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SYSTEMS ANALYSIS OF DECOMPOSER FUNCTIONS IN THE GRASSLAND ECOSYSTEM

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ABSTRACT

Experimental data were derived regarding the seasonal responses of Pawnee grassland soils to variations in temperature, soil water, nitrogen addition, and treatment with varied types and levels of carbonaceous materials. Response of differing microbial types, oxygen uptake, carbon dioxide evolution, and other parameters were evaluated. Seasonal responses of soils indicate that the respiratory quotient (RQ) is a sensitive indicator of carbon flow. Marked decreases in RQ values observed later in the summer are indicative of a change in the nutrient status of microbes from carbohydrate to amino acid metabolism. Studies of plant leachate influences in soil processes have indicated that even a 15-min leaching event can transport nutrients from plant material to the soil, which cause major shifts in soil respiration and microbial populations. Amoebal components of the decomposer ecosystem have been investigated, and amoebae respond to soil perturbations related to changes in microbial populations and nutrient status of the soil. Initial statistical analyses and synthesis of data have been completed to allow application of these data to development of an expanded decomposer model.

OBJECTIVES

1. Development and testing of an integrated system for measuring oxygen uptake, CO_2 evolution, and microbial population activities and enumerations, with regard to nitrogen, temperature, substrate, soil water, and root biomass variables.
2. Integration of these assays with assays for residual substrate, dehydrogenase, plant counts, amoebal protozoans, and nitrifiers.
3. Assays for inorganic N, other N forms, and root biomass evaluations in respiring soils.
4. Beginning of computer simulation to allow a mathematical description of the above-described parameters on a laboratory investigation scale.
5. Study of plant leachate effects on soil respiration and of availability with sequential extractions to relate to precipitation variables.
6. Beginning of work on field scale procedures which will embody the laboratory principles elucidated in the 1971 research year.

LOCATION

Soil cores used in this study were obtained from plot 26 of the IBP Grassland Biome Intensive Site. Samples were taken at approximately 3- to 4-week intervals, from December 1970 through August of 1971, for use in this research.

METHODS

Collecting Field Samples

Soil cores were removed from the field area by use of the IBP power corer, and cores 6.5 cm in diameter were approximately 10 cm in length.

Only the surface soil was utilized in this series of experiments, and after removing the surface litter the core was cut to a length of 8.0 cm, weighing approximately 220 to 250 g per sample.

Processing Field Samples

Samples were transferred to the laboratory after packaging in polyethylene. Samples were used for experimental runs within 1 to 2 days of collection, except for the winter season, where sufficient cores were collected for two runs to assure that materials would be available in the event of a storm. Cores were stored in the laboratory at 10°C in containers to which moist paper was added to maintain the original soil water levels of the cores.

Analysis Procedures

Integrated Studies

Soil cores were analyzed for responses by use of procedures summarized in Klein (1971). In addition, the respirometer unit used in these studies has been described in a publication by Klein, Mayeux, and Seaman (1972). Soil chemical analyses were carried out by the Soil Testing Laboratory at Colorado State University using standard procedures, and root biomass estimates were carried out using the procedures described in Uresk and Sims (1969).

Plant Leachate Studies

Blue grama hay samples (2.5 g) were extracted with 250-ml portions of cold water, at the following sequential time intervals, to be able to estimate the effects of varied length precipitation events on removal of plant constituents:

Fraction	*Extraction Duration*
F1	0- 15 min
F2	15- 30 min
F3	30- 60 min
F4	60-120 min
F5	120-180 min

These fractions were analyzed chemically for carbon and nitrogen and added to soils under laboratory conditions to evaluate microbial responses.

Standard procedures for nitrogen and carbohydrate levels were used in these studies, and leachates were added to soil cores removed from the Pawnee Site as described for the integrated experiment procedures. Analysis of soil responses was carried out using procedures listed in Klein (1971).

Soil Amoebal Responses

Soil treatments. Control cores, having approximately 5% soil water, were examined as received without modification. For soil water additions, 20 ml of water were added to cores to give approximately 13% soil water. The glucose plus water treated cores had 20 mg of glucose dissolved in 20 ml of water added; hay plus water treated cores had 0.3 g of chopped blue grama hay placed on the core upper surface, with 20 ml of distilled water slowly added to create a leaching effect.

