. It is known that microbial populations, like those of plants and animals, do not all peak at the same time, so an overall measurement should smooth out population peaks. It has been shown that it is possible by means of plate counts to demonstrate differences in microbial numbers in different soils (Sauerland and Marzusch-Trappmann, 1962), but plate counts do not necessarily reflect the biological state of the organisms as they exist in soil (Casida, 1968).

In the present research, the only significant correlation obtained was between pectinolyase activity and the number of pectinolytic microorganisms (Table 34 and Figure 14). This correlation suggests that this specific group of microorganisms is a metabolically active group in the soils studied and since their numbers correlated with activity, it is possible that these measurements are very useful in terms of the transformations occurring in the soil and the ecological state of the soil. It is interesting to note (Table 34) that this correlation is higher for native soil than spoils, and thus, this correlation might prove an interesting factor to follow as the spoils progress to climax states.

The other two parameters, respiratory and phosphatase activity, do not significantly correlate with each other or pectinolyase activity and the numbers of pectinolytic bacteria. This lack of correlation

may be due to correlations of these parameters with other factors such as the soil physical and chemical properties or seasonal temperature fluctuations and others. With respect to these parameters, an examination of the soil physical and chemical properties, and temperature fluctuations in conjunction with each of the seasonal measurements of phosphatase and respiratory activity might prove useful. In addition, measurement of the number of bacteria with phosphatase activity by selective plating might also shed some light on the parameter of phosphatase activity.

Although the Chi-square values of native soil for respiratory and phosphatase activities are different than those for spoils (Table 35), this difference cannot be interpreted as significant. The Chi-square total was significant for all microbiological parameters, which shows that for each test the variances were not homogeneous from plot to plot. However, the ratio of pooled variances for native soils and spoils (Table 36) are not significant (with the exception of the pectinolytic plate count which was due only to a large variance at Gouge plot) which shows that neither the spoils nor the native soil were more heterogeneous than the other. In summary of the analyses of variance due to plant species and times for each parameter (Tables 13,14,18,20,23,25, 30 and 32), the effects of time were significant in all cases whereas the effects of plant species were only slightly significant for measurements of phosphatase activity. This

suggests that these parameters of microbiological activity are generally useful regardless of the diversity of the vegetation at a particular site, but the effects of seasonal variation must always be taken into consideration.

All four microbiological parameters were observed to exhibit significant seasonal variation 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. Although the significance of these differences cannot be explained on the basis of this research, this finding of significant seasonal variation shows that such variation may exhibit interrelationships.

Most studies on soil bacteria have been concerned either with the counting of the total flora or of certain categories. It is very difficult to provide information of the taxonomic kind. Identification is dependent on a large number of often cumbersome biochemical tests. In recent years, various new techniques have been developed based on such characteristics as fatty acid components of cell walls and DNA base ratio of Guanine:Cytosine. These techniques are potentially much more reliable for positive identification of microorganisms and are actually not any more difficult to perform than the standard

biochemical tests used in the past.

The determination of the thermal melting point of DNA led to the conclusion that the unidentified soil organism isolated during this study contained a G:C base ratio of 81.78%. This value closely agrees with the findings of Bowie, et. al. (1972), that the genus Arthrobacter falls into the range of 80-83%G:C.

This method of identification may prove to be much more reliable for soil organisms which often tend to be variable in nutritional requirements and pleomorphic than the standard biochemical tests for identification.

Chapter 5

SUMMARY

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 were spoils plots 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. These analyses included: pH, phosphorus, potassium, nitrate-nitrogen, conductivity, organic matter, calcium, magnesium, sodium, percent available water at 0.3 atmospheres and percent sand, silt and clay. Experimental water-holding capacity, mean bulk density, fertilizer treatment and above ground production were also determined.

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 O₂ 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 variations 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 nature of the clay, a montmorillonite type with magnesium associated in the inner lattice, which may be adsorbing phosphatase enzymes onto its surface where the enzyme competes with other cations for adsorption sites. The absence of this correlation in spoils could be related to the low percent clay found in spoils. Respiratory activity in native soil correlated with only one soil characteristic, water-holding capacity, and that was only for native soil. This relationship, although not correlated with organic matter as is usually seen, is related to the pore space of the soil and particle size since the pores contain the water and air available for soil biological processes. Spoils did not exhibit this correlation, lack of which could be attributed to the more heterogeneous textural types found in spoils.

