COMPARATIVE RESPIRATION OF GRAZED AND UNGRAZED PRAIRIE SOILS

by 6221

A. V. SAMUEL

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Approved by:

John O. Harris

Major Professor

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Introduction

Although the phenomenon of increased metabolic activity following partial sterilization in soils was first observed during the first years of this century, it was only recently that soil scientists have begun to probe into this area of inquiry. It has been obvious that this increased soil metabolic activity is correlated with increased soil fertility, which constantly inspired the scientists to isolate the specific factor or factors responsible for the enhanced soil fertility. Such an effort has been unsuccessful to the present time.

Of the many metabolic activities in the soil, respiratory activity was the first studied. It still offers one of the simple but reliable methods of investigation in the soil by estimating oxygen uptake or carbon dioxide evolution.

This study was undertaken to compare the respiratory activity of grazed and ungrazed short grass prairie soils as part of soil decomposer studies conducted at Osage site of the Grasslands Biome, International Biological Program.

Review of Literature

Russell, as early as 1905, worked on soil respiration by directly estimating its oxygen uptake. He employed manometric techniques similar to Warburg respirometers. His aim was to correlate soil respiration to its fertility. Prior to his work the soil analytical chemists knew that drying the soil caused chemical changes in it (Richter, 1896). Otto Rahn (1907) observed that drying and remoistening of soil increased the soil metabolic activity more than in the non-dried soils. He observed that the rich, fertile

garden soils showed a marked boost of physiological activity on drying and remoistening while the poor sandy soils showed less activity. He also noted that drying of soil increased fertility as measured by plant growth. However, he could not explain this phenomenon as he noted a great fall in the microbial populations in the dried soil. Rahn's investigations on bacteriological activities were based on determination of carbon dioxide evolution, acid production in sugar solutions and ammonia production in peptone solution.

The observation that dried soils showed increased fertility prompted the soil microbiologists of that period to isolate some specific factor or factors that might have practical application on soil fertility. Howard and Howard (1910), and Russell (1910) published their investigations on the fertilizing effects of sunlight. Howard and Howard thought that the partial sterilization reduced the microbial predator protozoan population in the soil to cause an increase in the bacterial numbers. Russell expressed the view that the solar rays removed some factor from the soil that suppressed the soil fertility. Greig-Smith (1911) suggested that bacteriotoxins in the soil were denatured by sunlight and permitted the microbial populations to increase.

Fischer (1912) confirmed the work of Rahn and others with his results indicating that the activity in soil was of biological nature. He noted that oxidation was a principal factor, since nitrates increased in the soil when it was air-dried. The fact that drying killed the nitrifying organisms in the soil made him think that soil colloids and surface tension must play an important role in this oxidation. Klein (1915) did work on soil physiology and wrote a good review of work on the physiology of dry soils. He observed the increased carbon dioxide evolution of air-dried

and remoistened soil decreased to a constant and steady level about 35 days after the treatment.

Waksman and Starkey (1923) did extensive work on soil respiration and discovered that the carbon-dioxide evolution reached a peak in about 24 hours after treatment and then fell faster than the decrease of microbial numbers. They were of the opinion that the results were due to the changes created in the soil, its organic matter and modifications of microbial population groups rather than the removal of any one group of organisms. They proposed that the rapid germination of the resting spaces and the mycelial growth took place when the soil was remoistened thus contributing to enhanced production of carbon dioxide. They reported that longer drying periods to the extent of 519 days gave progressively more activity. Working with other partial sterilization methods, they heated the soil at 65°C for one hour and after remoistening observed an immediate decrease in populations followed by rapid increase. Treatment with volatile disinfectants had a similar effect.

Khabil (1929) confirmed the decrease in bacterial numbers after drying the soil. Subsequent remoistening and incubation gave higher bacterial populations than in the permanently moist-soil. He also confirmed the idea that the treatment does not improve the metabolic efficiency of bacteria after the treatment of the soil.

Rovira (1953) suggested the application of the use of Warburg respirometers to soil respiration studies. Working with air-dried remoistened soil, he observed a very short lag period followed by an increased respiration in spite of low microbial numbers. This, he suggested, was due to the existence of some microbial populations in a resting or in an inhibited

state, which when exposed to moisture, were able to respond without significant multiplication or adaptation to improved moisture contents. Following this, Bunt and Rovira (1955) used the apparatus to investigate the temperature of incubation and heat treatment of soil metabolism. They observed the ratio of carbon dioxide evolved to oxygen consumed was always below 0.4 in heat sterilized soils while in those of unsterilized soil it was around 1.0. They attributed this difference to a different type of oxidation in heat sterilized soils. They found a high correlation between organic matter content and oxygen uptake of soils. They further attempted to find if the activity was due to living organisms or residual enzymes from killed organisms or possibly from chemical oxidation. Their results appeared to be that the oxidative enzymes of the dead organisms were chiefly responsible for the immediate burst of respiration on wetting an air-dried soil.

To learn if organic matter in the soil could be exposed to microbial attack by means other than drying, Rovira and Greacen (1959) devised equipment for extreme tillage and aggregate disruption of the soil and found that such exposure showed an increase in oxygen uptake of soil microorganisms in a qualitative manner. Addition of sugar to tilled and untilled soil gave similar results and this excluded the possibility that aeration of the soil was the reason for the former observation. Biological inhibitors like sodium azide, sodium chloride, mercuric chloride had strongly depressive effects on respiration, indicating the soil respiration was of biological origin.

Working with paper chromatography, Stevenson (1956) demonstrated the presence of some amino acid fractions in the extract of air-dried soils while the fresh untreated soils did not show such fractions. He felt this

to be representative of increased nutrients in air-dried soils.

The stimulation of respiration in remoistened air dried soils has been variously ascribed to the physiological youth of surviving organisms (Birch 1956; Soulides and Allison 1960), newly available nutrients (Stevenson 1956, Birch 1959, Soulides and Allison 1960), germination of spores and rapid mycelial growth (Waksman and Starkey 1923), destruction of inhibitory substances (Greig-Smith 1911), free enzymes in the soil (Bunt, Rovira 1955) or combination of all these. Use of volatile antiseptics or mild heating also produced similar results (Waksman and Starkey 1923; Birch 1959; Funke and Harris 1968).

Birch (1959) reaffirmed that the longer the soils were dried, the greater was the subsequent decomposition and nitrate formation following remoistening the soil. Since no more moisture was lost after the third day from the air-dried soil, the drying must have been related to a factor in the dry state which increased with time. The increase in nitrate formation can be expressed as a function, for some highly organic soils, of the log of the time the soil dries. No appreciable differences were observed between the effects of air and vacuum drying of the soil. He tested the possibility of increased solubility of organic matter on drying by addition of charcoal powder to dried soil and then moistening it. The addition of charcoal did reduce the rates of respiration. Birch (1959) also worked with ether vapour and found it to work the same way as drying the soil, but this was probably not related to the solubility of organic matter as evidenced by charcoal not having been able to influence oxygen uptake process in this case.

Birch (1959) drew some interesting conclusions from previous work. He

observed that the drying effect on the humus fraction was little affected by the soil with which it was associated. This he thought to show that drying must involve the organic fraction, probably in colloidal form. He speculated that the heat treatment led to opening of the gel-pores which lead to a greater area of contact with added water. Reversability would involve swelling which closes the spaces, returning the colloidal gel to a condition characteristic of most gels. In a mortar, he ground dried and remoistened soil after its flush of activity declined, to expose more organic matter to obtain renewed increase in microbial activity. The results were inconclusive to think that soil organic matter was directly involved. Funke and Harris did not find any improvement in microbial respiration on remoistening overnight air dried soils ground in a mortar to expose more organic matter or from soil slurries made in a Waring blender and lyophilized immediately.

On the other hand, Birch (1959) stated that an argument against this concept was the fact that heating without drying caused a similar effect but would not cause the postulated changes in the colloidal gels. The treatments with volatile antiseptics were also not explained. This latter problem came to the attention much earlier of Greig-Smith (Waksman 1932, page 760) who had proposed that organic matter was protected from microbial attack by a fatty material from the plant residues. Birch argued that the volatile antiseptics were acting as fat solvents, exposing organic material for microbial attack. Birch (1959) concluded that treatments causing increased activity eliminate large portions of populations. Part of the increased activity could be due to cell material of the dead population now available to the survivors.

Birch (1960) reported that the flush of decomposition occurred through 105 treatments, although in a decreased magnitude. The projection of this decreased rate of activity, Birch expressed, mathematically could be extended to take place through 250 such cycles, mineralizing 73% organic matter originally present in the fresh soil, and the rest would be microbially unavailable. He speculated that some mechanism is operating that protects the organic matter from complete microbial decomposition.

Olsen and McCalla (1960) working with respiration of soils subtilled or ploughed over a 20-year period found that respiration in the subtilled plots was more than that of ploughed plots. They observed the rate of respiration in both soils decreased with depth of sampling. Drying and remoistening soil in both samples increased respiration. Working with various organic material (starch, mannitol, dextrose, lignin), herbicides (2, 4-D, Dalapon, carbonates), mineral nutrient solutions (at several concentrations) and a biological inhibitor (KCN), they observed generally high rates of respiration in subtilled plots than in ploughed plots regardless of the additive except in the case of KCN which progressively reduced the respiratory activity. They concluded that the nature of the microbial respiration in both tillage systems was qualitatively similar, although, samples from subtilled surface soil gave greater respiration quantitatively.

Gaarder (1963) noted that heath soils containing raw organic matter and forest soils under alder showed that microorganisms may thrive in uncultivated soils on very scant supplies of nutrients. He based this conclusion on the observation that when he added nitrates and phosphates to those soils, there was an increased rate of respiration. He found trace

elements did not have much effect.

Beckman, Scharpenseel (1964) working with soils sterilized at 140°C, speculated that carbon dioxide may be released not only by biological activity, but also by chemical decarboxylation of unidentified organic components. The reaction started at pH 6.5 to 7 and its magnitude increased with increasing pH indicating that the increase in soil respiration following liming was partially associated with the process of alkaline decarboxylation.

Shuichi, et. al. (1964) working with volcanogenous cultivated and virgin soils observed the latter to contain fewer microorganisms than the cultivated soil. The speculation was that high organic matter content of the virgin soil with high acidity had some factor or factors that proved detrimental to the microorganisms. Addition of lime increased soil metabolic activity in virgin soil, while the cultivated soil did not respond significantly.