Soil cores, set up in triplicate, were analyzed by use of standard procedures described in Klein (1971), except for analysis of amoebal populations.

Amoebal plating and enumeration. The following technique was developed for the isolation and enumeration of some small soil amoebae. The technique was based on the overlay technique used by virologists. The base medium containing 0.5% NaCl w/v and 1.4% w/v agar per liter was poured into Petri

dishes and allowed to solidify. The overlay agar consisted of the following mineral salts:

$MgSO_4 \cdot 7H_2O$	4.0 mM
$CaCl_2$	0.4 mM
$FeSO_4$	50.0 M
K_2HPO_4	5.0 mM
KH_2PO_4	5.0 mM
$(NH_4)_2(SO_4)_2$	50.0 mM

0.8% w/v agar per liter

pH adjusted to 6.5 with 1 N NaOH

An *Aerobacter aerogenes* suspension was added to this overlay agar. The *A. aerogenes* was grown in peptone broth, harvested by centrifugation, washed with phosphate buffer, and frozen until needed. Upon thawing, the culture was washed again in phosphate buffer, weighed, and sufficient buffer added to make a 1:1 w/v solution. Of the solution, 0.1 ml was added to 4 ml of the overlay agar before being poured over the soil dilution on the basal agar. The soil dilutions were made in buffered distilled water. Shaking was done by hand, as higher counts were obtained than when a mechanical shaker was used. Dilutions (0.1-ml each) were plated, spread with a glass spreader, and the soil water allowed to absorb into the basal medium to minimize air bubble accumulation between the basal and overlay agars. Dilutions were plated in triplicate.

Plates were incubated in an upright position for 7 to 9 days at 30°C in a humid incubator. A water bath with the platform above the water level was found to be an ideal incubator. Plates were read daily, starting on

the second day after plating, until no further new plaques could be observed. Plates were read by holding them up to an incandescent light and counting the clear zones. It was assumed that each plaque was initiated by a single organism; the amoebal numbers were determined by the number of plaques and the dilution. All counts were expressed on a soil dry weight basis. Countable plates had 5 to 20 plaques by the end of the incubation period. If plates with confluent lysis were read, it was assumed that the plaques had not increased in number but only in size.

Amoebal identification procedures. The amoebae were isolated from the plaques on the enumeration plates and maintained on streaks of *A. aerogenes* on NaCl agar. Following several enumeration runs, plaques were randomly picked and microscopically observed to assure that representative amoebae forming plaques would be observed and identified.

The tentative identification of the amoebae was made by use of microscopic techniques. Phase contrast microscopy was used for the gross physical details and for cell measurements.

The criteria used in the identification of these amoebae was obtained from two papers by Page (1967*a*,*b*). Page redefined the genus *Acanthamoeba* and proposed some taxonomic criteria for limax amoebae, using physical and encystment characteristics, nuclear division, and several chemical tests to aid in classification. The described physical characteristics were used in the tentative identification of the amoebae isolated from the Pawnee grassland soil.

Amoebal feeding studies. Potential host bacterial cultures were grown in peptone broth and harvested by centrifugation, followed by washing and freezing until use. Stock cultures were maintained on peptone slants.

Unknown bacterial cultures were randomly picked from sodium caseinate total bacteria plates.

The Petri plate cultures for feeding studies were prepared using NaCl agar as the basal medium. A 2-cm strip of agar was removed from the center of each plate, thus allowing two organisms to be tested on each plate. A 5.5-cm streak of the bacteria was placed on the NaCl agar, and a small portion of NaCl agar containing amoebae in the encysted form was placed at one end of the streak. The plates were incubated in the 30°C water bath incubator for 7 days. They were observed daily and discarded when the streak was completely consumed. The interval, in days, required for the streak to be fully consumed was used as a measure of the amoebal feeding rate.

Data Processing and Analysis

Data derived from these studies were analyzed for individual statistical validity and significance of treatment-response interaction by standard multivariate analysis procedures in the Natural Resource Ecology Laboratory central data processing facility.

Sample Dates and Numbers of Samples Taken

Samples used in these studies were taken on the following listed dates (Table 1). Sufficient cores were taken to allow completion of studies on integrated experiments, leachate studies, and analysis of amoebal responses to soil perturbations.

Status of Samples

All sample respirometry and biological analyses have been completed. Chemical analyses for organic matter, total and nitrate nitrogens, and

Table 1. Amoebal feeding studies data.