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. Although the significance of these differences cannot be explained based on the results of this research alone, this finding of significant seasonal variation shows that such variation must be considered in similar ecological studies evaluating the biological activity of soil. 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. This finding suggests that the general nature of these parameters allows them to be applicable to areas of diverse vegetation.

The possibility of other, unidentified, factors contributing to the variation found with the microbiological parameters must not be overlooked.

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 significantly different 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 soil were effective in showing differences. The importance of these differences is a question which requires further research. Further research must take into consideration both variations due to seasons and soil physical and chemical properties, but the influence of various plant species need not be considered. The trend in the correlation between pectinolyase activity and the number of pectinolytic bacteria could be important in discerning ecological differences.

APPENDIX

Table 38. Mean values of all four microbiological parameters for all sites and plants sampled

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
Native Range Un- fertilized(NRU) #1	9-12-73	None	2.2563	118	-- ^e	--
NRU - #12	9-12-73	None	1.7089	30.5	0.254	--
NRU - #33	9-12-73	None	2.2590	40.5	0.231	--
Native Range Fertilized (NRF) - #23	9-12-73	None	0.5003	79.5	0.185	--
NRF - #22	9-12-73	None	0.3526	39	0.240	--
NRF - #5	9-12-73	None	3.9608	410	--	--
Topsoil Gradient (TG) - #14	9-13-73	None	1.6898	77	0.172	--
TG - #27	9-13-73	None	0.2832	44	0.219	--
TG - #37	9-13-73	None	2.7500	23	0.222	--
Gouge (G) - #7	9-12-73	None	2.7875	75	--	--

a = respiration as uliters of O₂ per hour per gram of soil

e = not determined

b = Phosphatase as umoles phenol per gram of soil

c = Pectinolyase as optical density at 550nm

d = Pectinolytic Plate Count as number of bacteria x 10⁴ per gram of soil

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
G - #18	9-12-73	None	0.8494	27.5	0.468	--
G - #38	9-12-73	None	1.6670	13	0.215	--
Demo (D) - #15	9-12-73	None	1.5611	40	0.155	--
D - #11	9-12-73	None	1.0414	4	0.270	--
D - #35	9-12-73	None	1.4950	13	0.186	--
BN - #29	9-13-73	None	0.2541	47	0.108	--
BN - #30	9-13-73	None	1.7104	325	0.084	--
BN - #32	9-13-73	None	1.6065	345	0.100	--
Cape Oliver(CO) - #10	9-13-73	None	1.4350	9.2	0.284	--
CO - #21	9-13-73	None	0.9085	15.5	0.298	--
CO - #26	9-13-73	None	0.3460	74	0.291	--
Dryland Pasture (DP) - #2	9-13-73	None	0.3708	85	--	--

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
DP - #6	9-13-73	None	2.5709	45	--	--
DP - #24	9-13-73	None	0.3054	21	0.298	--
McDonald(MD) - #9	9-13-73	None	1.6720	16	0.243	--
MD - #3	9-13-73	None	1.9391	645	--	--
MD - #28	9-13-73	None	0.2756	290	0.174	--
MP - #4	9-13-73	None	4.7811	155	--	--
MP - #19	9-13-73	None	1.2644	52	0.425	--
MP - #36	9-13-73	None	2.0346	250	0.201	--
Raw Spoils 1 month old (RSM) - #20	9-13-73	None	0.3256	34.5	0.415	--
RSM - #25	9-13-73	None	0.6794	41.5	0.229	--
RSM - #39	9-13-73	None	0.6811	2.3	0.225	--
Raw Spoils 1 year old (RSY) - #13	9-13-73	None	0.7608	36	0.148	--
RSY - #31	9-13-73	None	0.8831	25.5	0.145	--