Ross and Roberts (1965) noted the oxygen uptake of soil to increase two to three fold on addition of glucose to Tussock grassland soil and the rate of native organic matter decomposition markedly decreased (Keefer, Mortexen - 1963--in Ross and Roberts - 1965) in the grassland soils.

The sources of carbon dioxide in the soil were respiration of soil microorganisms, soil fauna, and the roots of higher plants. Some carbon dioxide was released during the production of H_2CO_3 in soil chemical reactions (McLaren and Peterson 1967). Most carbon dioxide, however, came from microbial respiration. Even in identical soil types, the various metabolic activities in the soil vary with the type of plant association.

Funke and Harris (1968) noted that soil respiration was stimulated more by mild oven heating followed by moistening than drying alone. They attributed this to possible induced germination of bacterial spores present in the soil.

Stout and Dutch (1968) differentiated soil respiration into "basal respiration" which may be inhibited by moisture deficiency or its excess; and "zymogenous respiration" caused in response to glucose amendment of soil. Working on grassland soils with different levels of productivity under different types of vegetation and a pasture land soil under whey irrigation, they noted the basal respiration was not directly related to the productivity of the soil and therefore, they stated, was not a total reflection of metabolism. The zymogenic respiration appeared to have significant differences in the two soil treatments. This they related to the differences of animal (particularly earthworm) and microbial populations. This may be important in assessing soil metabolism.

Ross and Roberts (1968) working with Tussock grassland soils from different altitudes found that sucrose and starch hydrolysing enzyme activity without added substrate generally increased with the increase in the altitude of the soil. They noted enzyme activity to be strongly correlated with the organic carbon and to a lesser extent to the soil moisture content. They also found that the oxygen uptake values were positively related to these factors and to the numbers of viable bacteria. Consistent differences between soils were not found. Measuring the oxygen uptake rates in two irrigated white clover pastures, Rixon (1968) observed the highest rate was at a moisture level of pF2 for the soil and pF 2.8 for the organic mat on the soil. He noted that at moisture tensions greater than pF2, the highest rate

of O_2 uptake per gram of organic C was of the same degree of magnitude for both mats and soils despite their highly different organic carbon contents. He speculated this due to having the same C/N ratio in mats and soils. Korah and Singte (1969), after incorporating finely ground inedible oil cakes in soils, observed the rate of CO_2 evolution to be inversely proportional to the content of crude fibre in the oil cake.

Owens, L. D. et. al. (1969) and Gilbert and Griebel (1969) working with alfalfa and other plant volatiles on soil respiration found these volatiles increased it three to eight fold. Owens identified the volatiles and tried them separately with varying results. He noted acetaldehyde alone at distillate level of concentration was alone responsible for half the respiration increment of treated soils. Gilbert and Griebel observed that the volatiles diffused into the soil readily and caused a rapid burst of respiration. They noted the respiration depended on the concentration of the volatiles. At high concentrations, the respiration rate fell after only two hours indicating that the volatiles were toxic at high concentrations.

Conducting respiratory studies of soils fumigated with formalin or methyl bromide for six months to 5 1/2 years, Jenkinson (1970) noted that fumigated and unfumigated soils respired similarly when incubated in the laboratory. After they were exposed to chloroform vapour, the fumigated soils respired less rapidly and mineralized less nitrogen than the unfumigated soils. Irradiation (2.5 Mrads) was broadly similar to chloroform vapour in its effect on soil respiration and mineralization. The results, he attributed, to the elimination of a section of the soil biomass during field fumigation. Recovery was not complete even after several years.

Clark (1969) concluded that a great majority of microorganisms in the soil are dormant or inactive at any given time. In the periods of their intense activity, they have tremendous influence on diverse biotic and abiotic components of the grassland ecosystem. With regards to grassland fauna, Paris (1969) expressed that Saprophagous primary decomposers play the same role, to a degree, as do bacteria and fungi in the soil. They affect the decomposition of dead plant and animal material, thus dissipating the energy and returning nutrient elements to the soil. Microfloral grazers may serve to recirculate energy and nutrients in grasslands. The soil fauna hastens the process of decomposition by bacteria by communiting and scavenging the raw and crude plant and animal debris, reducing the size suitable for speedy bacterial action. Populations of Saprovores and microbiovores typically fluctuate in abundance both seasonally and from year to year.

The available literature considers that some mechanism serves to protect the soil organic matter from complete decomposition to avoid complete depletion. Attempts were made to explain how drying the soil exposes additional soil organic matter to microbial action. Great difficulty has been faced in formulating a concept that would adequately explain the fact that a number of treatments, widely different in nature, cause an increase in soil respiratory activity.

Materials and Methods

Soils used in these experiments were obtained from grazed and ungrazed (short grass) prairie on the International Biological Program's Osage site on the Adams' ranch near Grainola, Oklahoma. The Labette silty clay loam soils in both cases were collected to a depth of 6 inches,

uniformly mixed removing all the undecomposed root and other plant material, and passed through a #10 mesh sieve. The soil that passed through the sieve was sealed in plastic bags and stored at 8 C.

Water (moisture) holding capacity of the soils was determined by placing 100 g of oven dried (at 105-110 C for 36-48 hours) soil in moistened cones of filter paper in funnels. A known volume of distilled water was slowly added to the soil in the funnel while plugging the stem of the funnel to facilitate complete saturation with water. Water was added until the soil was fully submerged in the cone of filter paper. The water was drained out and collected in a measuring cylinder over a period of 30-40 min until no water dripped from the soil. The difference in the volume of water added to the soil and that drained out represented the moisture holding capacity of the soil.

Loss of moisture on air drying and oven drying were also determined to establish moisture optima for microbial activity in the soil. The loss of moisture from fresh soil on air drying was determined by drying a known weight of fresh soil on a sheet of aluminum foil of known weight for four days at constant temperature of 30 C.

Oven dried samples were heated at 105-110 C for 36-48 hr; cooled in a desiccator and reweighed.

Bacterial and mold mycelial counts were determined for the grazed and ungrazed soils. For thorough mixing, 11 g of soil was homogenized in an Osterizer in 99 ml of sterile distilled water for 5 min. The homogenate was serially diluted from 10^{-1} to 10^{-6} in sterile distilled water. Aliquots of the above were plated in disposable sterile plastic plates with plate count agar (Difco), to give dilutions of 10^{-4}, 10^{-5}, and 10^{-6}. The plates were incubated at 30 C for 48 hr. The counts were made on a Quebec colony

counter (Spencer Lens Company, Buffalo, N.Y.) using a hand tally. For the estimation of the mycelial lengths, the dilutions of 1/10 and 1/100 were used. To 1 ml aliquots of the dilutions, 0.8 ml of sterile distilled water and 0.2 ml of plate count agar were added and shaken. Of this, 0.01 ml was carefully spread over five 1 cm^2 areas on a clean microscopic slide. After they dried, the slides were stained with methylene blue and the lengths of the mycelial hyphae were measured under oil (1000 X) with a Camera Lucida (Spencer American Opticals) attached to the microscope. An inch counter (copyright Western Germany) was used to trace and calculate the hyphal lengths.

Moisture optima for soil microbiological activity in terms of oxygen uptake was determined by increasing soil moisture content from 15-60% in 5% increments in 4 g soil samples, both fresh and air dried, taken in Warburg respirometers.

For determining rate of CO_2 evolution from soils with closed system, plastic leakproof disposable 18 quart capacity garbage bags (Union Carbide) were used. The soil samples of known weight were placed in beakers of suitable size, moistened to the optimal biological activity and enclosed air tightly in the plastic bags along with KOH taken in 50 ml beakers and incubated at 30 C for 24 hr. After incubation, the excess alkali was titrated with standard HCl with phenolphthalein as the indicator. Just before titration 30% $BaCl_2$ was added to the alkali. Both fresh and air dried soils with and without cellulose were used in this investigation.

Moist heating of soil was done by placing 4 g soil in the Warburg flasks. Before heating, substrates, if required, were added and moisture content was increased to the optimal saturation by uniformly adding distilled water to the soil with a fine tipped pipette (1 ml in 1/100). The flask and its side arm were then closed with aluminum foil. After allowing

the flasks to stand for 15-20 min for moisture equilibration, they were placed in a hot water bath at 80± 1 C for 30 min. The flasks were transferred to a freezer at -20 C and kept for 5 min to cool them rapidly.

Cellulose used in the experiments was obtained by grinding small pieces of Whatman #1 filter paper with distilled water in an Osterizer. Excess water was squeezed from the pulp which was then dried in an electric oven overnight at 105-110 C. The dry pulp was again beaten in the Osterizer to separate the individual cellulose filaments into a loose fluff. This loose fluff was suitable for providing maximum soil-cellulose contact.

Oxygen uptake of soils was measured in duplicates by Warburg respirometers at an incubation temperature of 31 C. Four gram samples were used in all Warburg respirometers. Soil in the flasks was uniformly spread, moistened uniformly with a required amount of distilled water or liquid substrate with a fine tipped pipette. In experiments using cellulose as substrate, 0.1 g of loose cellulose fluff was uniformly mixed with soil in the flasks and then the moisture content was brought up to the optimum.

The equilibration and incubation periods varied from 30 min to 10 days based on the nature of the experiments. For experiments with incubation periods more than 24 hours, the flasks were left immersed in the reservoir keeping the stopcocks open. Before each measurement period, flasks were detached from the manometers for an additional aeration. Alkali (10% KOH--0.2 ml) was changed every day in the central wells. After attaching the flasks to the manometers, the stopcocks were kept open for a period of 20-30 min for equilibration before the readings were taken.

To check the separate effects on soil respiration, the following were added with a pipette to the soil in the respirometers to give an optimal moisture saturation and a concentration of 100 ppm of N, PO_4, SO_4 and Ca

(nitrogen as NH_4NO_3, PO_4 as K_2HPO_4, SO_4 as KSO_4 and Ca as $CaCl_2$). In addition to these, the effect of 0.1 M glucose was also investigated.

A pure culture of yellow pigment forming Cellulomonas isolated from prairie soil was used to test if an increased cellulose utilizing bacterial population in the soil will give greater oxidation of native organic matter or pure cellulose added to the soil. The bacteria were grown in Ashby's mineral salt solution with cellulose as the sole carbon source. For preparing a suspension to be used for respirometer additions, the organism was grown in nutrient broth in 250 ml Erlenmeyer flasks fitted on a shaking machine for 36 hours. The bacteria were washed by centrifuging and suspending in sterile distilled water alternately 3-4 times. Finally a suspension of $8.3X10^{12}$/ml population density was added to the soil as described.

