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MICROBIAL DECOMPOSER ACTIVITIES AT THE PAWNEE SITE: INTEGRATION OF EXPERIMENTAL APPROACHES WITH PROGRAM MODELLING REQUIREMENTS

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ABSTRACT

Experimental procedures which will allow modelling of Pawnee soil decomposer responses have been tested during this research period, and results are discussed in relation to general soil biochemical considerations and specific characteristics of the grassland ecosystem. Procedures for measurement of respiration and mineralization which are recommended include macro-respirometry and an *in situ* spatially-oriented dehydrogenase assay. Combined with measurement of evolved carbon dioxide, these procedures give baseline responses for test soils. Plating enumerations for total bacteria, actinomycetes, and fungi have been tested together with Most Probable Number (MPN) nitrifier and amoebal protozoan assays. Soils have been perturbed by variations in temperature, and by moisture, glucose, control hay, and extracted hay additions. Results are discussed in relation to parameters of nitrogen and phosphorus additions planned for future experiments. Progress to date in modelling of decomposer functions is presented.

Results from this research period indicate that sensitive, inexpensive procedures are now available for characterization and modelling of grassland decomposer functions.

INTRODUCTION

This report describes research which has been carried out in the Department of Microbiology, Colorado State University, in conjunction with the U.S. IBP Grassland Biome study regarding characterization of decomposer activities at the Pawnee Site.

First studies from this laboratory, concerned with the enumeration of microbial populations in Pawnee Site soils in relation to site use and depth, are summarized by Mayeux and Jones (1969) in Technical Report No. 22.

During 1970 the major efforts have been devoted to evaluation and testing of experimental approaches which would yield maximum information interfacing with modelling aspects of the biome effort. The soil decomposer function is one of the most difficult with which to work, in that conventional biomass activity relationships cease to have meaning. This results from the ability of microbial cells to respond to restricting nutritional conditions by approaching a stasis condition which can be rapidly reversed in the event that additional nutrients become available.

The objective of this report is to present research procedures which have been developed together with interpretation and discussion of their relationships to program modelling objectives.

BACKGROUND AND GENERAL DECOMPOSER CONSIDERATIONS

In development of procedures for the evaluation of decomposer processes at the Pawnee Site, the climatological and productivity characteristics of the site must be considered.

From a climatological viewpoint, there is only a relatively short period of the year when optimal microbial metabolism might take place. On a modelling basis, this has been summarized in "Grasslands management, research, and training viewed in a systems context" by George M. Van Dyne (1969) where active microbial respiration may be considered to occur over a relatively short interval of the entire year. Active respiration takes place only over a 30-day period of optimal substrate and rainfall and lags somewhat behind the time of development of standing live vegetation. Microbial biomass and respiration indices are assumed to respond after the peak of plant respiration and standing live vegetation accretion has been reached, followed by a short period of increased microbial activity.

Carbon turnover on the Pawnee Site must also be considered in generation of decomposer modelling information. D. N. Hyder (1969) has noted an average net herbage production of 670 kg/ha/year, equivalent to a productivity of 6.7 mg/cm^2/year. Of this, 43% is considered to return directly to the soil, giving a direct addition of 2.88 mg/cm^2/year, an extremely low rate of carbon addition. This low rate of carbon addition would indicate that microbial respiration, decomposer functions, and consequent biomass responses may proceed at rates which approach minimal subsistence levels. This research report summarizes research procedures which should provide information on relationships between microbial biomass and activities under varied environmental conditions in Pawnee grassland soils.

RESEARCH APPROACHES

Macro-Respirometry

Initially, electrolytic respirometry as described by McGarity, Gilmour, and Bollen (1958) was planned for evaluation of whole soil core oxygen uptake

over extended periods of perhaps one or two weeks. In the initial evaluation of this approach using Pawnee soil cores, it was found that barometric variations damped out the low daily oxygen uptake rates making this procedure unsuited for use under these experimental conditions.

To establish the respiration index by a more reliable procedure in which the barometric variation could be compensated for, combined with minimal expense and ease of operation, a macro-respirometer was designed in this laboratory for use specifically with soil cores (Klein, Mayeux, and Seaman 1971).

Respiration apparatus. The respiration apparatus consists of a one-quart, wide-mouth canning jar and two 25-cm pieces of glass tubing (8 mm diameter) which are connected by a 20-cm length of rubber tubing to form a manometer. The metal lid of the jar is modified by boring two 12-mm holes. This is best done by drilling a series of covers simultaneously to form a turned lip which will allow rubber stoppers to be firmly fitted into the cover. For each unit, size 0 one-hole stoppers are placed in the holes, and short lengths of glass tubing are placed in the stoppers. One of the tubes is connected to a 3-cm length of 3/36 × 1/16 rubber tubing which is clamped off using a pressure clamp. The clamp is opened after reading to allow pressure equilibration. The other outlet is connected by a piece of similar rubber tubing or approximately 30 cm in length to the manometer. One leg of the manometer is attached vertically to the side of the jar on a small strip of wood approximately 20 cm in length by use of fiber glass tape. The other leg is held in place by a rubber band. The manometer is filled with distilled water to a mark on the stationary log. To read the pressure change the moveable log of the manometer is adjusted until the water level

in the other leg returns to its original mark. The distance between the water levels in the two legs is then measured in millimeters. Readings are made at approximately 24-hour intervals.

Respiration units were calibrated using standard manometric constants involving gas volume (Vg, µl), liquid volume (Vf, µl), temperature (°K), the dry soil volume (µl), liquid volume (alkali plus soil moisture in µl), and the oxygen absorption coefficient at a given temperature (°C). This allows a direct calculation of oxygen uptake in µl (× 1000 = ml) after correcting the reading for thermobarometer changes. Sealed vials are placed in the thermobarometer to displace the approximate soil value used in the test units, and an amount of water equivalent to the moisture in the soil cores is added. A set of sample calculations for k is illustrated, based upon standard procedures (Umbreit, Burris, and Stauffer 1970).

$$k = \frac{Vg(\mu l)\ \frac{273}{T} + Vf(\mu l) \times \alpha}{Po}$$

$$k = \frac{835{,}000 \times \frac{273}{298} + 65{,}000 \times 0.02822}{10{,}336 \text{ mm (Po of distilled water)}}$$

$$k = 74(\text{in } \mu l) \text{ or } 0.074 \text{ (in ml) } O_2$$

The corrected change in uptake (mm) is multiplied by k to give oxygen uptake directly in milliliters under the desired test conditions.