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
RSY - #34	9-13-73	None	0.7643	0.64	0.239	--
Streeter(S) -#16	9-13-73	None	2.5878	208	0.129	--
S - #8	9-13-73	None	2.6486	172.5	--	--
S - #17	9-13-73	None	2.8066	230	0.354	--
CO - #40	4-10-74	<u>Agropyron smithii</u> (Agsm)	1.5091	21.67	0.121	--
CO - #41	4-10-74	<u>Gutierrezia sarothrae</u> (Gusa)	1.4339	36.67	0.123	--
CO - #42	4-10-74	<u>Chrysothamnus nauseosus</u> (Chna)	1.3212	40.67	0.150	--
MD - #43	4-10-74	<u>Stipa comata</u> (Stco)	1.7643	60	0.364	--
MD - #47	4-10-74	<u>Koeleria cristata</u> (Kocr)	2.0820	65.67	0.234	--
BN - #44	4-10-74	Agsm	4.1940	193.33	0.354	5.920
BN - #45	4-10-74	<u>Artemisia frigida</u> (Arfr)	1.4392	446.67	0.370	10.630
BN - #48	4-10-74	Stco	1.8758	120	0.207	2.256
MP Lower - #46	4-10-74	Kocr	3.2357	74	0.262	--
MP Lower - #51	4-10-74	Gusa	2.3101	80.33	0.293	--

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
MP Upper - #49	4-10-74	<u>Schizachyrium scoparium</u> (Scsc)	2.3269	51.33	0.211	--
MP Upper - #50	4-10-74	<u>Bouteloua curtipendula</u> (Bocu)	2.4503	48	0.257	--
MP Upper #52	4-10-74	<u>Rhus trilobata</u> (Rhtr)	1.3577	32.33	0.303	--
G - #53	4-127-74	<u>Agropyron elongatum</u> (Agel)	0.8723	18.67	0.311	5.147
G - #54	4-27-74	<u>Agropyron cristatum</u> (Agcr)	0.7045	27.67	0.271	17.813
G - #55	4-27-74	<u>Bromus inermis</u> (Brin)	1.1414	15.17	0.225	35.373
G - #56	4-27-74	<u>Artemisia cana</u> (Arca)	1.1868	13	0.227	17.827
NRF - #57	4-29-74	<u>Carex</u> spp.	1.6902	41.33	0.216	5.947
NRF - #58	4-29-74	Gusa	1.7008	81	0.137	9.427
NRF - #59	4-29-74	Stco	1.7386	75	0.118	5.567
NRU - #60	4-29-74	<u>Carex</u> spp.	1.4181	115	0.126	7.696
NRU - #61	4-29-74	Stco	0.3572	103.67	0.296	8.183
NRU - #62	4-29-74	Gusa	0.2577	33.33	0.275	1.833
DP - #63	4-27-74	Agel	0.1914	16.23	0.277	--

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
DP - #64	4-27-74	<u>Dactylis glomerata</u> (Dagl)	0.2257	41.67	0.427	--
DP - #65	4-27-74	Agcr	0.2061	75	0.382	--
S - #66	4-27-74	Stco	0.4017	253.33	0.396	--
S - #67	4-27-74	<u>Pinus ponderosa</u> (Pipo)	3.9205	142.33	0.272	--
S - #68	4-27-74	<u>Agropyron spicatum</u> (Agsp)	1.9737	156.67	0.282	--
D - #69	4-27-74	Agcr	1.5253	108.33	0.290	11.147
D - #70	4-27-74	Brin	0.3478	56.33	0.293	6.513
D - #71	4-27-74	Melilotus officinalis (Meof)	0.3722	73.67	0.346	19.953
30-40 year old spoils						
(OS) - #72	4-27-74	Bare	0.1306	23	0.203	0.833
OS - #73	4-27-74	Oryzopsis hymenoides (Orhy)	0.2187	29.33	0.161	0.393
OS - #74	4-27-74	Chna	0.8686	21.67	0.204	5.600
RSY - #75	4-27-74	Bare	0.6537	17.33	0.241	0.175
RSY - #76	4-27-74	Bare	0.6600	12.17	0.185	0.100

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
RSM - # 77	4-27-74	Bare	0.5288	11.33	0.180	0.927
NRU - #78	8-17-74	Gusa	2.4189	116.80	0.196	19.020
NRU - #79	8-17-74	Stco	3.0962	206	0.147	33.763
NRU - #80	8-17-74	<u>Carex</u> spp.	2.9247	259	0.156	20.593
NRF - #81	8-17-74	<u>Carex</u> spp.	2.2638	149	0.197	4.007
NRF - #82	8-17-74	Gusa	2.4062	106	0.385	25.593
NRF - #83	8-17-74	Stco	2.3108	170.60	0.363	3.850
TG - #84	8-17-74	Brin	4.4725	90.80	0.294	32.093
Ridge above TG (RTG) - #85	8-17-74	Bare	1.0775	35.2	0.239	1.847
G - #86	8-17-74	Arca	1.8036	37.40	0.290	44.393
G - #87	8-17-75	Agel	1.7202	45	0.231	166.613
G - #88	8-17-74	Brin	1.8709	39.80	0.254	174.140
G - #89	8-17-74	Agcr	1.6996	16.60	0.217	25.080