Results

Prior to the first series of the experiments in this investigation, the moisture holding capacity of the grazed and ungrazed soils was determined and was found to be 78% and 75% respectively.

Bacterial numbers and mold hyphal lengths were determined with plate count agar. The mean of 8 samples from a depth of 0-10 cm in the grazed soil was $6.20X10^6$ for bacteria and 498 m of mycelia, while in the ungrazed soil, $8.00X10^6$ bacteria and 728 m of mycelia in a gram of soil. The actinomycete population constituted less than 1% of the total soil microflora. This low count may be due to high acidity of the soils.

Using 4.0 g of soil in Warburg flasks, the optimum soil moisture saturation for maximum microbiological activity in the soil in the form of oxygen uptake was determined for fresh and air dried samples of grazed and

ungrazed soils. To do this, the uniformly moistened soil samples were preincubated overnight at room temperature and then the oxygen uptake was measured with Warburg apparatus at hourly intervals for 7-8 hr.

As shown in plate I, the soil microbiological activity was maximum at 45% soil moisture saturation for all soil samples. It can also be noted that the respiratory activity in the air dried soils was far greater than in the respective fresh soils. In both fresh and air dried samples, the grazed soils showed more respiration than the ungrazed soils.

Many workers have reported that glucose added to the soil readily increases the microbial activity. An investigation was undertaken to see how addition of 0.1M glucose would effect the respiratory activity in the grazed and ungrazed soils. The glucose solution was uniformly distributed in the soil until the soil was moistened to 45% saturation and preincubated overnight at room temperature along with a control which was moistened with distilled water to bring it to optimum moisture. To determine the lag period of microbial activity, glucose was added to another set of samples just before running the experiment. The results presented in plate II show that samples amended with glucose, after preincubation show an 8 and 5 fold increased microbial activity over their respective controls in fresh and air dried soils, respectively. It can also be noticed that nonincubated soils with glucose showed a lag period of 5-6 hours. The grazed soil showed a higher rate of respiration than the ungrazed soil.

The speculation that drying of soil exposes more soil organic matter to microbial activity (Birch (1959) and others) led to check if the addition of purified cellulose to the soils would increase soil respiratory activity.

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EXPLANATION OF PLATE I

Optimum moisture saturation for maximum microbial respiration fresh and air dried samples of grazed and ungrazed prairie soils. No substrate was added. Before the run, the samples were equilibrated overnight at room temperature.

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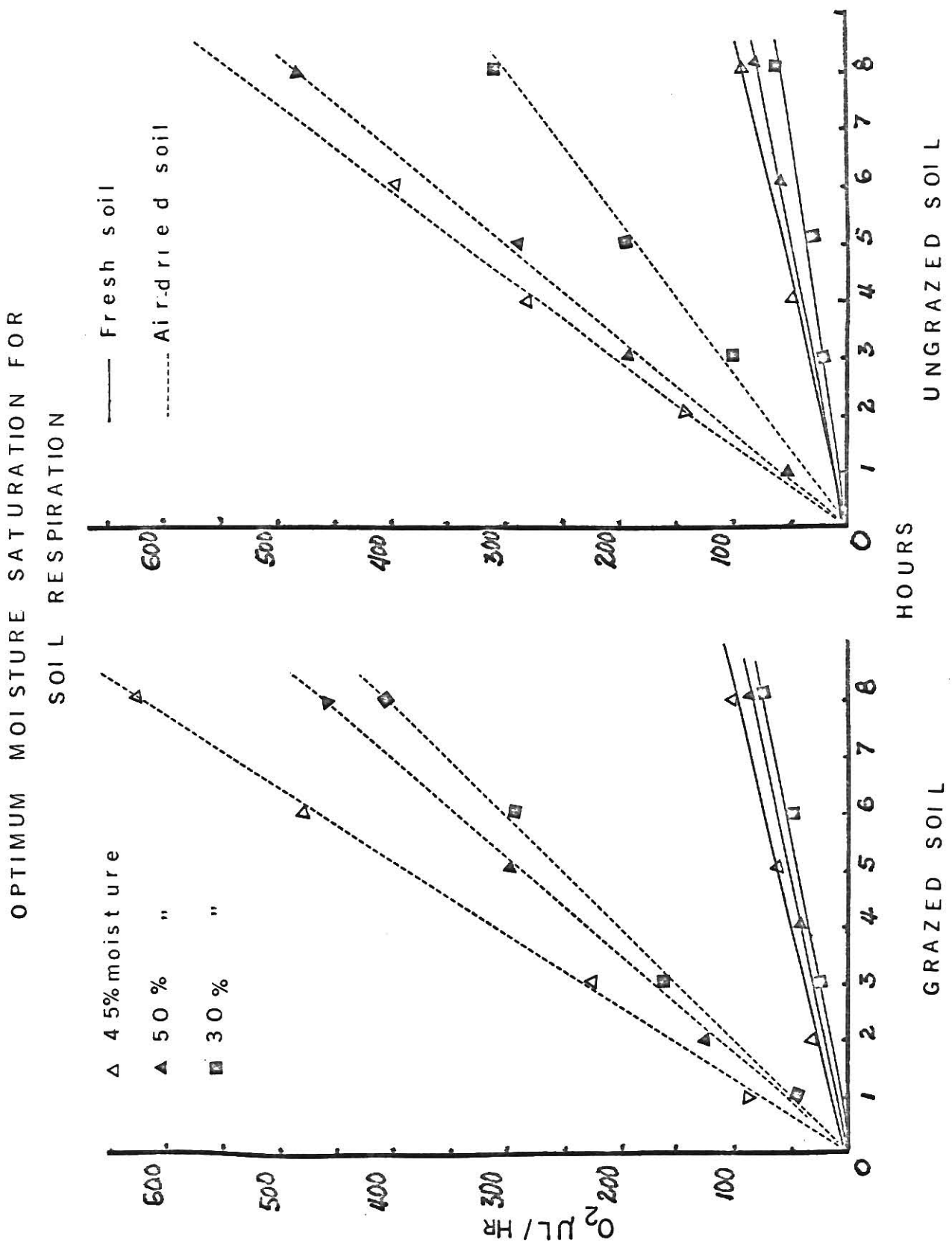
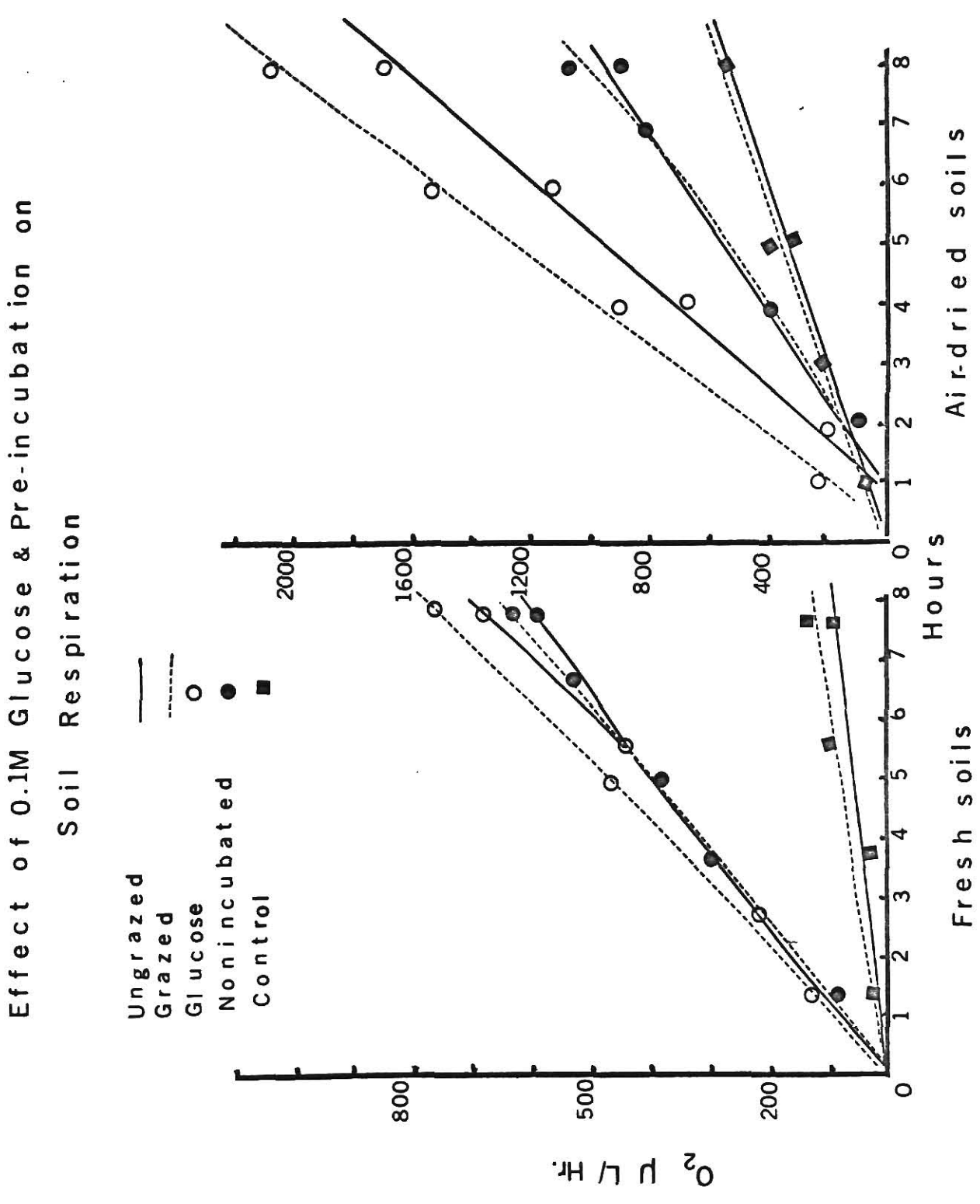

OPTIMUM MOISTURE SATURATION FOR SOIL RESPIRATION
Fresh soil
Air-dried soil
45% moisture
50 % "
30 % "
O_2 µL / HR
HOURS
GRAZED SOIL
UNGRAZED SOIL

EXPLANATION OF PLATE II

The effect of 0.1M glucose and preincubation on the microbial respiration of fresh and air dried samples of grazed and ungrazed prairie soils.