Run Number	Soil Water (%)	Date	Treatment Variables	Number of Cores Taken
08-09	5.0	12-09-70	Nitrogen, soil water, glucose, hay	80
08-30	4.0	12-29-70	Temperature (15 and 25°C), soil water, glucose, hay	--
08-42	8.0	01-21-71	Temperature (15 and 25°C), soil water, glucose, hay	60
17-01	5.0	02-11-71	Nitrogen, soil water, glucose, hay	75
17-11	5.0	03-04-71	Nitrogen, soil water, glucose, hay	60
17-21	5.0	03-31-71	Soil water level variations, hay	100
17-32	10.0	04-30-71	Soil water level variations, hay	40
17-42	9.0	05-20-71	Carbon pulse variations of glucose and hay	40
17-52	8.0	06-10-71	Soil water, varied glucose, varied hay	50
17-61	2.0	07-01-71	Soil water, varied glucose, varied hay	50
17-71	2.0	07-22-71	Soil water, varied glucose, varied hay, higher levels additional	50
17-80	2.6	08-12-71	Soil water, dry nitrogen, wet nitrogen	50

other inorganics have been completed by the soil testing laboratory and are included in this report (Appendix I). Analysis for statistical relationships have been summarized in Campion and Francis (1971*a*,*b*) and Campion (1972*a*,*b*).

SYNTHESIS AND INTERPRETATION OF RESULTS

Integrated Experiments

Raw data available for the integrated experiments carried out during this experimental period are given in Fig. 1 through 13 and Tables 2 through 23. All activities have been analyzed in relation to the basic oxygen uptake activity curve carried out over a 21-day experimental period. Analyses of biological populations were carried out at 7, 14, and 21 days to provide an envelope of responses under the oxygen uptake curve.

Runs 8-9, 8-30, 8-42, and 17-1 were carried out using an initial model of the respirometer which read oxygen uptake in changes in water column density instead of pressure. These data, having been summarized in Campion and Francis (1971*a*), are available at the Natural Resource Ecology Laboratory. This statistical summary covers runs 8-9 and 8-30 in depth. A statistical analysis was not completed for experimental runs 8-42 and 17-1, as late runs in this series allowed evaluation of effects of treatment variables with the respirometer described in Klein (1971).

Run 17-11

This run, the first carried out using the respirator unit as described in Klein (1971), was set up to analyze the response of a 5% water content soil, sampled the first part of March 1971, to the following treatment parameters: moisture, glucose, hay, and nitrogen.

This run (Table 2 and 3) indicated the additive effects of having a hay plus nitrogen treatment. However, over the experimental period used in this study, it was found that the nitrogen addition to either moist soil, or moist soil plus glucose, caused a depression of respiratory activity (Fig. 1). Initial stimulation had been indicated in the first week with half of these variables; however, by the second and third weeks, nitrogen-containing soils had depressed the oxygen uptake rates in comparison with the related controls.

The statistical analysis for this run (Campion and Francis, 1971*b*) indicated that the soil water, nitrogen, and time (week) effects were generally nonsignificant while the treatment effect was significant. There were never any significant interactions of soil water with either nitrogen or time. The nitrogen treatment showed significance 50% of the time. The most commonly significant covariant was the core percent soil water.

Run 17-21

Run 17-21 was designed to be a first attempt to evaluate the effects of variations in soil water level on the ability of grassland soil to respond to additions of a hay carbon pulse. For this purpose, 300 mg of chopped blue grama hay were placed on the surface of test cores before addition of water to soil water levels of 5, 10, 15, 20, and 25%. Incubation was at 25°C for all samples.

This run generated an excellent series of responses of soil respiration to the presence of increased levels of soil water (Fig. 2). This response was reflected in the tabular data where the highest bacterial levels, CO_2 evolution, and MPN (most probable number) levels for nitrifiers were found

Table 2. Run 17-11, respiration parameter responses to soil treatments.