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
D - (Random Plant Location)						
(DRPL) - #90	8-17-74	Brin	1.5084	32.20	0.264	--
DRPL - #91	8-17-74	Brin	1.0675	27.6	0.232	--
DRPL - #92	8-17-74	Brin	2.3383	38.80	0.205	--
DRPL - #93	8-17-74	Brin	2.3780	31	0.218	--
DRPL - #94	8-17-74	Brin	2.1927	29.2	0.480	--
DRPL - #95	8-17-74	Brin	1.9350	23.40	0.440	--
DRPL - #96	8-17-74	Brin	2.4610	40.60	0.333	--
DRPL - #97	8-17-74	Brin	2.0897	41.80	0.349	--
D - Upper Soil Horizon						
(DUSH) - #98	8-17-74	None	1.3880	29.50	0.340	2.167
D - Lower Soil Horizon						
(DUSH) - #99	8-17-74	None	1.0554	20.60	0.245	2.200
DRPL - #100	8-17-74	Brin	2.3626	43.60	0.391	--
DRPL - #101	8-17-74	Brin	2.5019	64	0.361	--

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
D General Plot Variation - (DGPV) - #102	8-17-74	Agcr	1.7363	35.20	0.284	--
DGPV - #103	8-17-74	Bare	1.7683	34	0.282	--
D Staked Variation - (DSV) - #104	8-17-74	Brin	2.0710	69.20	0.354	7.380
DSV - #105	8-17-74	Brin	1.6660	46.40	0.279	4.767
DSV - #106	8-17-74	Brin	2.8009	43.20	0.204	4.160
DSV - #107	8-17-74	Brin	2.9748	43	0.222	4.680
DSV - #108	8-17-74	Brin	2.8226	59.60	0.283	9.433
D - #109	8-17-74	Meof	2.8103	124.80	0.294	29.380
D - #110	8-17-74	Agcr	2.2236	50.40	0.327	12.853
DGVP - #111	8-17-74	Meof	1.2656	12.68	0.455	--
DGVP - #112	8-17-74	Agcr	2.0573	33.20	0.262	--
NRU - #113	10-3-74	Gusa	2.980	94	0.0874	47.947
NRU - #114	10-3-74	Stco	3.1460	109.60	0.088	22.800

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
NRU - #115	10-3-74	<u>Carex</u> spp.	2.7054	123	0.102	38.200
NRF - #116	10-3-74	<u>Carex</u> spp.	0.6562	35.40	0.103	211.533
NRF - #117	10-3-74	Gusa	4.0040	71.60	0.089	47.867
NRF - # 118	10-3-74	Stco	3.8164	88	0.090	70.667
TG - #119	10-3-74	Brin	3.8749	66	0.107	65.800
RTG - #120	10-3-74	Bare	1.6789	20.60	0.141	22.067
G - #121	10-3-74	Arca	3.1650	39	0.169	68.133
G - #122	10-3-74	Agel	2.8307	39.20	0.186	97.533
G - #123	10-3-74	Brin	3.2673	31.80	0.141	219.233
G - #124	10-3-74	Agcr	2.5815	26.60	0.160	172.133
D - #125	10-3-74	Brin	3.7490	54.60	0.163	8.087
D - #126	10-3-74	Meof	3.9631	78.80	0.184	44.706
D - #127	10-3-74	Agcr	3.3424	56.40	0.161	23.787

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
NRU - Quartered Variation						
(NRUQV) - #128	10-3-74	Stco	4.5015	125	0.144	33.067
NRUQV - #129	10-3-74	Stco	5.9092	187.40	0.147	8.927
NRUQV - #130	10-3-74	Stco	4.9934	173.20	0.151	9.660
NRUQV - #131	10-3-74	Stco	5.3567	130.40	0.104	9.630
NRUQV - #132	10-3-74	Stco	4.9806	137.60	0.109	18.153
NRU - Staked Variation						
(NRUSV) - # 133	10-3-74	Stco	3.8721	183	0.149	15.013
NRUSV - #134	10-3-74	Stco	3.9976	123	0.170	10.434
NRUSV - #135	10-3-74	Stco	3.8174	144	0.152	11.157
NRUSV - #136	10-3-74	Stco	3.5410	206.60	0.141	8.607
NRUSV - #137	10-3-74	Stco	3.8421	110.80	0.229	10.073
NRUQV - #138	10-3-74	Gusa	5.6762	98	0.271	--
NRUQV - #139	10-3-74	Gusa	7.5553	118	0.221	--