Any evaporation of water from the manometric tube is replaced by injection of distilled water through the lower rubber tube.

Carbon dioxide evolution. For absorption of carbon dioxide, each respiration unit contains a 50-ml beaker of alkali (20 ml of 30% w/v KOH, 5.36 N) which is seated in a ring-type stand made of stainless steel tubing. This

allows the alkali to be changed without removing the soil core either by removing the jar cover or by simply removing one of the rubber plugs and withdrawing the sample. Carbon dioxide absorption in sodium hydroxide is determined by titration of residual alkali by a double end point procedure. H_2SO_4 (1.0 N) is used to titrate the samples to a phenolphthalein end point. The second titration to a methyl orange end point is carried out with 1/12 N H_2SO_4. Each milliliter of the 1/12 N H_2SO_4 is equal to 1.0 mg of absorbed carbon in the sample.

Soils and Soil Treatments. For this study, soil cores of approximately 6.35 cm in diameter and of 8.0 cm in depth from the Pawnee Site were used. These cores were taken from the surface horizon and were prepared for use by removal of free standing litter material. The sides and bottoms of the cores were coated with a layer of paraffin to simulate the somewhat limited gas exchange of such a soil sample in its normal field environment. The cores were of approximately 200 g weight, and the field moisture level was 5% to 7% the total weight. For cores receiving moisture, 20 g of distilled water was added uniformly over the surface area and allowed to equilibrate. For glucose addition, 0.1 g was dissolved in the 20 ml of water before addition to the surface of the core. Blue grama hay was used in all experiments, and for extraction this was soaked in cold distilled water for 24 hours, followed by rinsing and drying at room temperature.

Hay, either regular or extracted, was cut into approximately 2- or 3-mm lengths, and 0.3 g was spread as uniformly as possible over the core surface. Water, if required, was added after spreading the hay on the core surface in an effort to simulate normal rainfall addition and leaching. All experiments in this series were carried out at 20°C.

In situ Dehydrogenase

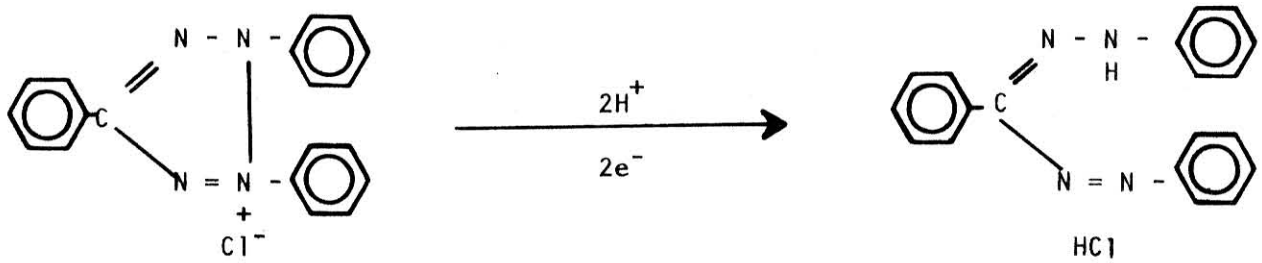
Dehydrogenase assays (Stevenson 1959; Casida, Klein, and Santoro 1964; Skujins 1967; Glathe and Thalmann 1970) have been found to be an index of soil respiration which, although not generally correlating with microbial numbers in unamended soils, do show correlations in soils which have been amended with nutrient sources. This observation of varying relationships between numbers and respiration activity again emphasizes that under minimal nutritional conditions found in unamended soils conventional concepts of biomass or number-activities relationships may not be applicable.

The formula for 2, 3, 5 Triphenyltedrazolium chloride (TTC) is given in Fig. 1. In the enzymatic dissimilation of substrates by microorganisms, electrons are accepted by this material causing reversion to the water-insoluble formazan. The half-cell potential of TTC in a system at pH 7 is also given. TTC lies below both oxygen and nitrate, implying that per mole of available electrons, there is a decreased energy yield for the micro-organisms.

The standard reference procedure (Casida, Klein, and Santoro 1964) involves weighing 3.0 g of a test soil into a test tube followed by addition of 0.5 ml of 3% TTC in distilled water, and 1.25 ml of water. This is then mixed with a glass rod and sealed with a rubber stopper. Care is taken to assure that the liquid level is above the soil. After incubation at an appropriate or desired temperature, the soil is transferred with methanol to a cotton stoppered funnel, and extracted with methanol (50.0 ml) to assure complete formazan extraction. The optical density of the extracted methanol solution is read at 485 nm.

T T C

2, 3, 5 TRIPHENYLTEDRAZOLIUM CHLORIDE



LEUCO FORM

HOH - SOLUBLE

FORMAZAN

HOH - INSOLUBLE (RED)

485 nm. max.

POTENTIALS:	OSYGEN	+ 0.816 volt Eo^1
	NITRATE	+ 0.421 volt Eo^1
	TTC	+ 0.300 volt Eo^1

Fig. 1. Structure and reduction of TTC.

To extend this procedure to possible field applications, 11-mm soil cores taken from the surface to a depth of 3.0 cm were incubated with and without surface addition of ground hay. The 0 to 1, 1 to 2, and 2 to 3 cm sections were assayed by the standard procedure after removal of the surface material to avoid influencing the subsequent dehydrogenase index. Samples were assayed in relation to hay addition and temperature (26° and 37°C) over a three-day period, with moisture added to approximately one-third bar water tension.

The final *in situ* dehydrogenase procedure recommended for use in routine soil core evaluations was carried out by removing an 11-mm diameter by 30-mm length core from the sample, incubating this at 27°C directly with addition of 3% TTC solution, and extracting three 1.0-cm portions by use of methanol.

Soil Microbial Activity Correlation

Both *in situ* dehydrogenase and soil core respiration characteristics can be related by considering the electron flow which takes place from a substrate added to soil to either TTC or oxygen, respectively. Dehydrogenase activity measurement is capable of giving short-term rates which can then be related to allover core respiration as given by oxygen uptake. The concept of substrate electron content (Mayberry, Pruchazka, and Payne 1968) is suggested for following energy flow in these systems. This approach can be used to give activity estimations in moles of electrons or potential energy available (in calories) over time for a particular unit of soil.