Parameter	Time (weeks)	Dry (1)[a]	Dry + Hay (2)	Wet + Glucose (3)	Wet (4)	Wet + Hay (5)	Dry (6)	Dry + Hay (7)	Wet + Glucose (8)	Wet (9)	Wet + Hay (10)
O_2 uptake (ml)	1	18.648	64.676	31.820	32.116	74.592	19.610	52.836	45.806	42.328	78.688
	2	26.022	91.020	63.492	44.770	127.132	46.324	100.122	74.370	60.162	140.748
	3	41.218	119.770	89.762	77.922	139.490	62.900	138.232	87.000	69.264	163.244
CO_2 (mg)	1	[b]	124.78	55.05	146.80	55.05	33.03	89.55	88.08	107.77	106.43
	2	47.71	154.14	102.76	7.34	168.82	73.4	161.48	84.41	66.06	161.48
	3	80.74	183.50	183.50	148.18	234.88	102.76	223.87	117.44	124.78	220.20
Formazan (mg)	1	.0049	.0029	.0021	.0018	.0032	.0043	.0042	.0023	.0025	.0025
	2	.0032	.0053	.0051	.0027	.0039	.0051	.0026	.0045	.0039	.0029
	3	.0046	.0031	.0031	.0031	.0040	.0045	.0045	.0044	.0009	.0029
CO_2 (ml)	1	--	63.638	28.076	74.868	28.076	16.845	45.671	44.921	54.963	54.279
	2	24.332	78.611	52.408	3.743	86.098	37.434	82.355	43.049	33.691	82.355
	3	41.197	93.585	93.585	101.072	119.789	52.408	114.174	59.894	63.638	112.302
RQ	1	--	0.984	0.882	2.331	0.376	0.859	0.864	0.981	1.299	0.690
	2	0.935	0.864	0.825	0.084	0.677	0.808	0.823	0.579	0.560	0.585
	3	0.999	0.781	1.043	1.297	0.859	0.833	0.826	0.688	0.919	0.688

[a] See Appendix Table 1 for soil chemical analysis.

[b] Data point not available.

Table 3. Run 17-11, microbial population responses to soil treatments.

Plate Counts	Time (weeks)	Dry[a/] (1)	Dry + Hay (2)	Wet + Glucose (3)	Wet (4)	Wet + Hay (5)	Dry (6)	Dry + Hay (7)	Wet + Glucose (8)	Wet (9)	Wet + Hay (10)
Total × 10^5	1	183	387	221	197	220	107	188	262	279	191
	2	258	304	165	152	307	260	240	215	160	212
	3	186	276	219	269	233	86	395	175	139	194
Actino × 10^5	1	67	58	58	42	66	40	40	59	56	45
	2	65	57	56	51	47	65	51	62	56	59
	3	69	76	75	68	67	32	100	62	66	62
Fungi × 10^3	1	25	29	49	54	190	30	35	180	39	54
	2	81	63	27	15	200	160	200	24	77	100
	3	57	240	45	26	180	43	210	200	98	41
MPN for nitrosomonas	1	110	790	170	23	330	2	2	240	31	1300
	2	6.8	2	23	79	49	7.8	23	240	13	13
	3	23	170	70	23	23	13	33	33	7.8	23
Percent root biomass per sample	1[b/]	0.51	0.85	--	--	--	0.72	--	0.56	--	--
	2	--	0.65	0.58	0.48	0.49	0.62	0.29	0.59	0.55	0.74
	3	0.66	0.86	0.57	0.60	0.89	0.92	1.03	0.63	0.76	0.68

[a/] Sample number, see Appendix Table 1 for soil chemical analysis.

[b/] Tubes no. 3, 4, 5, 7, 9, and 10 were not numbered.