Effect of 0.1M Glucose & Pre-incubation on
Soil Respiration
Ungrazed
Grazed
Glucose
Nonincubated
Control
O_2 µL/Hr.
Hours
Fresh soils
Air-dried soils

Cellulose was added to the samples of fresh and air dried soils from grazed and ungrazed localities. After thoroughly incorporating cellulose with the soil in the respirometer flasks, the moisture saturation was adjusted to 45% and the samples were incubated for 10 days. The oxygen uptake was measured each day for 4 hours and the second hourly readings are presented in plate III.

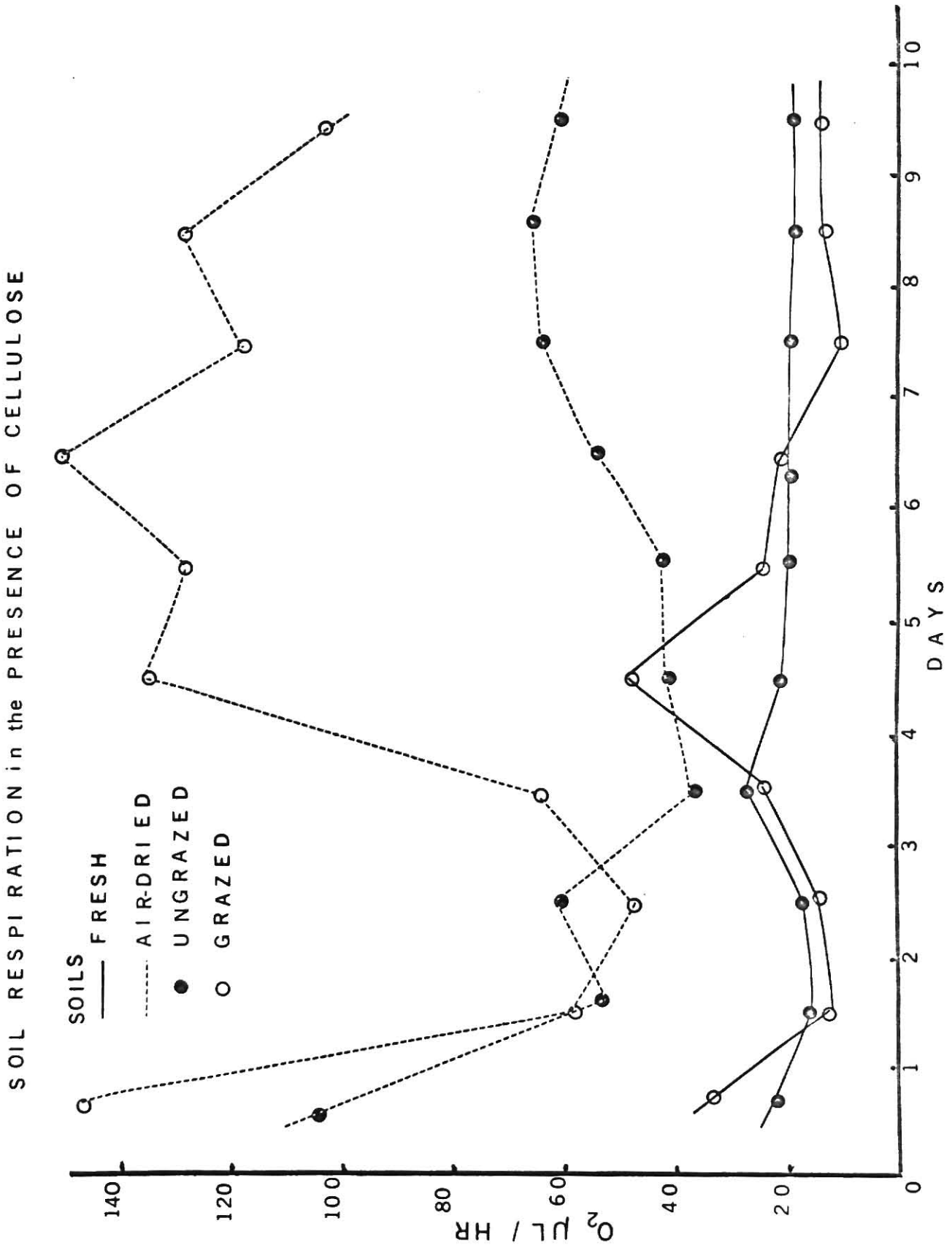
The results show that the cellulose decomposers in all the samples needed an adaptation period of three to four days before they can decompose the purified cellulose. In fresh soils, although this observation is evident, it is not pronounced. The decomposition of cellulose in air dried grazed soils is more than twice as much as it is in the air dried samples of ungrazed soil. The adaptation period for grazed air dried and fresh soils seems shorter for microbial activity than in the ungrazed soil.

Following the work of Funke and Harris (1968), on the effect of moist heat shocking on soil respiration, an experiment was designed to check whether purified cellulose could be utilized in soils after giving a moist heat shock at 80 C for 30 min, followed by a 10 day incubation period.

The results presented in plate IV show a high rate of respiration for 24 to 36 hours following the shock. The respiration rate falls rapidly until the third day and then gradually over the rest of the incubation period. However, the grazed soils shows slightly higher rates of respiration than the ungrazed soil both in fresh and air dried samples. This data suggest that the cellulose decomposers are inactivated almost entirely as indicated by the gradual tapering of the respiratory curves. The organisms that remain after the shock do not seem to break down cellulose.

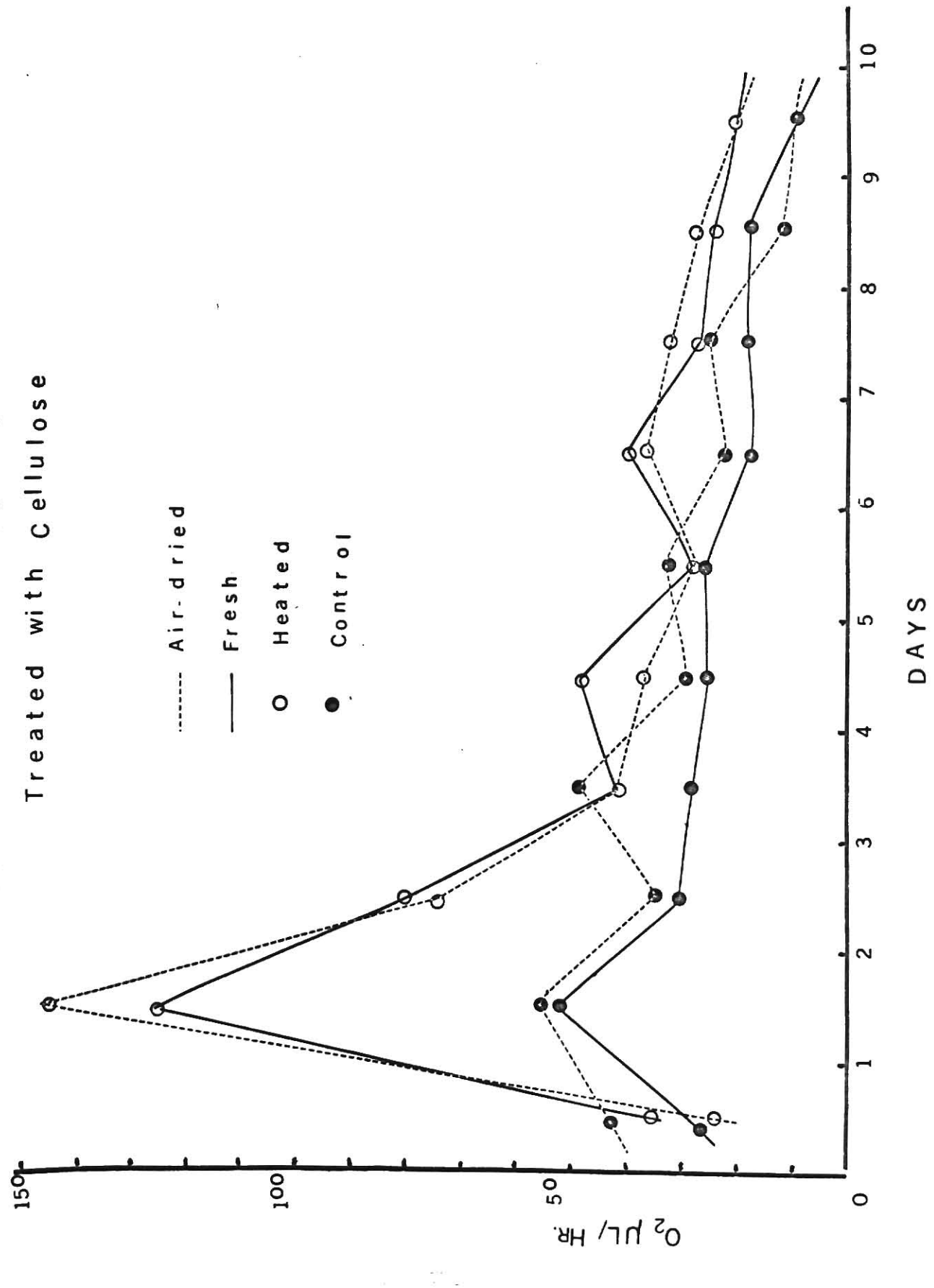
EXPLANATION OF PLATE III

The effect of purified cellulose on the respiration of fresh and air dried samples of grazed and ungrazed prairie soils during ten days incubation period.


SOIL RESPIRATION in the PRESENCE OF CELLULOSE
SOILS
FRESH
AIR-DRIED
UNGRAZED
GRAZED
O_2 µL / HR
140
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
DAYS

EXPLANATION OF PLATE IV

Effect of moist heat shock at 80 C for 30 minutes on the respiration of fresh and air dried grazed soil treated with purified cellulose during an incubation period of ten days.


Effect of Moist-heat Shock on Grazed Soil
Treated with Cellulose
Air-dried
Fresh
Heated
Control
O_2 µL/ Hr.
150
100
50
0
1
2
3
4
5
6
7
8
9
10
DAYS

A pure culture of cellulose decomposing bacterial suspension (1.4 ml) was added to the soils treated with purified cellulose to see if the increased cellulolytic bacterial population in the soil could increase the decomposition of added cellulose or native organic matter. The incubation period lasted for 10 days.

The data collected for air-dried soils is presented in plate V and that for fresh soil in plate VI. Comparing the data for air dried soils in plates III and V, it could be seen that the added bacteria were able to break down the purified cellulose to a significant extent in both grazed and ungrazed soils. The increased bacterial populations also seemed to utilize the native organic matter reasonably well.