Plating Enumeration

Although the worth of soil microorganism enumeration procedures is sometimes questioned, this approach can be of value when correlated with

broader shifts in microbial distribution and when carried out in conjunction with microbial activity evaluations. Based on initial considerations of the nutritional hierarchy which exists between the various classes of microorganisms in the soil environment, an evaluation of time- and substrate-related microbial responses can be a major analytical tool.

In this study, the following assays were carried out:

Total Count - Soil extract agar

Total Count - Sodium caseinate agar

Actinomycetes - Sodium caseinate agar

Fungi - Rose Bengal agar

MPN for nitrifiers. (Nitrifier results were not available in time for incorporation in this report.)

Appropriate dilutions were made using buffered distilled water, and 0.1-ml aliquots were transferred to the appropriate media. All plates and tubes were incubated at 27°C with soil extract and Rose Bengal plates read at four days, and sodium caseinate read at seven days.

Protozoal Enumeration

In conjunction with enumeration of more conventional microbial types, a technique for evaluation of protozoal responses has been developed. Special concern is being given to the small soil amoebal forms which may play a substantial role in decomposer relationships in this environment. Assays are being carried out using two basic approaches, both of which involve preparation at a mineral salts medium semisolid agar layer. The "normal" or standard procedure involves adding the desired inoculum plus washed *Aerobacter aerogenes* to the molten overlay medium, in which the

amoebal forms must be able to develop in the agar matrix. In comparison, the surface technique is carried by spreading the *Aerobacter* plus inoculum on the previously-prepared semisolid layer. This latter procedure is similar to Singh's "ring procedure" while avoiding many of the experimental and manipulatory limitations of that approach. Plates are read daily until all plates in an experimental run have given two consecutive similar plaque levels. This usually requires between 7 and 10 days of incubation.

RESEARCH RESULTS

Macro-Respirometry

Soil respiration over a 192-hour period for cores perturbed by additions of varied combinations of moisture, glucose, and regular and extracted hay are given in Fig. 2, 3, and 4.

Respirometry experiment #1--Addition of moisture and moisture plus glucose. Soil core respiration with addition of water, or water plus glucose, in comparison with the control core are given in Fig. 2. Over the 192-hour duration of the experiment, the soil core endogenous uptake was observed to proceed at a steady rate over the course of the experiment, indicating the level of basal respiration for this sample. In comparison, moisture addition had a marked effect on core respiration, which did not return to the control rate during the duration of the experiment. If the rates are considered from a differential viewpoint, microbial activity continues to increase, indicating an often-observed response of soil microbes to increased available moisture.

Addition of glucose had marked effects on the respiratory capability of this soil. After approximately 100 hours of observation, the glucose-treated

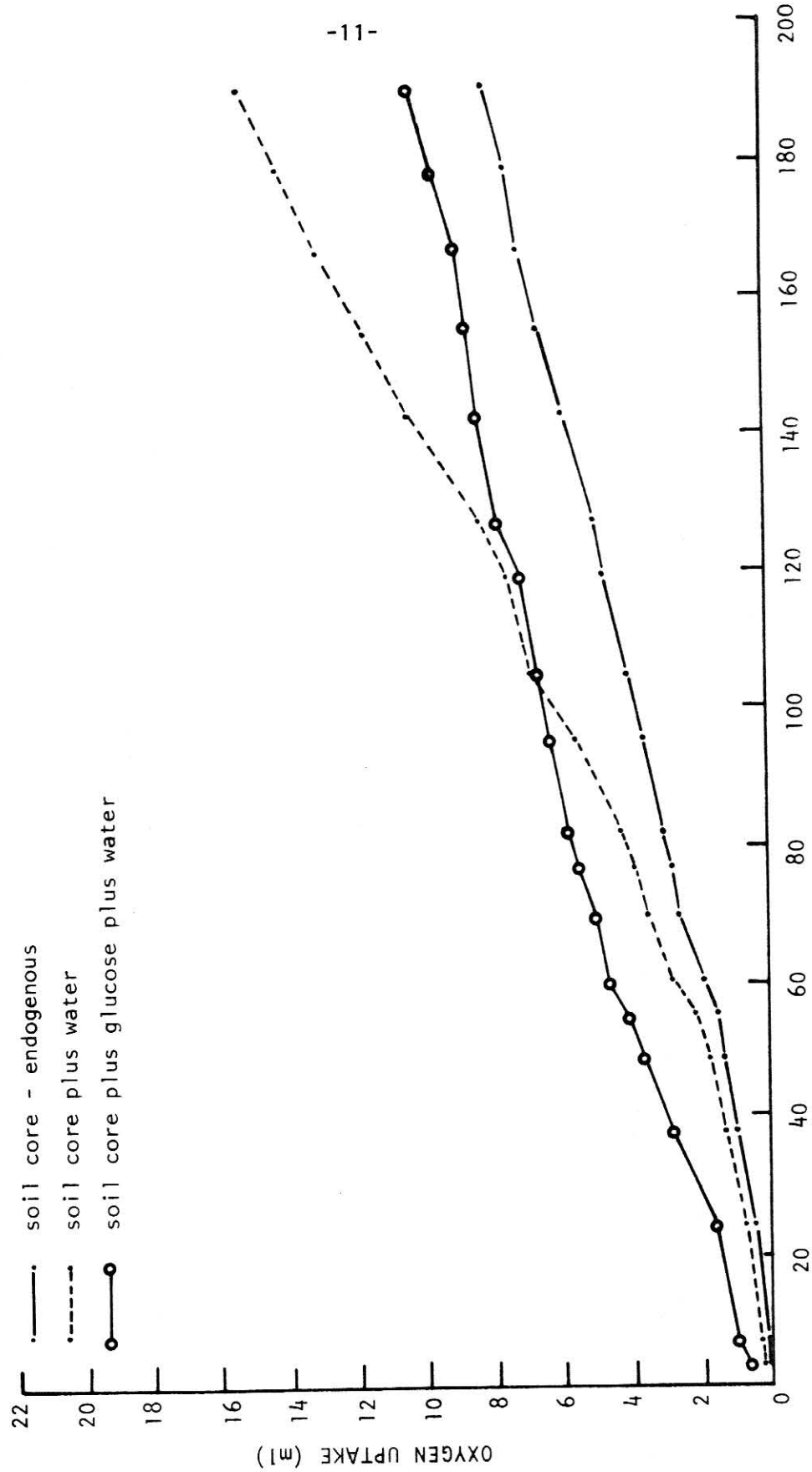


Fig. 2. Effect of moisture and glucose on soil core respiration rates.