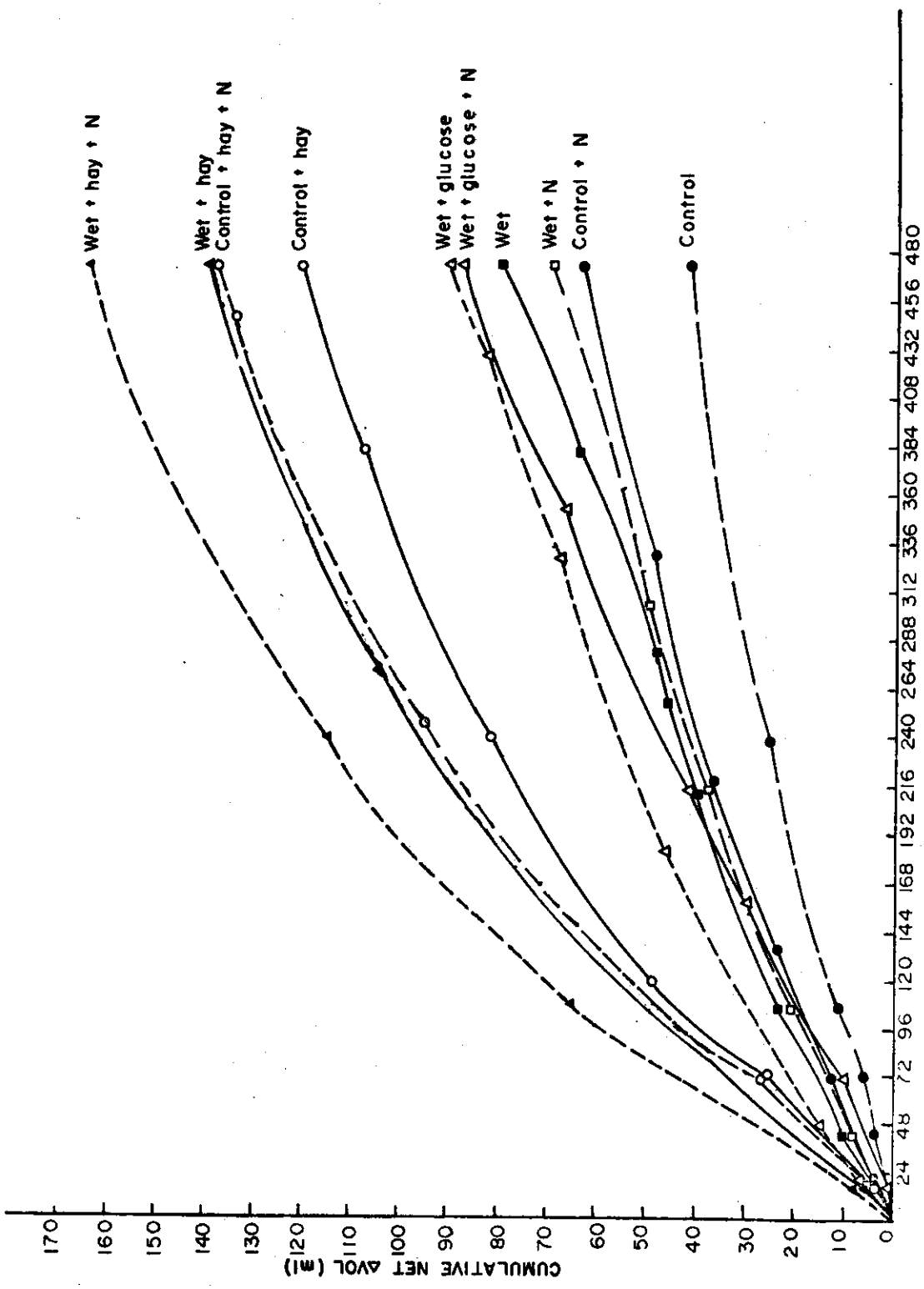

Fig. 1. Run 17-11, oxygen uptake responses.