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
NRUQV - #140	10-3-74	Gusa	7.5007	129	0.202	--
NRUQV - #141	10-3-74	Gusa	4.8807	119	0.275	--
NRUQV - #142	10-3-74	Gusa	5.3904	123.80	0.274	--
NRUSV - #143	10-3-74	Gusa	1.1323	161.40	0.120	--
NRUSV - #144	10-3-74	Gusa	5.3145	190.60	0.125	--
NRUSV - #145	10-3-74	Gusa	2.2781	214	0.110	--
NRUSV - #146	10-3-74	Gusa	1.6711	115.60	0.115	--
NRUSV - #147	10-3-74	Gusa	2.4121	84.60	0.121	--
Demo Quartered Variation						
(DQV) - #148	10-3-74	Brin	2.8206	86.20	0.151	8.247
DQV - #149	10-3-74	Brin	1.7137	66.10	0.263	7.967
DQV - #150	10-3-74	Brin	1.7197	59.40	0.270	9.480
DQV - #151	10-3-74	Brin	3.5216	85.70	0.237	6.860
DQV - #152	10-3-74	Brin	3.1657	76.80	0.248	15.020

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
DSV - #153	10-3-74	Brin	2.3986	30.10	0.113	2.713
DSV - #154	10-3-74	Brin	2.4268	41.20	0.119	3.440
DSV - #155	10-3-74	Brin	2.9533	44.70	0.138	2.427
DSV - #156	10-3-74	Brin	2.2438	47.90	0.139	3.007
DSV - #157	10-3-74	Brin	2.3602	35.90	0.148	2.413
DQV - #158	10-3-74	Agcr	2.6183	57.40	0.150	--
DQV - #159	10-3-74	Agcr	2.8925	87.20	0.308	--
DQV - #160	10-3-74	Agcr	3.3336	91.2	0.296	--
DQV - #161	10-3-74	Agcr	1.8060	66.40	0.294	--
DQV - #162	10-3-74	Agcr	1.6741	69.40	0.331	--
DSV - #163	10-3-74	Agcr	1.9418	80.80	0.242	--
DSV - #164	10-3-74	Agcr	1.9283	70.60	0.230	--
DSV - #165	10-3-74	Agcr	2.1422	79	0.190	--

Table 38. (continued)

Plot and Sampling #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
DSV - #166	10-3-74	Agcr	1.7500	62.80	0.196	---
DSV - #167	10-3-74	Agcr	7.1071	65	0.234	---
BN - #168	10-3-74	Arfr	4.9373	296	0.216	11.120
BN - #169	10-3-74	Stco	1.5085	146	0.274	4.367
BN - #170	10-3-74	Agsm	1.3194	194.40	0.250	7.407
NRU - #171	12-5-74	Gusa	0.4731	44.60	0.360	11.420
NRU - #172	12-5-74	Stco	0.8760	11.30	0.419	12.133
NRU - #173	12-5-74	<u>Carex</u> spp.	1.4981	75.80	0.323	4.840
NRF - #174	12-5-74	<u>Carex</u> spp.	0.7013	43.70	0.323	35.600
NRF - #175	12-5-74	Gusa	6.2967	102.40	0.341	6.567
NRF - #176	12-5-74	Stco	2.9949	96.20	0.320	6.967
TG - #177	12-5-74	Brin	2.9978	53.20	0.292	3.967
RTG - #178	12-5-74	Bare	0.2993	26	0.266	4.900
G - #179	12-5-74	Arca	3.0482	17.30	0.304	00.487

Table 38. (continued)

Plot and Sample #	Date	Plant	Resp ^a	Phos ^b	Pec ^c	PecPL ^d
G - #180	12-5-74	Agel	2.7531	26.40	0.338	3.433
G - #181	12-5-74	Brin	1.6596	54.20	0.297	8.280
G - #182	12-5-74	Agcr	0.7009	31	0.311	4.247
D - #183	12-5-74	Brin	2.7140	45.40	0.222	1.280
D - #184	12-5-74	Meof	2.4215	66.40	0.234	13.660
D - #185	12-5-74	Agcr	1.6610	57.60	0.252	7.473
BN - #186	12-5-74	Arfr	1.3465	273	0.213	11.067
BN - #187	12-5-74	Stco	2.3203	112.60	0.229	4.340
BN - #188	12-5-74	Agsm	1.8750	193	0.244	7.193