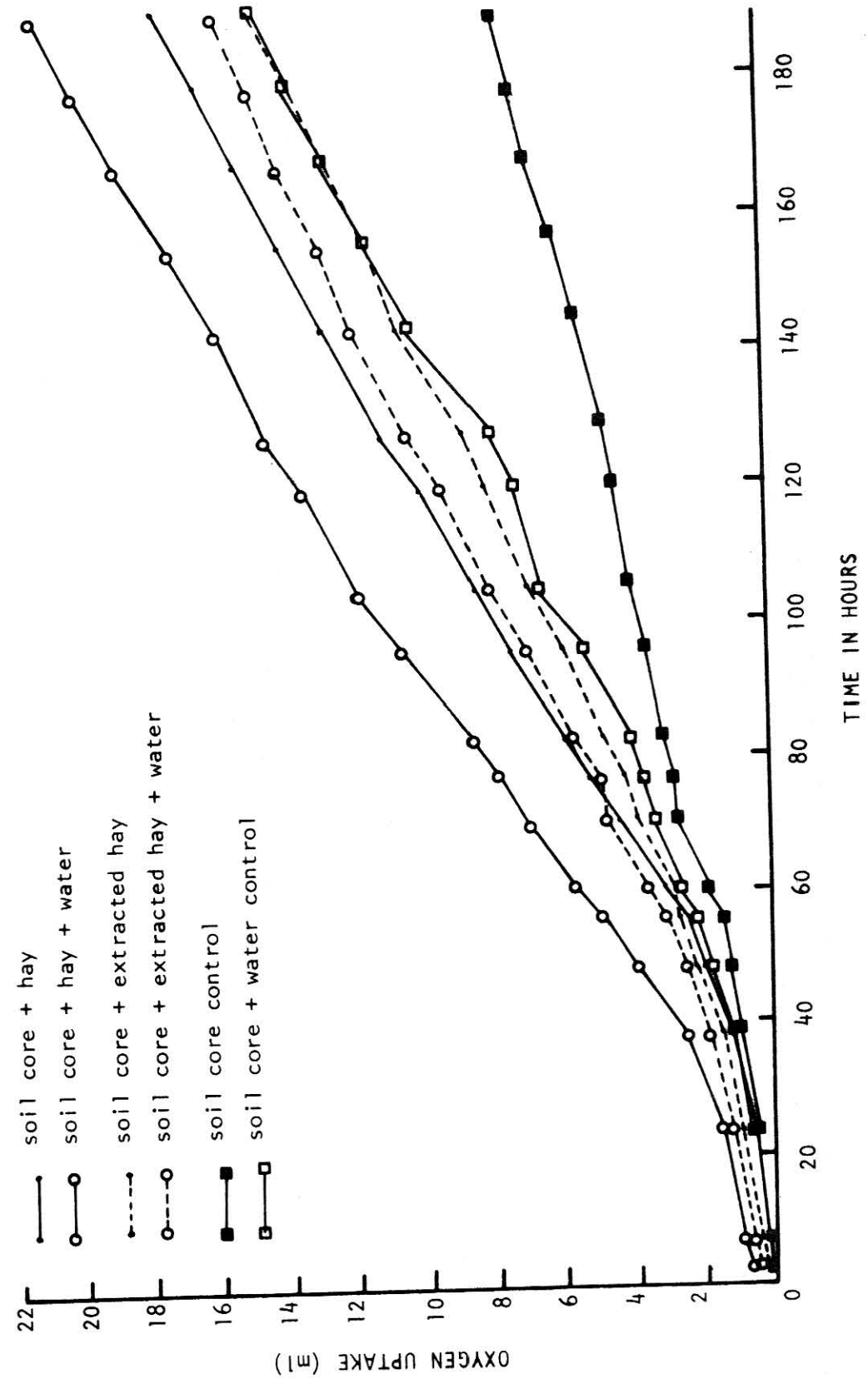


Fig. 3. Effects of hay, extracted hay, and water on soil core respiration rates.