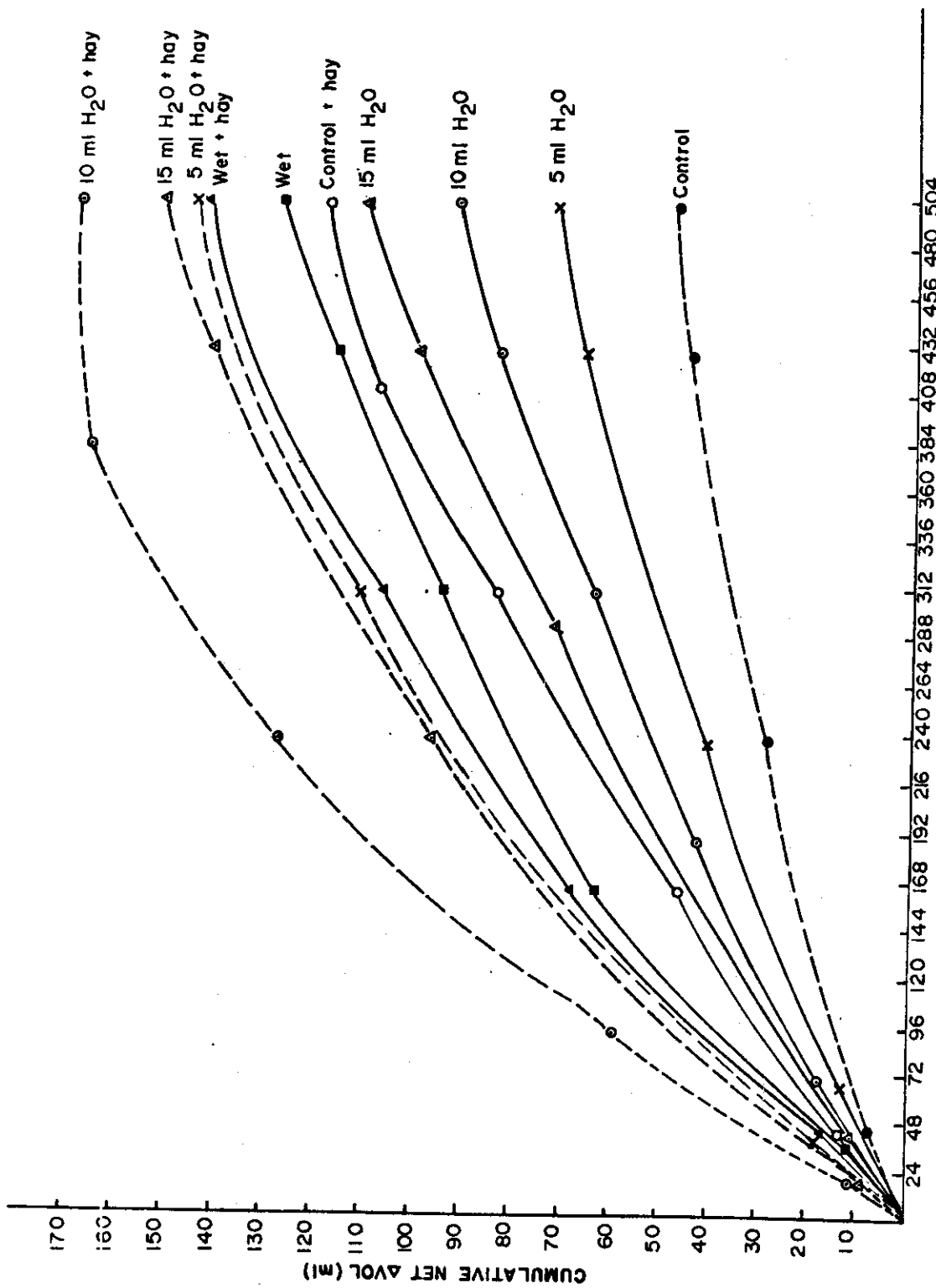

Fig. 2. Run 17-21, oxygen uptake responses.

at 25% soil water (Table 4 and 5). With the addition of a carbon pulse, the increase in respiration demands caused decreased activities at equivalent 20 and 25% soil water levels. This would indicate that both carbon pulse and soil water levels interact in these soils to yield a lower water level where maximal activity is observed.

The tabular data also reflected this additional stress in that maximal responses are seen on microbial levels at the 15% soil water treatment. The summary of statistical relationships for this run are given in Table 6.

Of these early season soils, the respiratory quotient is observed to shift to higher levels towards the end of the experimental period.

Run 17-32

This run was carried out with a format similar to that used in 17-21, except that the first spring rains at the end of April 1971 had increased the average soil water content to 10%. The results were similar to those seen with 17-21 in that increased soil water levels in the presence of a carbon pulse resulted in a maximal microbial activity at 20% water level (Fig. 3). However, this maximal level was observed at 25 to 30% in soils which had not received the carbon pulse.

The nitrifier MPN values were higher than those observed in earlier runs, although it was not possible to observe distinctive changes as a result of the treatments used (Table 7 and 8). The summary of statistical relationships for run 17-32 are given in Table 9.

In comparison with runs 17-11 and 17-21, the basal respiration level of the soil had increased from 120- to 125-ml oxygen over the test period by this later time in the season. This resulted in the respiratory response which is related to added soil water being much more limited than as observed in earlier runs.

Table 4. Run 17-21, respiration parameter responses to varied soil water level and hay addition (0.3 g) of soil treatments.