Table 39. Physiochemical properties of soils as determined April, 1974

Sample #	pH	P ^a	K ^b	NO ₃ ^c	Salt ^d	ORM ^e	Ca ^{++f}	Mg ^{++g}	Na ^h	H ₂ O - 0.3 ⁱ
#44	8.2	0.35	216	3.03	0.4	2.76	14.93	4.53	0.33	16.77
#45	8.0	0.65	186	2.49	0.6	2.66	15.33	4.96	0.33	25.01
#48	8.2	0.65	246	3.03	0.4	2.66	12.97	3.95	0.33	21.60
#53	8.8	5.70	97	1.44	0.4	0.30	25.41	1.99	0.59	13.73
#54	8.8	0.65	97	1.44	0.6	0.37	23.77	2.61	0.59	14.80
#55	8.8	1.20	87	0.93	0.5	2.15	21.73	2.45	0.55	11.18
#56	8.4	5.40	156	1.44	1.2	0.58	24.18	2.45	0.68	12.68
#57	8.2	6.80	475	10.02	0.9	2.86	30.38	3.51	0.89	30.56
#58	8.2	6.40	395	3.03	0.6	0.84	24.18	3.21	0.63	22.10
#59	8.1	3.30	385	23.93	0.8	3.18	23.77	3.51	0.68	21.39
#60	8.4	1.20	385	2.49	0.5	4.13	29.96	3.51	0.76	20.54
#61	8.3	0.90	435	1.97	0.7	0.77	20.52	3.06	0.72	30.16

a = phosphorus in ppm

b = Potassium in ppm

c = nitrate in ppm

d = conductivity in millimhos

e = organic matter in percent

f = calcium in meq/100g

g = magnesium in meq/100g

h = sodium in meq/100g

i = % available H₂O at 0.3 atm

Table 39. (continued)

Sample #	pH	P	K	NO ₃	Salt	ORM	Ca ⁺⁺	Mg ⁺⁺	Na	H ₂ O - 0.3
#62	8.4	0.90	325	1.44	0.6	1.62	32.47	3.36	0.68	21.39
#69	8.2	8.10	156	1.97	0.7	1.75	11.02	2.30	0.76	10.11
#70	8.3	3.50	176	1.44	0.4	1.68	11.40	1.99	0.55	16.61
#71	8.6	3.50	126	0.93	0.5	0.71	30.79	2.30	0.59	7.59
#72	8.4	0	107	1.44	4.6	0.90	27.88	6.25	0.98	18.40
#73	7.9	0	107	1.44	6.1	2.66	39.29	3.06	0.95	20.02
#74	7.9	0.35	117	1.44	6.4	4.77	35.44	2.61	0.68	21.66
#75	9.0	0.35	87	1.44	0.4	0.15	20.93	2.45	0.63	7.28
#76	8.9	0	77	2.49	0.5	0.37	20.52	2.15	0.63	10.36
#77	8.8	0	77	1.97	0.6	0.15	24.59	2.15	0.55	8.58

Table 39b. Additional physiochemical properties of soils as determined April, 1974

Sample #	%Sand	%Silt	%Clay	Fertilized	MBD ^a	AGP ^b	experimental whc ^c
#44	43.00	29.00	28.00	no	-- ^d	1,539	.6651
#45	43.00	27.00	30.00	no	--	1,539	.5468
#48	43.00	31.00	26.00	no	--	1,539	.5394
#53	71.00	16.00	13.00	yes	1.66	4,376	.3608
#54	61.00	24.00	15.00	yes	1.66	4,376	.3725
#55	73.00	16.00	11.00	yes	1.66	4,376	.4579
#56	73.00	12.00	15.00	yes	1.66	4,376	.4225
#57	47.00	35.00	18.00	yes	1.42	3,365	.4866
#58	49.00	33.00	18.00	yes	1.42	3,365	.4742
#59	47.00	34.00	19.00	yes	1.42	3,365	.5385
#60	51.00	33.00	16.00	no	1.42	1,589	.5234
#61	49.00	35.00	16.00	no	1.42	1,589	.5496
#62	45.00	37.00	18.00	no	1.42	1,589	.4894

a = mean bulk density in g/cc

b = Above Ground Production in kg/ha

c = experimental water-holding capacity

d = not determined

Table 39b. (continued)