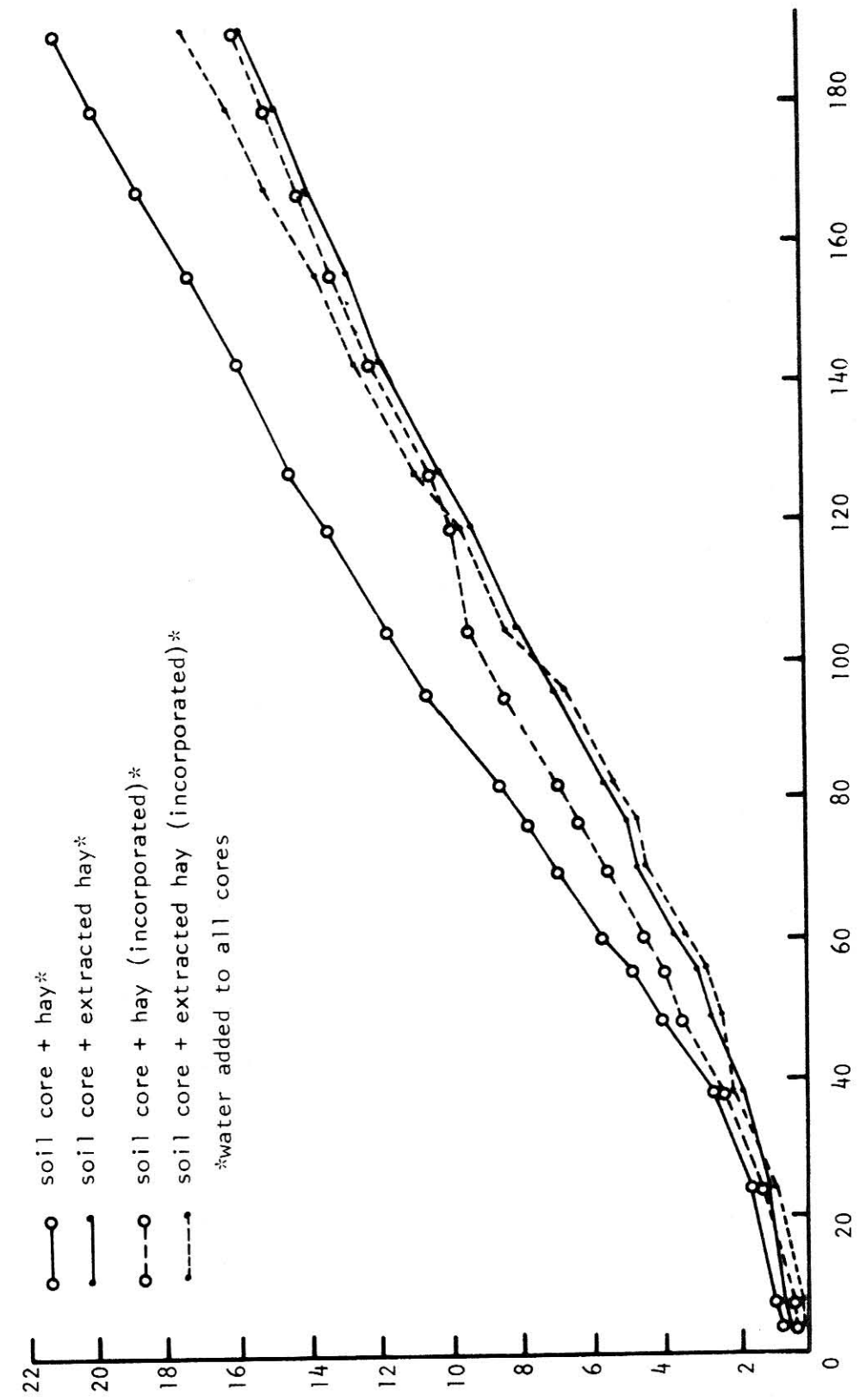


Fig. 4. Effect of incorporated hay on soil core respiration rates.

sample showed decreased respiratory response. This may have been due to the particular glucose addition level used, in that the carbon source was present in an amount which exceeded the aerobic respiratory capacity of the soil. Under these conditions fermentation products were accumulated, causing a decreased soil pH and concomitant decreased metabolic capability. From a procedural standpoint, this observation illustrates the hazard of using glucose, normally only available in soils at low and continuing levels, in a pulse type of experiment. Such a carbon source, if used at all, should only be added at levels which will not cause major shifts in soil metabolism.

Respirometry experiment #2--Addition of control and extracted hay with and without moisture. Fig. 3 shows the responses of the Pawnee soil to the addition of regular and extracted hay, both with and without added moisture. In considering the basal response of the soil plus water, approximately 14.5 ml oxygen uptake, the addition of extracted hay with or without water did not greatly exceed this basal uptake. The addition of water to the extracted hay also had a minimal effect indicating that leaching removed soluble materials from the hay. Only with the addition of unextracted hay was an increased response shown, with the added water providing an environment where the readily soluble materials were able to leach into the soil microbial zone. When this response is viewed from a differential rate standpoint, this soil microbial respiration rate returns to the basal extracted hay uptake rate within approximately 120 hours. This would indicate a short-term response of the soil within this period due, perhaps, to depletion of these readily-soluble components. Similar results for agricultural soils have been obtained by Brown and Fredericks (1968) and by Grabert and Matschke (1970), and such a sequential availability of hay

components would be expected. It will be critical in the present study to determine specific parameters which can affect the course of Pawnee soil decomposer processes. From the results which have been obtained, it would appear that the macro-respirometer designed in this laboratory provides sufficient sensitivity to allow generation of meaningful information.

Respirometry experiment #3--Subsurface addition effects on respiration activity. In an additional experiment regular and extracted hay were tested as surface and subsurface additions. Incorporation of test hay was carried out by splitting the core horizontally, placing the hay on the open surface and reassembling the core. After this was completed, the required water was added to the core surface. Results are given in Fig. 4. With extracted hay similar results were obtained with both addition methods, indicating that physical contact was not limiting microbial activity. When the oxygen uptake curves for unextracted hay are examined, marked differences are observed. Oxygen uptake appears to be limited in the incorporated sample, indicating that oxygen diffusion into the core center may have been limited, or the formation of anaerobic zones in the vicinity of the hay particles had occurred. This experiment would indicate that subsurface incorporation should be used with caution in examining Pawnee soil decomposer processes, where most decomposition can be assumed to occur at the soil surface. In an additional context, however, the role of invertebrates in aiding incorporation of plant materials directly into the soil, or as fecal matter, must be considered.

In evaluating the total significance of these responses in relation to existing decomposer models, it is evident that even dry soils taken from the

Pawnee Site in the late fall are capable of respiration; thus, decomposer models should reflect this observation.

Dehydrogenase Assays

Dehydrogenase experiment #1--Pawnee soil standard dehydrogenase assay. Results of assaying the Pawnee soil for dehydrogenase activity using the standard procedure of Casida, Klein, and Santoro (1964) are shown in Fig. 5. In this experiment, samples were held at temperatures of 5°, 15°, 26°, and 37°C, using unamended samples and samples where three sections of blue grama hay were inserted in the soil-TTC mixture. It is possible with time to observe the effects of straw addition at the 37°, 26°, and 15°C temperatures; however, at 5°C it was not possible to observe dehydrogenase activity over the time period assayed. As TTC reduction can be related directly to electrons which have been passed through the biological system, it should be possible to extend this approach to a meaningful field-assay.

Dehydrogenase experiment #2--Soil cores with surface hay treatments. Again using the standard TTC procedure, assays of TTC reduction in soil cores with and without surface addition of hay have been carried out (Fig. 6) to determine if surface addition of hay can be detected by this procedure.