Parameter	Time (weeks)	Water Minus Hay					Water Plus Hay				
		0 (1)[a]	5 ml (2)	10 ml (3)	15 ml (4)	20 ml (5)	0 (6)	5 ml (7)	10 ml (8)	15 ml (9)	20 ml (10)
O_2 Uptake (ml)	1	37.074	31.376	36.778	38.258	135.716	66.230	87.098	75.036	79.006	79.698
	2	48.470	57.646	79.180	68.598	100.308	100.640	138.380	112.998	132.238	139.490
	3	48.026	71.854	92.278	110.112	127.650	118.474	141.784	167.832	151.182	144.448
CO_2 (mg)	1	30.38	38.90	33.03	--[b]	47.71	62.39	112.30	79.27	--	108.63
	2	80.74	108.63	154.14	102.76	150.47	163.68	215.06	182.03	209.92	220.20
	3	86.61	132.12	128.35	157.81	168.82	194.51	212.86	260.57	209.19	187.70
Formazan (mg)	1	.0051	.0037	.0030	.0045	.0031	.0031	.0041	.0033	.0024	.0029
	2	.0002	.0006	.0003	.0021	.0017	.0017	.0002	.0004	.0034	.0018
	3	.0036	.0040	.0042	.0036	.0022	.0022	.0041	.0031	.0035	.0024
CO_2 (ml)	1	15.494	19.839	16.845	--	24.332	31.819	57.273	40.428	--	55.40
	2	41.177	55.401	78.611	52.404	76.740	83.477	109.681	92.835	107.059	112.302
	3	44.171	67.381	65.459	80.483	86.098	99.200	108.559	132.891	106.687	95.727
RQ	1	0.418	0.632	0.458	--	0.179	0.480	0.658	0.539	--	0.695
	2	0.850	0.961	0.993	0.764	0.765	0.829	0.793	0.822	0.810	0.805
	3	0.920	0.938	0.709	0.731	0.674	0.837	0.766	0.792	0.706	0.663

[a] Sample numbers, see Appendix Table 1 for soil chemical analysis.

[b] Data point not available.

Table 5. Run 17-21, microbial population responses to varied soil water level and hay addition (0.3 g) of soil treatments.

Plate Counts	Time (weeks)	Water Minus Hay					Water Plus Hay				
		0 (1)[a]	5 ml (2)	10 ml (3)	15 ml (4)	20 ml (5)	0 (6)	5 ml (7)	10 ml (8)	15 ml (9)	20 ml (10)
Total $\times 10^5$	1	178	132	128	152	520	181	224	222	261	193
	2	227	183	137	197	326	1099	197	176	153	177
	3	144	194	198	50	105	196	172	403	184	86
Actino $\times 10^5$	1	62	50	46	46	32	33	64	65	79	44
	2	86	75	55	66	58	222	54	40	44	27
	3	44	76	54	23	35	46	52	77	52	38
Fungi $\times 10^3$	1	15	12	18	10	38	300	73	25	190	200
	2	48	18	270	63	77	37	39	55	32	140
	3	31	240	25	42	71	180	260	250	32	27
MPN for nitrosomonas	1	130	350	70	46	240	540	5400	9200	1700	9.3
	2	2800	130	33	79	9200	130	5400	350	1100	23
	3	23	230	790	79	130	22	79	3500	23	220
Percent root biomass per sample	1	--[b]	--	--	--	--	--	--	--	--	--
	2	--	--	--	--	--	--	--	--	--	--
	3	--	--	--	--	--	--	--	--	--	--

[a] Sample number, see Appendix Table 1 for soil chemical analysis.

[b] Data point not available.

Table 6. Run 17-21, statistical analysis.

Source	Parameters							
	CO_2	Formazan	MPN	Total Plate Count	Actino	Fungi	Cumulative O_2	Net O_2
Treatment	NS	NS	NS	**	NS	NS	***	**
Hay	***	NS	NS	***	**	***	***	***
Water	***	***	NS	***	***	***	***	***
Treatment + Hay	NS(p)	NS(p)	NS(p)	**	NS	***	***	***
Treatment + Water	NS(p)	NS(p)	NS(p)	***	***	***	***	*
Hay + Water	NS(p)	NS(p)	NS(p)	NS	**	***	***	***
Dry wt	NS	NS	NS	**	***	***	NS	**
Percent soil water	NS	NS	NS	***	***	***		
Cumulative hours							***	

NS = nonsignificant for $\alpha = .10$.

NS(p) = nonsignificant for $\alpha = .25$.