Sample #	%Sand	%Silt	%Clay	Fertilized	MBD	AGP	experimental whc
#69	77.00	11.00	12.00	yes	1.70	1,966	.4765
#70	79.00	10.00	11.00	yes	1.70	1,966	.4788
#71	79.00	8.00	13.00	yes	1.70	1,966	.4316
#72	31.00	49.00	20.00	no	--	--	--
#73	29.00	45.00	26.00	no	--	--	--
#74	33.00	42.00	25.00	no	--	--	--
#75	83.00	8.00	9.00	no	--	--	--
#76	75.00	12.00	13.00	no	--	--	--
#77	81.00	8.00	11.00	no	--	--	--

Figure 16. Soil microbiology field sampling data sheet

SAMPLE NUMBER: _____ NAME OF SITE: _____ DATE: _____
 SITE DESCRIPTION: _____
 DATE ESTABLISHED: _____ MECHANICAL TREATMENT: _____
 FERTILIZER USED: KIND _____
 AMOUNT _____
 DATE _____
 PRINCIPAL PLANTS NOW GROWING, INCLUDING THOSE SEEDED IN NATURALLY: _____
 CHECK HERE IF GENERAL SITE PHOTO TAKEN: _____
 SOIL MOISTURE RELATIONSHIP AT SITE, CHECK APPROPRIATE SPACES:
 WET AND PUDDLED _____ WET, MUDDY TO WALK ON _____
 SPONGY, SPRINGY _____ FIRM BUT NOT HARD _____
 HARD AND DRY _____ SUN BAKED, VERY HARD _____
 DRY, NOT ENOUGH CLAY TO BAKE HARD _____
 PARTLY FROZEN _____ DRY, CLODDY _____
 CHECK HERE IF SAMPLE LOCATION PHOTO TAKEN _____
 SAMPLE DESCRIPTION:
 IDENTITY OF NEAREST PLANT: _____
 GROWTH STAGE OF THIS PLANT, CHECK APPROPRIATE SPACES:
 SPRING GROWTH NOT YET BEGUN _____ IN LEAF BUT LITTLE GROWTH _____
 GROWTH WELL UNDERWAY _____ MATURE OR NEARLY MATURE _____
 LATE MATURITY _____ AFTER END OF GROWING SEASON _____
 IN BLOOM _____
 AMOUNT OF SOIL LITTER:
 COMPLETELY COVERS SOIL FROM VIEW _____ NO LITTER, BARE SOIL _____
 INCOMPLETE COVERAGE BUT APPRECIABLE AMOUNT _____ SCANTY LITTER _____
 DEGREE OF SLOPE, APPROXIMATE _____
 TAKEN FROM THE CREST OF A TROUGH _____ TAKEN FROM THE BOTTOM OF A TROUGH _____
 PLANT ROOTS IN SAMPLE:
 PROBABLY ONLY THE NEAREST PLANT NAMED ABOVE _____ NO VEGETATION _____
 MAY HAVE ROOTLETS OF ONE OR TWO OTHER PLANTS _____ NAME THESE: _____
 DENSE VEGETATION, PROBABLY MANY PLANT SPECIES ROOTS _____
 DIRECTIONS: SCRAPE ALL LITTER ASIDE, TAKE A 6 INCH DEPTH CORE NEAR
 ENOUGH TO THE MAJOR PLANT SPECIES TO INCLUDE SOME OF THE FINER ROOTS.
 TAKE ANOTHER SAMPLE WITH A DIFFERENT SAMPLE NUMBER ON THE SAME SITE
 BY THE SECOND MOST PREDOMINANT PLANT.
 DESCRIBE HERE HOW SAMPLE EXACT LOCATION CAN BE IDENTIFIED:
 TWO PHOTOS ARE NEEDED: 1. GENERAL VIEW OF SITE SHOWING VARIETY OF
 VEGETATION, EXPOSURE, SLOPE, ANY SPECIAL FEATURES. 2. CLOSE UP OF
 SAMPLE LOCATION SHOWING PLANT, LITTER SCRAPED INTO A PILE BESIDE PLANT,
 BARE SOIL.

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LITERATURE CITED

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