Over the assay period, the clearest indication of spatial distribution of responses is shown by the 0- to 1-cm layers in relation to time, temperatures, and hay additions. During this period, the lower soil layers showed only minimal responses which were generally accentuated by the higher temperature. The conceptualization of a surface zone stimulation of microbial activity by biomass decomposition correlates well with the natural decomposition conditions in the Pawnee Site area, and use of such a differential

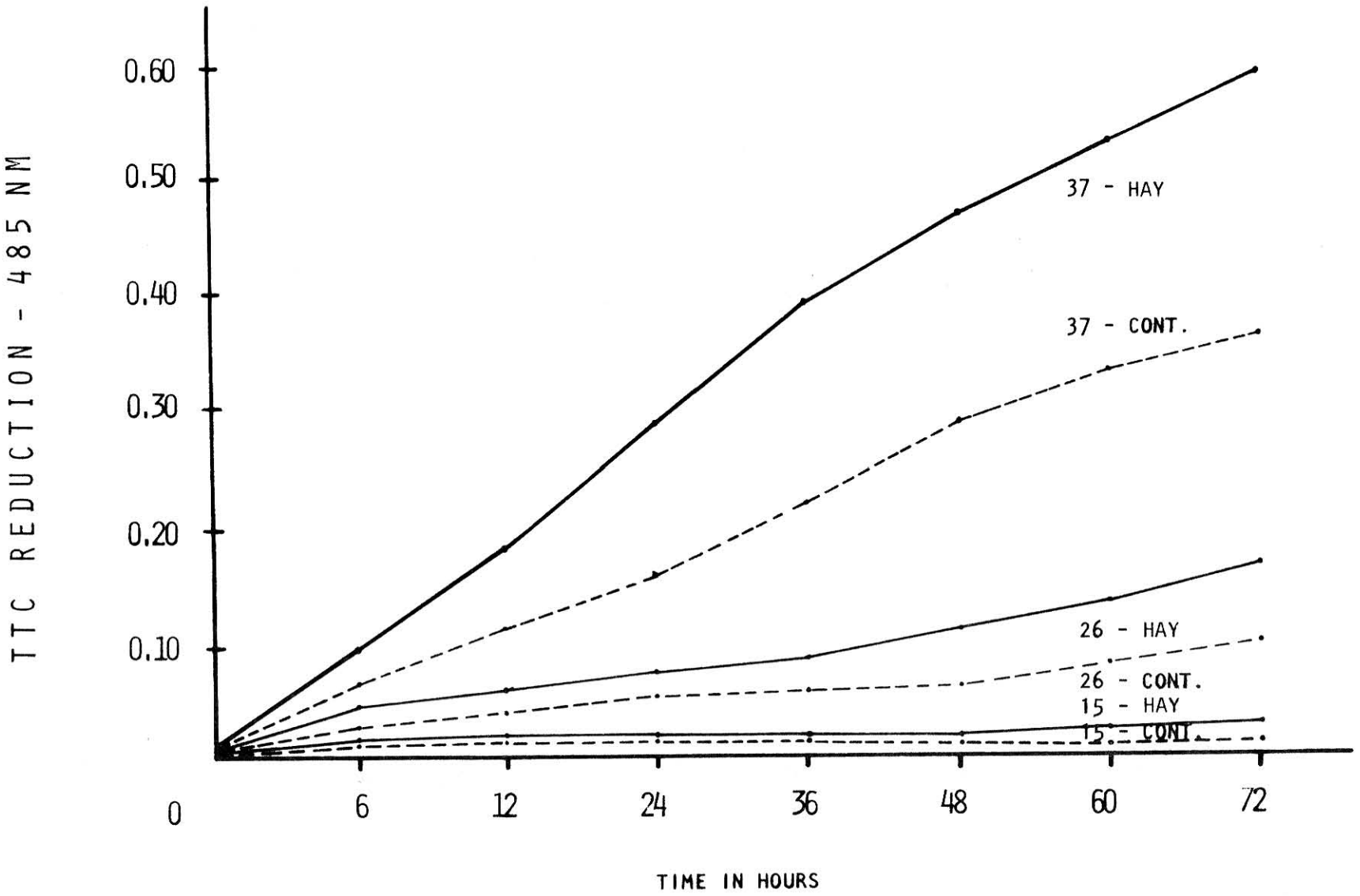


Fig. 5. Standard Dehydrogenase Procedure - effects of temperature and hay variation.