In the case of fresh soils, as presented in plate VI, the added bacterial populations seem to digest purified cellulose and native organic matter in the ungrazed soils better than in the grazed soils. In the grazed soils, native organic matter seemed to be more readily available for utilization than the added cellulose.

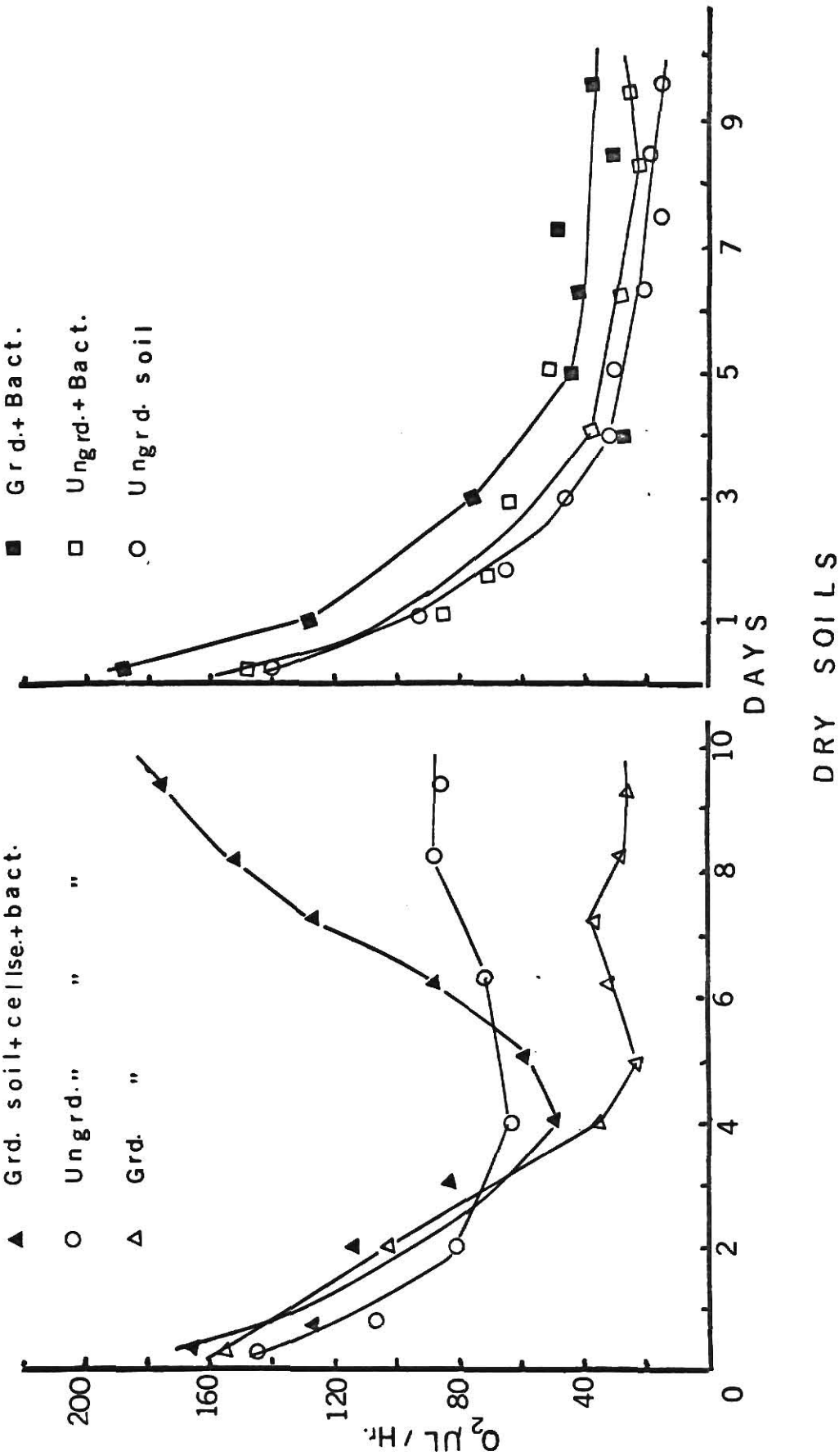
Respiration of soils treated with cellulose and nitrogen, phosphate, sulphate, and calcium at 100 ppm was studied separately on air dried grazed soil. The solutions of the above nutrients were added to the soil and incubated for 10 days.

The data presented in plate VII and VIII show that an adaptation period of 4 days was needed before the microbes could decompose the added cellulose. It can be noticed that there is no marked difference in oxygen consumption rates in the presence of increased available nitrogen in the soil. It is interesting to note that in the presence of added nitrogen the adaptation period for cellulose decomposition is prolonged and the rate of respiration

EXPLANATION OF PLATE V

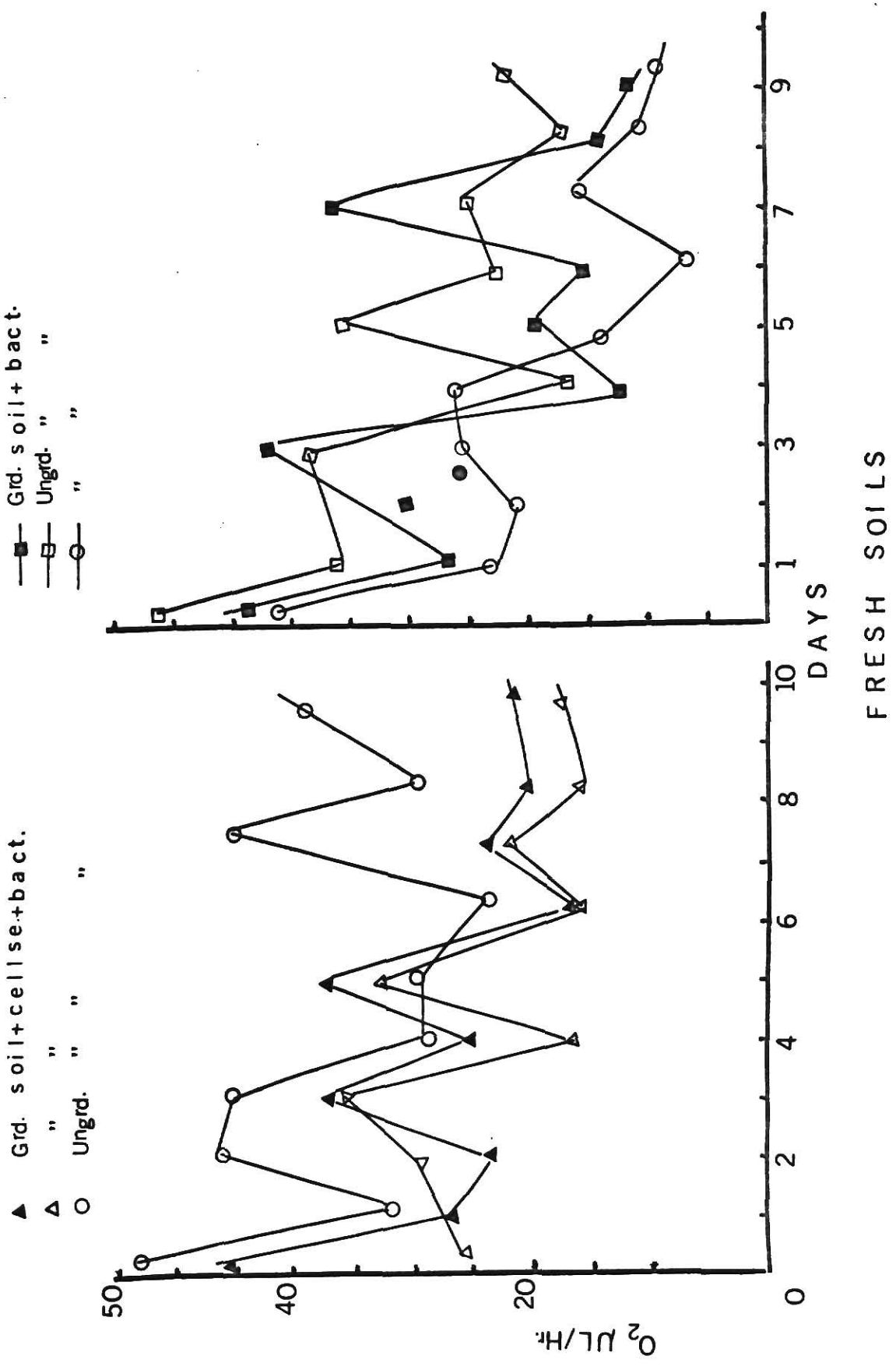
Effect of cellulolytic bacterial suspension of $1.162 \times 10^{13}/1.4$ ml added to the soils to check the rate of digestion of purified cellulose added to air dried samples (plate V) and fresh samples (Plate VI) of grazed and ungrazed prairie soils. Incubation period was ten days.

Air-dried & fresh soils with added cellulose & bacteria.



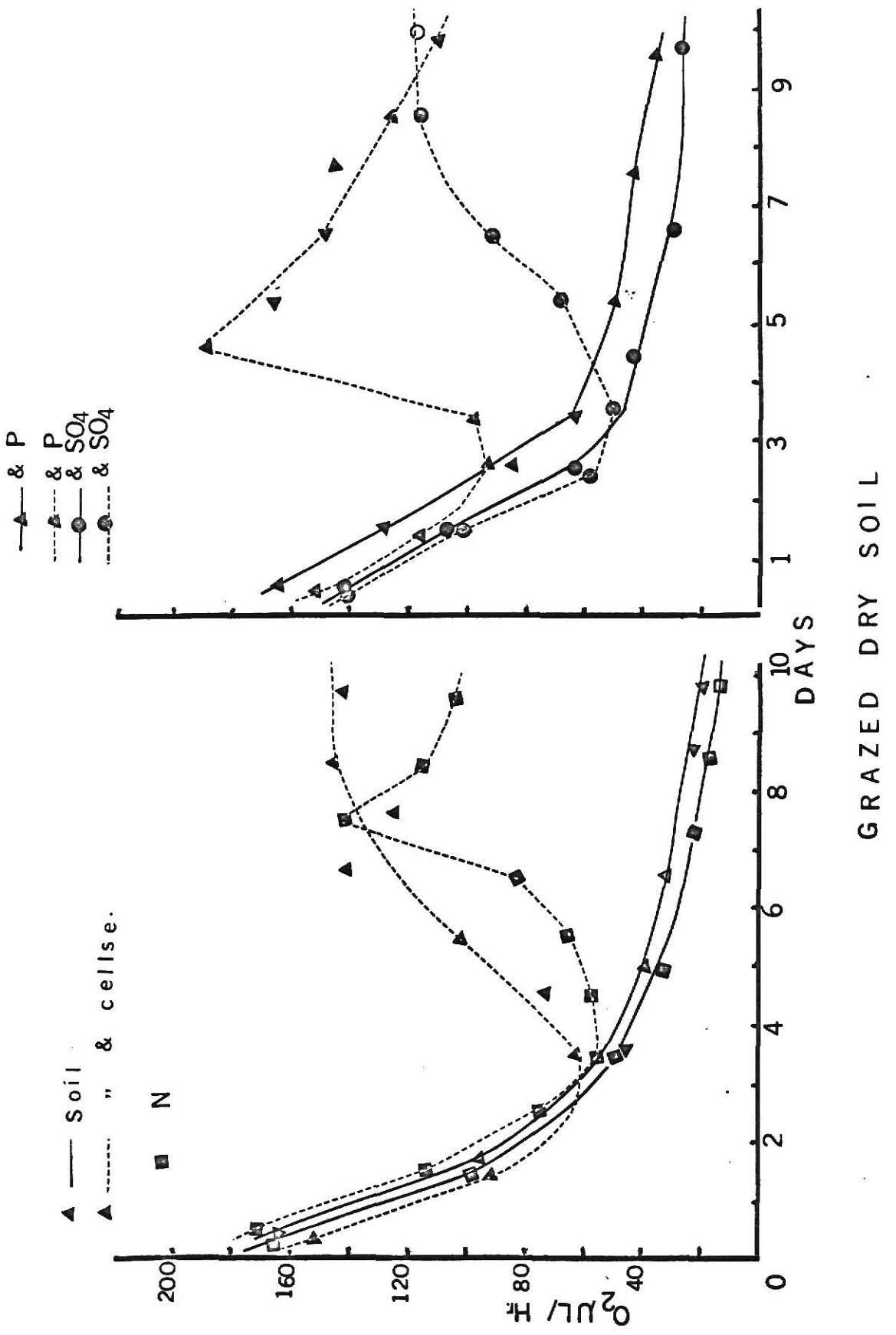
EXPLANATION OF PLATE VI

Effect of cellulolytic bacterial suspension of $1.62 \times 10^{13}/1.4$ ml added to the fresh gra. ungra. soils treated with purified cellulose to check cellulose digestion rate during a ten days incubation period.


Grd. soil+cellse+bact.
" " "
Ungrd. " "
Grd. soil+ bact.
Ungrd. " "
" "
O_2 µL/Hr.
0
20
40
50
2
4
6
8
10
1
3
5
7
9
DAYS
FRESH SOILS

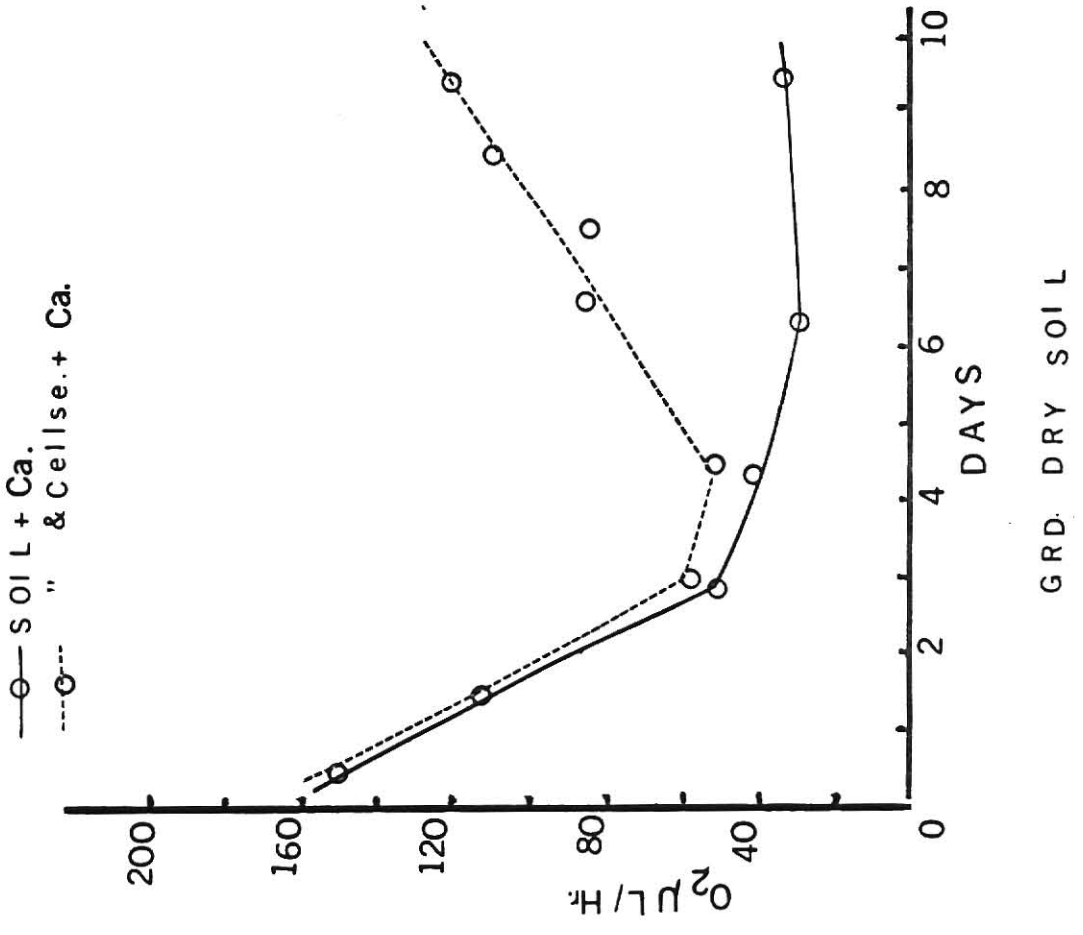
EXPLANATION OF PLATE VII

Microbial respiration of grazed air dried prairie soil treated with purified celluose and 100 ppm separately each of available nitrogen, phosphate, and SO_4 as compared to untreated controls.


GRAZED DRY SOIL
DAYS
O_2 µL/ hr
Soil
" & cells.
N
& P
& P
& SO_4
& SO_4

EXPLANATION OF PLATE VIII

Microbial respiration of grazed and air dried prairie soil treated with purified cellulose and 100 ppm of Ca^{++} as compared to untreated control.



GRD. DRY SOIL

seemed to be slower. Addition of nitrogen did not seem to increase the rate of decomposition of native organic matter, but on the contrary, it seemed to have an inhibitory effect on it.

Phosphate seems to have increased the soil microbial decomposition of cellulose both in purified and native forms. It can be noted that unlike nitrogen, phosphate has a more positive effect on soil respiration right from the beginning of incubation. Both native organic matter and purified cellulose are decomposed rapidly and at a higher rate in the presence of increased available PO_4 than with N.

Sulphate seemed to aid the decomposition of native organic matter better than pure cellulose in the soil. As with added nitrogen the adaptation period with sulphate is prolonged and it does not seem to be a limiting factor in the soils. As presented in plate VIII, Ca does not increase the decomposition of cellulose in both the cases.

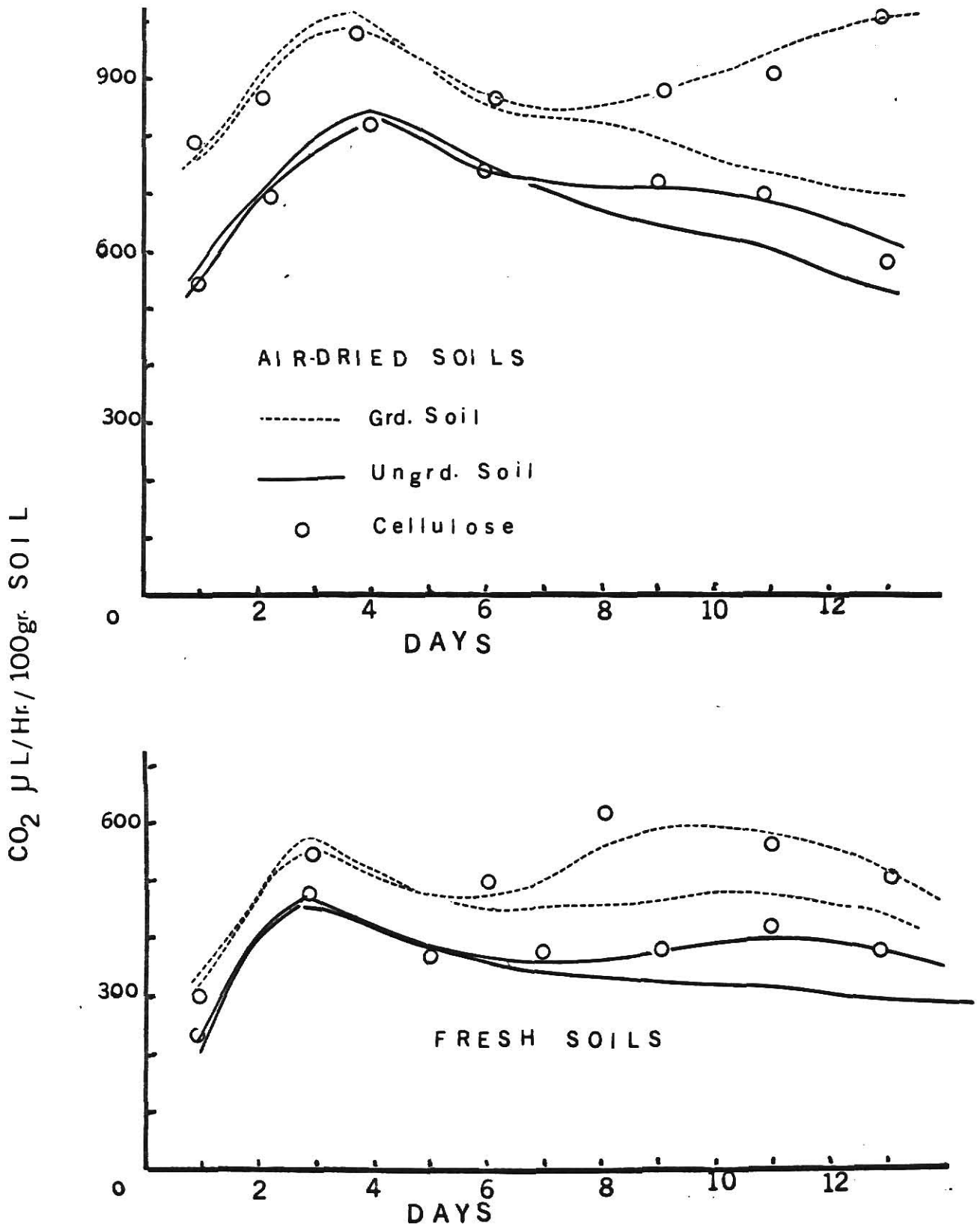
Data presented in table IX indicate the mode of respiration in grazed and ungrazed soils on a 100 g fresh and air dried sample basis. CO_2 was absorbed in KOH and the excess alkali was titrated against standard HCl. The data indicates that in both the soils cellulose seems to be decomposed in fresh and air dried soils after a period of adaptation, while control samples show a fall in the rates of CO_2 production after a maximum. No effort was made to correlate the evolution of CO_2 with oxygen consumption.