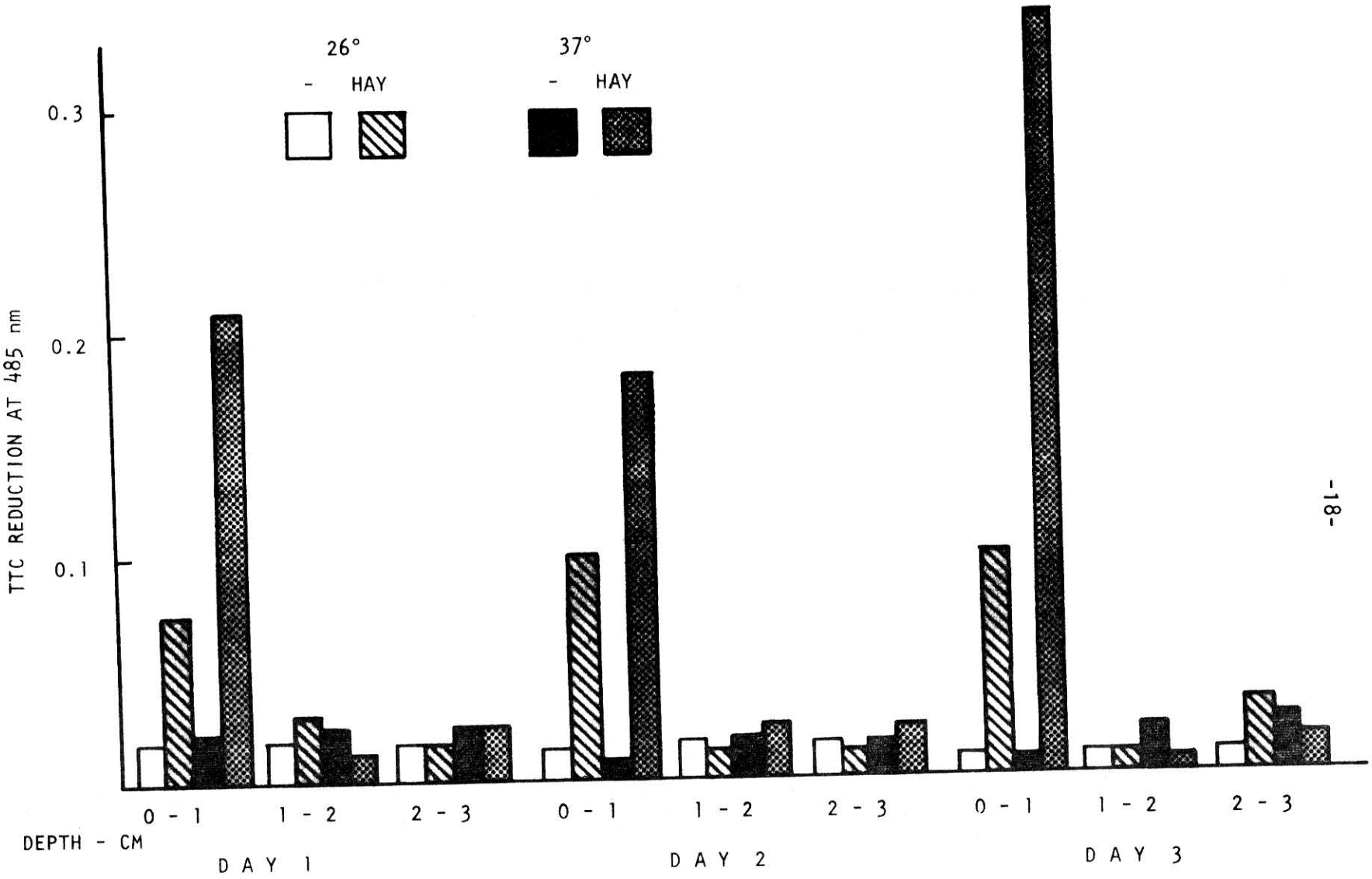


Fig. 6. Dehydrogenase activity - Soil core responses to surface hay additions.

approach can lend itself to development of a more spatially-oriented conceptualization of decomposer processes.

Dehydrogenase experiment #3--Assay of soil cores modified by moisture, glucose, and hay additions--192-hour samples from macro-respirometry experiments. Both standard and *in situ* dehydrogenase assays have been run on selected soil cores from samples given in Fig. 2, 3, and 4, with results presented in Table 1. Using the standard procedure, it is possible to observe respiratory responses with moisture additions and with some organic matter treatments. Responses in relation to surface activity were also observed under these test conditions, with the core plus water, water plus glucose, and the core plus normal hay being of particular interest. The *in situ* procedure developed in this study should reflect the respiratory activity of undisturbed soil cores by avoiding mixing, which is required for the standard procedure.

Carbon Dioxide Evolution

Carbon dioxide evolution from soils tested for macro-respirometric response has indicated that this parameter will tie in well with other respiration indices.

Results for the treatment variables given in Fig. 2, 3, and 4 are summarized in Table 2. Moisture addition again is a major detriment in carbon mineralization, with the differences between normal and extracted hay being evident in all vessels where these are used. The somewhat lower final level of carbon dioxide evolved with glucose additions appears to be correlated with the decreased respiration rate observed at the latter part of the incubation period (vessel 3). The highest carbon dioxide yield was

Table 1. TTC responses of selected Pawnee Site soils--dehydrogenase.

Treatment	Standard Procedure	*In situ* Dehydrogenase
	OD @ 485 nm	OD @ 485 nm
Plain core	.390	.498
Core and water	.710	.883
Core, water, and glucose	.532	1.025
Core and hay	1.475	1.090
Core, hay, and water	.845	.719
Core and extracted hay	.552	.327
Core, extracted hay, and water	.822	.846

Table 2. Carbon dioxide evolution in relation to soil treatment.[a/]

Vessel No.	Treatment	CO_2 Absorbed (mg)
1	Core	154
2	Core and water	308
3	Core, water, and glucose	352
4	Core and hay	264
5	Core, hay, and water	440
6	Core and extracted hay	170
7	Core, extracted hay, and water	308
8	Hay and water	220
9	Extracted hay and water	170
10	Core, hay, and water (incorporated)	308
11	Core, extracted hay, and water (incorporated)	264
12	Thermobarometer control	0.0

[a/] Soils derived from Fig. 2, 3, and 4 respirometry experiments.

given by the core plus hay and water, indicating that hay-soluble materials available over a longer leaching period allowed efficient respiration and a higher level of total evolved carbon dioxide. This difference between the control and extracted hay is also shown in field moisture cores, vessels 4 and 6.

Plating Enumeration

Plate count data derived from soils tested for macro-respirometric responses are given in Table 3 on a wet weight basis. The only major shifts which can be observed in soils held over this test period in relation to treatments occur in the total counts on soil extract and sodium caseinate, comparing the dry core and the core containing water and glucose. Other treatments and plating media do not indicate major microbial population responses, in spite of the observed changes in basal respiration given by dehydrogenase, macro-respirometry, and carbon dioxide mineralization.

These results again may illustrate the ability of microbes stressed by steady-state nutritional limitations to increase respiration in the presence of low bid increased nutrient levels derived from hay extracts; whereas only a major nutritional pulse, as with the addition of glucose, where there was a sufficient nutritional "overshot" allows detectable increases in microbial numbers.

Protozoal Enumeration

Examination of protozoal populations in soils tested for macro-respirometry has indicated that amoebal numbers may be a sensitive indicator of changes in nutrient and moisture status.

Table 3. Plate count enumeration on selected test soils checked for respiration capabilities.[a/,b/]

Treatment	Sample Depth (cm)	x 10^6 Total SE[c/]	x 10^5 Total NaC[c/]	x 10^5 Actino.[c/]	x 10^3 Fungi
Plain core	1	1.4	13.9	2.5	7.9
	2	.54	4.6	1.5	2.3
	3	.84	6.6	2.5	2.4
Core and water	1	1.5	15.8	8.2	3.0
	2	1.1	9.0	4.0	3.7
	3	1.0	9.4	3.7	2.2
Core, water, and glucose	1	1.8	20.0	3.5	7.5
	2	1.2	14.4	2.4	4.9
	3	1.3	12.9	3.8	3.7
Core and hay	1	1.6	15.2	1.8	7.8
	2	.48	4.8	1.6	4.0
	3	.49	5.5	2.8	4.1
Core, hay, and water	1	1.5	16.0	2.5	4.5
	2	.85	7.9	2.5	2.4
	3	.86	7.4	1.7	2.4
Core and extracted hay	1	.93	8.9	1.8	4.0
	2	.76	7.8	1.6	7.8
	3	.30	1.6	.6	3.5
Core, extracted hay, and water	1	1.3	11.5	2.2	5.7
	2	.69	5.4	1.9	3.3
	3	.86	7.5	2.3	3.1

a/ $\frac{(\bar{x}\ \text{count})(\text{diln})}{11}$ = count/gram (field moisture)

$\frac{(\bar{x}\ \text{count})(\text{diln})}{11 - \text{water fract.}}$ = count/gram (field moisture)

b/ Soils tested were tested for respiration in Fig. 4, 5, and 6.

c/ SE = Soil extract, NaC = Sodium caseinate, Actino. = Actinomycetes.

Using the normal incorporated inoculum amoebal enumeration procedure, the dry soils generally had 5-6 × 10^3 amoebal forms/g in surface and subsurface samples; whereas treatment with water, water plus glucose, or water plus hay brought at least a one log unit increase in recoverable numbers, in the range of 5-6 × 10^4/g soil. The moisture effect was generally more pronounced than any of the specific nutrient treatments, which may represent an increased general ability to excyst under improved moisture conditions.

This general trend of amoebal responses was also observed in the surface assay procedures; however, recoverable levels approach 10^{5-6}/g in treated samples with perhaps one log unit lower recoveries in the control core. These results would indicate that these two approaches to amoebal enumeration may yield markedly different populations. To date the major forms recovered by both procedures have been considered as either *Hartmannella* or *Naegleria*.

Amoebal forms appear to be present, active, and responding to the perturbation of Pawnee Site soils. Our future work will be concentrated on improving assay techniques and gaining information on the nutritional cross reactions of these isolates in relation to microbial feeding patterns.

Soil Microbial Activity Correlations

Correlation of these varied activity assays is essential if a unified concept of energy flow in the decomposer ecosystem is to be made. Such a correlation should be able to provide information on soil unit respiration and relate this to substrate utilization on an energy or heat basis, either expressed in moles of electrons passed through the system or in terms of calories of heat liberated.

Results of such an initial activity correlation on the test soils examined in this study are given in Table 4, using arbitrary units of activity expression. Normally, all units would be translated into either electron flow or calorimetric values. It has not been possible to complete this before preparation of this report due to the unavailability of several primary activity standards and standard curve reagents (standard reduced formazan, etc.).

In evaluating these results, it is critical to consider that oxygen uptake and carbon dioxide evolution are total accumulated values; whereas TTC reduction is a short-term rate of expression. Close numerical relationship between oxygen uptake and carbon dioxide evolved are shown for the plain cores, core and water, and the core plus hay and water. This relationship of activity correlations on these three samples also extends to the TTC values, although these are not as well related.

Relations for the core plus water and glucose indicate the problems which can be involved in use of this readily utilizable substrate. High carbon dioxide evolution is observed without a corresponding level of oxygen uptake, although the short-term TTC uptake indicates a good biological potential. This may be due to the formation of anaerobic acidic conditions in this soil. Under anaerobic conditions, carbon dioxide could be evolved from substrate dissimilation with electrons normally transferred to oxygen being added to metabolic intermediates. Thus, one would observe carbon dioxide evolution without concomitant oxygen uptake.

In relating these activity values to the observed microbial numbers, all treatments, whether moisture, hay, or combination, did show small increases in numbers over the control sample levels. However, the only

Table 4. Soil microbial activity correlations,[a/] using arbitrary units.

Treatment	Assays				
	O_2 Uptake (ml O_2)	CO_2 Evolved (mg)	TTC Reduction OD @ 485 nm	Total Count Soil Extract x 10^6	Total Count NaC x 10^5
Core	1.0	1.0	1.0	1.0	1.0
Core and water	1.94	2.0	1.77	1.07	1.13
Core, water, and hay	2.81	2.85	1.44	1.07	1.15
Core and hay	2.30	1.72	2.18	1.14	1.09
Core, water, and glucose	1.4	2.28	2.06	1.28	1.43

[a/] Soil cores from Fig. 2, 3, and 4 incubated 192 hours @ 20°C.

major response was in the sample containing glucose, again indicating the sensitivity of microbial populations to this readily available substrate.

RECOMMENDED RESEARCH PROCEDURES

Research of this period has indicated that several basic respiration assay procedures are now available for characterization of Pawnee soil decomposer functions. In relation to these basic indices of activity, secondary enumeration and population assays have been developed which will allow generation of information on microbial decomposer processes. The following assays are recommended for use in characterizing activity-population relationships in Pawnee soil samples:

i. Soil core respirometry.

Measurement of oxygen uptake per unit of soil over short- and long-term periods and corresponding assays of evolved carbon dioxide.

ii. TTC reduction assay of electron flow.

Measurement of electron flow in selected samples over short time periods, applicable to independent analysis of field samples.

iii. Plating enumeration of total microorganisms, actinomycetes, fungi, amoebal protozoans, and MPN nitrifiers.

Measurement of these members of the microbial populations, when carried out in conjunction with activity measurements, should provide a meaningful indication of number-activity relationships. As it is possible that nutritionally-limited soil microbes can increase their metabolic activity, respiration, and carbon mineralization before actually responding by increasing in

numbers, plating enumerations may then reflect numerical lags and responses under varied nutritional conditions.

iv. These assays should be correlated with chemical analyses of organic and inorganic nitrogen, root biomass, and residual substrate wherever possible, assuming sufficient laboratory capabilities are available. Assays for inorganic and organic nitrogen forms are especially critical as nitrogen additions are planned for later experiments.

FUTURE RESEARCH PLANS

Variables of time, temperature, moisture levels and moisture response shifts, substrate additions and levels, and influences of inorganics such as N and P on these processes will be studied using these recommended procedures. To aid in generation of meaningful time-related information, at least three weekly assays will be run per variable to provide an envelope of responses, which will then be subjected to statistical and modelling analysis.

Based on our laboratory-level procedures, field evaluations of respiration will be attempted during the summer of 1971. This will then allow a further extrapolation of soil core data to the field environment in a process modelling content.

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