Discussion

Microbiological activity in soil is influenced by the nutrient status and circulation in conformity with other pedological and ecological factors that directly or indirectly affect the soil biomass. In the case of prairies,

EXPLANATION OF PLATE IX

Soil respiration in terms of CO_2 evolution from fresh and air dried samples of grazed and ungrazed prairie soils treated with purified cellulose, as compared to untreated controls during 13 days incubation at 31 C. Soil moisture saturation was maintained at 45% throughout incubation.


900
600
300
0
AIR-DRIED SOILS
Grd. Soil
Ungrd. Soil
Cellulose
2
4
6
8
10
12
DAYS
CO_2 µL/Hr./100gr. SOIL
600
300
0
FRESH SOILS
2
4
6
8
10
12
DAYS

the equilibria in bioecosystems are not as often drastically altered as in cultivated soils that are repeatedly subjected to tillage operations. Hence the circulation of plant nutrients and changes in microbial populations are more rapid in the cultivated soils than in the prairie soils. However, the chances of nutrient circulation in grazed prairie soils are comparatively greater than in the ungrazed as a result of grazing and the activity of macrofauna associated with grazers. This would cause a more favorable environment for microbial activity and higher rate of oxidation of soil organic matter in the grazed soils than in the ungrazed soils. In the ungrazed soils, the activity of the soil macrofauna is comparatively less. The lower rate of incorporation of above ground litter into the soil may be the factor limiting the rate of oxidation of soil organic matter.

This investigation has been mainly concerned with comparative respiratory activity in terms of oxygen uptake in grazed and ungrazed prairie soils as estimated with Warburg apparatus. The soils, at large, were taken as two basic entities, i.e. grazed and ungrazed soils.

At 45% moisture saturation, the fresh and air-dried samples of grazed and ungrazed soils showed highest microbial respiration. At this moisture saturation, the capillaries and the interspaces between the soil aggregates must have been optimally occupied with air and moisture for the highest activity of soil microbes. The grazed soils showed higher respiratory activity than the ungrazed soils both in fresh and air-dried samples. This observation is not in agreement with plate count populations for those soils. The reason for this discrepency is not known. With grazing and detritus and communiting action of soil macrofauna, the nutrient circulation in

grazed soil has to be better than in the ungrazed soil. The oxygen consumption data obtained in this investigation is in agreement with the above assumption. Shuichi, et al. (1964) reported lower microbial counts in volcanogenic virgin soils as compared with the adjacent cultivated soils. They attributed this to the high content of organic matter in the virgin soils with low pH values. The prairie soils at Osage site showed a pH of 5.2 in ungrazed soil as compared to 5.8 in the grazed soil. This difference in pH range might partially explain the disparity in microbial activity in the two soils.

Glucose added to both grazed and ungrazed soils was found to be rapidly metabolized. Ross and Roberts (1965) reported a two to three fold increase in oxygen uptake when the Tussock grassland soils were treated with glucose. Treated with 0.1 M glucose, the soils from Osage site showed a five and eight fold increase in oxygen uptake in air-dried and fresh soils, respectively, in this investigation within a 20 hr incubation period.

Rovira (1953) and Clark (1969) suggested that a great majority of the microorganisms in the soil were dormant or inactive at any given time. When a proper stimulation was given, these dormant cells exhibit a prompt and high burst of metabolic activity in the soil. This phenomenon can clearly be seen in the prairie soil data recorded in this investigation. Stout and Dutch (1968), working with Tussock grassland soils, concluded that the fertility of a soil should not be determined on the basis of basal microbial activity of that soil but on the basis of zymogenous activity of soil microbes. All this information backs the conclusion that the grazed soils at Osage site may be more fertile than the ungrazed soils.

The disproportionate increase of respiration in fresh and air-dried

samples of both soils can be easily explained. The fresh soils, as Rovira and Clark expressed, must have a majority of the soil organisms in a dormant or static state which respond promptly and vigorously when stimulated with glucose. The cumulative effect is a burst of physiological activity in the soil. In air-dried samples most of the vegetative organisms are eliminated, while a few may survive assuming dormancy along with the resting spores. This drastic cut in active organisms causes extremely low microbial activity in dry soils. Birch (1959) reported that on remoistening the dry soil, the dormant organisms get into a physiological youth as a result of protoplasmic reorganization before they start multiplying. The spores promptly germinate. These two processes show a very high rate of respiration as compared to normal populations in the fresh soils.

In both fresh and dried samples in the presence of glucose, a lag period of 5-6 hr was required for maximum microbial activity. This suggests that the physiologically young populations in the air-dried samples and the mixed total populations in the fresh soils have a similar type of adaptational process for glucose utilization.

Birch (1959) found that the increased rates of respiration over a prolonged period of time in dried soil were due to more soil organic matter being exposed to increased microbial attack. An experiment was undertaken to determine if purified cellulose added to the prairie soil would cause an increased microbial activity. Results obtained from a 10 day incubation period showed a 2-3 day adaptation period, after which an increased rate of respiration indicated microbial digestion of cellulose faster in air-dried samples than in the fresh samples. This observation is in agreement with the observations made by Stewart et al. (1966b) for a 10 day incubation period. However, their data showed the maximum rate of cellulose digestion

to be around 15 days of incubation. In the fresh soil samples, the rate of cellulose digestion ceased or even dropped below the initial rate toward the end of the incubation period, indicating an interference of some suppressing factor as reported by Russell (1910) and Howard and Howard (1910) in their separate works. One may speculate that the suppressing factor operating in the fresh soils fails to work in the air-dried soils over a period of 10-15 days. In the case of ungrazed soil, a prolongation of the adaptation period followed by a comparatively lower rate of oxygen consumption suggests a low rate of cellulose digestion which may possibly be due to the effect of lower pH on the microbial populations.

Most cellulose digesters from both fresh and dried samples seem to be eliminated in the grazed soil when subjected to moist heat at 80 C for 30 min. Although the data show a continuous decline in O_2 uptake after an initial rise for a short period, the cellulose digestion was not altogether stopped. Keyan (1961) reported that although many soil organisms can decompose cellulose, each group of organisms does so under its own set of conditions. Tribe (1960) observed interdependence or mutual exclusion in certain organisms for biological degradation of cellulose. Such phenomena may have been operating in the soil treated with moist heat. From the data it can also be inferred that (in agreement with observation made by Keyan) more than one group of organisms digest cellulose and the majority of them seem to be sensitive to moist heat shock.

An increased population of cellulolytic bacteria in the prairie soils appeared to break down purified cellulose and as well as the native organic matter in the air dried samples during a 10 day incubation period. The rate of digestion was more in the grazed than in the ungrazed soil. In

the case of fresh soils, the added bacteria digested cellulose better in the ungrazed soil than in the grazed soil. This leads to speculate that some factor, possibly of biological nature is present in the grazed fresh soils has inhibited the activity of added bacteria. On air drying the soil, the inhibitor seemed to be made nonfunctional.

Increased available nitrogen in the air-dried grazed soil did not increase the rate of digestion of purified cellulose. On the other hand it prolonged the adaptation period and suppressed the digestion of native organic matter. It may be that the grazed soils have an adequate supply of available soil nitrogen.

Addition of phosphate reduced the adaptation period and increased the rate of cellulose digestion and also the digestion of native organic matter in the soils. The slow fall in the digestion rate of cellulose towards the end of the incubation period may have been due to the interference of some other limiting factors like moisture, etc. in the experimental samples. Based on this data it is possible to conclude that the soils are deficient in available phosphate. Field experiments with phosphate fertilization of the prairie soils may confirm this observation further.

Addition of sulphate slightly increased the digestion of native organic matter but did not increase the digestion of purified cellulose. Stewart et al. (1966b) reported that the digestion rates of glucose and cellulose in the presence of optimum N and PO_4, depended on the levels of sulphate in the soil. Sulphate was not limiting at the Osage site. Likewise, calcium did not increase the digestion of purified cellulose or of native organic matter in the soils.

As expected, the rate of CO_2 evolution from the remoistened air-dried

samples was more than that from the fresh samples. In the presence of purified cellulose the evolution of CO_2 increased over the respective controls to indicate microbial digestion of cellulose in both grazed and ungrazed soils. An adaptation period was needed before the microorganisms could attack cellulose. The ungrazed soils showed low activity. Contribution by alkaline decarboxylation does not arise in these soils as their pH is in the acid range. In spite of optimal moisture saturation maintained throughout the incubation period, the slow drop in the CO_2 production may be due to the formation of some biological inhibitory factors like antibiotics, reduced microbial growth rates, or domination of a particular group or groups of organisms which may be considerable reduced the other groups. Klein (1915) observed such a fall in CO_2 production in remoistened air-dried soil over a period of 35 days before it leveled off.

Summary

Microbial respiration in the prairie soils at Osage site of the Grasslands Biome, International Biological Program was found to be highest at 45% moisture saturation. With 0.1M glucose, fresh and air dried samples of both grazed and ungrazed soils showed 8 and 5 fold increase in respiration rates as compared to untreated controls. Air dried soils treated with cellulose showed higher rates of respiration than the fresh soils during a 10 day incubation period. Moist heat (80 C) eliminated most of the cellulose digesters from the grazed soil.

Addition of 1.162×10^{13} cellulolytic bacteria in 1.4 ml of suspension to air dried soils treated with cellulose showed an increase in respiration as compared to those with no added bacteria during a ten day incubation

period. With similar treatments, ungrazed fresh soil showed an increase in respiration, while, the grazed soil did not. In the absence of purified cellulose, the native organic matter was more rapidly digested in the grazed than in the ungrazed fresh soils as measured by oxygen uptake.

Addition of 100 ppm phosphate stimulated the rate of respiration in both the soils while 100 ppm of total available nitrogen, SO_4^{--} and Ca^{++} separately did not cause such an effect. One might conclude that the prairie soils at Osage site are deficient in available phosphorous.

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Literature Cited

Alexander, M. Introduction to Soil Microbiology. 1961. page 163- John Wiley and Sons, Inc. N.Y., London, Sidney.

Beckman, H. and Scharpenseel, H. W. Carbon Dioxide Release from Sterilized Soil. Plant and Fertilizers. 1964. Abstract. 3441.

Birch, H. F. Further Observations on Humus Decomposition and Nitrification. Plant and Soil. 1959. 11(3):262-286.

Birch, H. F. Nitrification in Soils after Different Periods of Dryness. Plant and Soil. 1960. 12(1):81-96.

Birch, H. F. and M. T. Friend. Humus Decomposition in East African Soils. 1956. Nature. 178:500-501.

Bunt, J. S. and Rovira, A. D. The Effect of Temperature and Heat Treatment on Soil Metabolism. 1955. Journal of Soil Science. 6:129-136.

Clark, F. E. The Microflora of Grassland Soils and Some Microbial Influences on Ecosystem Functions. The Grassland Ecosystem, IBP #17. Science Series #2. Nov. 1969. page 331 (Range Sci. Dept. Colorado St. Univ.)

Ellis, R. C. The Respiration of Soil Beneath Some Eucalyptus Forest Stands as Related to the Productivity of the Stands. 1969. Aust. J. Soil Research. 7(3):349-357.

Elkan, G. H. and Moor, W. E. C. A Rapid Method for Measurement of CO_2 Evolution by Soil Microorganisms. 1962. Ecology 43:775.

Fischer, H. Vom Trocknen des Bodens. 1912. Central Bakteriol Abst. II. 36:346-349.

Funk, B. R. and Harris, J. O. Investigation of the Oxygen Uptake of Dried, Remoistened Soils. 1968. Plant and Science. 28:38-48.

Gaarder, T. The Effect of Inorganic Nutrient Elements on Soil Respiration in Uncultivated Norweigian Soils. 1964. Bergen Mat. Nature Ser. 1963 #21. page 25.

Gilbert, R. G. and Griebel, G. E. Stimulation of Soil Respiration by Volatiles from Alfalfa. 1969. Soil Sci. Soc. of Am. Proc. 33(2):270-.

Greig-Smith. The Bacteriotoxins and Agricere of Soils. Bakteriol AGT.II. 1911. 30:154-156.

Howard, A. and G. L. C. Howard. The Fertilizing Influence of Sunlight. 1910. Nature 82:456-457.

Jenkinson, D. S. and Powlson, D. S. Residual Effects of Soil Fumigation on Soil Respiration and Mineralization. 1970. Soil Biology and Biochem. 2(2).

Keyan, A. et. al. Factors Influencing the Composition of Microflora of Cellulose Decomposition. 1961. Nature 191:307.

Khalil, F. The Effect of Drying on Microbiological Processes in Soil. 1929. Central Bakteriol. Abt. II; 79:93-107.

Klein, M. M. Studies on the Drying of Soil. 1915. Journal of American Society of Agronomy. 7:48-77.

Korah, P. A. and Singte, A. K. On the Effect of Non-Edible Oil Cakes on the Respiratory Activity of Soil. 1969. Agricultural Journal of Kerala. 6)2):95-97.

Levinson, H. S. and Hyatt, M. T. Correlation of Respiratory Activity with Phases of Spore Germination and Growth of Bacillus megatericum as influenced by Manganese and L. Alanine. 1956. J. of Bacteriology. 72:176-183.

McLaren, A. D. and Peterson, G. H. Soil Biochemistry. 1967. Marcel Dekker Inc. New York. pp. 405-406.

Niederpruem, D. J. Respiration of Basidiospores of Schizophillum commune. 1964. J. of Bact. 88:210-215.

Olsen, G. W. and McCalla, T. M. A Comparison of Microbial Respiration in Soils after a 20-year Period of Subtilling and Ploughing. 1960. Soil Sci. Soc. of Am. Proc. 24:349.

Owens, L. D., et. al. Identification of Plant Volatiles that Stimulate Microbial Respiration and Growth in Soil. 1969. Phytopathology. 59(10):1468-1472.

Paris, O. H. The Function of Soil Fauna in Grassland Ecosystem. 1969. The Grassland Ecosystem, IBP. #17. Science Series #2. Nov. 1969. page 331. (Range Sci. Dept. Colorado St. U.)

Rahn, O. Bacteriologische Untersuchungen Uber das Trocknen des Bodens. 1908. Central Bakteriol. Abt. II. 20:38-61.

Richter, L. Uber die Veranderungen Welche der Boden durch das Sterilisieren erleidet. 1896. Landwirtschaftlichen Veruchs-Stationen 47:269-274.

Rixon, A. J. Oxygen Uptake and Nitrification at Various Moisture Levels by Soils and Mats from Irrigated Pastures. 1968. J. of Soil Sci. 19(1).

Ross, D. J. and Roberts, H. S. A Study of Activities of Enzymes Hydrolysing Sucrose and Starch and of Oxygen Uptake in a Sequence of Soils Under Tussock Grassland. 1965. J. of Soil Sci. 16(1):73.

Ross, D. J. and Roberts, H. S. A Study of the Activities of the Enzymes Hydrolyzing Sucrose, Starch and Oxygen Uptake in a Sequence of Soil under Tussock Grasslands. 1968. J. of Soil Sci. 19(1):186.

Rovira, A. D. Use of Warburg Apparatus in Soil Metabolism. 1953. Nature. 172:29-30.

Rovira, A. D. and E. L. Greacen. The Effect of Aggregate Disruption on the Activity of Microorganisms in the Soil. 1957. Australian J. of Agr. Research. 8:659-673.

Russell, E. J. Oxidation in Soil, and its Connection With Fertility. 1905. J. of Agr. Sci. 1:261-279.

Russell, E. J. The Fertilizing Influence of Sunlight. 1910. Nature. 83:247.

Shuichi et. al. Microflora of Japanese (Volcanogenous) Soils. 1964. Soil Sci. and Plant Nutrition. 10(2):22.

Soulides, D. A. and Allison, F. E. Effect of Drying and Freezing on CO_2 Production, Available Mineral Nutrients, Aggregation and Bacterial Population. Soil Science. 91:291-298. (1960).

Stout, J. D. and Mary E. Dutch. Rates of Organic Matter Decomposition with Warberg Respiratory Experiments. 1968. 9th International Congress of Soil Science. 3:303.

Stewart, B. A. et. al. Sulfur Requirements for Decomposition of Cellulose and Glucose in Soil. 1966. Soil Sci. Soc. of Am. Proc. 30:453.

Tarr, H. L. A. Some Observations on the Respiratory Catalysts Present in the Spores and Vegetative Cells of Certain Aerobic Bacilli. 1930. Biochemical Journal. 27:136-145.

Viant, H. V. Jr. Influence of Moisture Content on Soil Respiration. J. of Forestry. 1967. 65:902-903.

Waksman, S. A. Principles of Soil Microbiology. 1927. Baltimore, William & Wilkins Co. 895 p.

Waksman, S. A. and Starkey, R. L. Partial Sterilization of Soil Microbiological Activities and Soil Fertility. 1923. Soil Science. 16:247-268.

COMPARATIVE RESPIRATION OF GRAZED AND UNGRAZED PRAIRIE SOILS

by

A. V. SAMUEL

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AN ABSTRACT OF A MASTER'S THESIS

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Abstract

Microbial respiration in the grazed and ungrazed Labette silty clay loam soils were compared by use of the Warburg microrespirometer. The grazed soil showed higher respiration than the ungrazed both in terms of oxygen consumption and CO_2 evolution. At 45% moisture saturation, both the soils showed maximum microbial respiratory activity. At this moisture saturation, the capillary pores and the interspaces between the soil aggregates may have been optimally filled with water and air for highest microbial activity. On treating with 0.1M glucose, the grazed and ungrazed soils showed 5 and 8 fold increase in oxygen uptake in fresh and air dried samples after a 20 hr incubation period.

Cellulose was digested faster in air dried than in the fresh soil samples during a 10 day incubation, as measured in terms of oxygen consumption. It is speculated that some factor operating in the fresh soils slows down the rate of cellulose digestion and on drying the soil it is made nonfunctional. However, the rate of cellulose digestion in the ungrazed soil was slower than that in the grazed soil. An adaptation period of 3-4 days was needed to detect microbial cellulose decomposition in the soils. Moist heat shock at 80°C for 30 minutes followed by rapid cooling eliminated most of the cellulose digesters from the grazed soil.

When a suspension containing 1.162×10^{13} cellulolytic bacteria was added to remoistened air dried soils treated with purified cellulose, an increase in soil respiration was observed as compared to samples that did not receive the bacterial suspension during a ten day incubation period. But similarly treated soils in the absence of purified cellulose resulted in an increase in respiration, indicating the ability of the added bacteria

to digest also the native organic matter. In the fresh soils, purified cellulose was digested at a higher rate in the ungrazed than in the grazed soil. Native organic matter was digested more rapidly in the grazed than in the ungrazed soil.

Grazed and ungrazed soil samples both treated and not treated with cellulose showed an increased rate of respiration on adding 100 ppm available phosphate. Similarly treated soil samples in the presence of 100 ppm of added total available nitrogen, SO_4^{--} and Ca^{++} separately, did not show any increase in soil microbial respiration. Hence it is pointed out that the prairie soils at Osage site are deficient in available phosphorous while available nitrogen, SO_4^{--} and Ca^{++} are not limiting.

Soil respiration measured in terms of CO_2 evolution showed higher rates in grazed than in the ungrazed soils both in fresh and air dried samples treated with or without purified cellulose during a 14 day incubation period. Microbial attack on purified cellulose was detected after an adaptation period of 4-5 days. Similar to respiratory activity measured in terms of oxygen uptake, the cellulose digestion in air dried soils was also found to be more rapid as measured in terms of CO_2 evolution.