* = significant for $\alpha = .10$.

** = significant for $\alpha = .05$.

*** = significant for $\alpha = .01$.

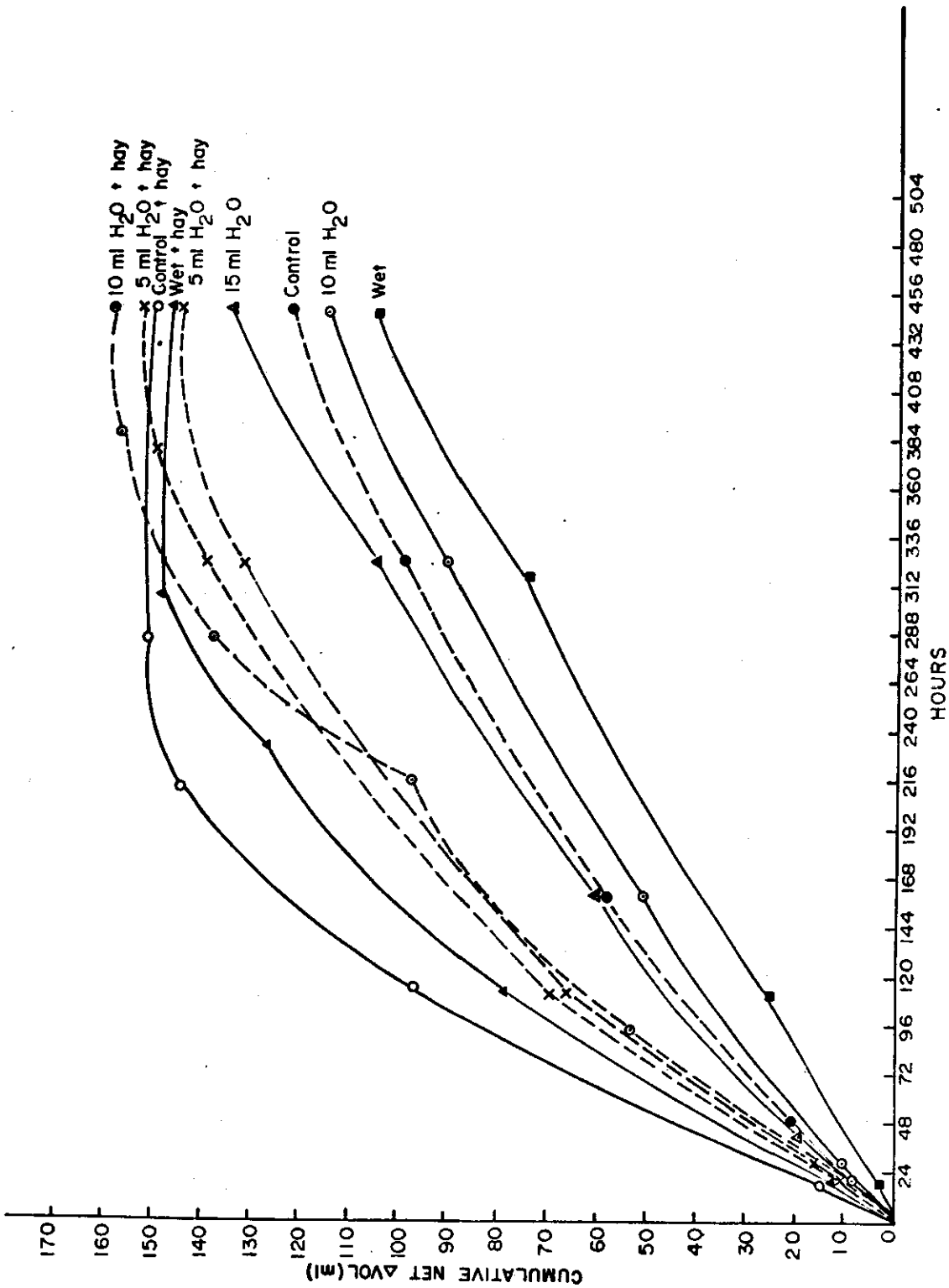

Fig. 3. Run 17-32, oxygen uptake responses.

Table 7. Run 17-32, respiration parameter responses to soil treatments.

Parameter	Time (weeks)	Water Minus Hay					Water Plus Hay (0.3 g)				
		0	5 ml	10 ml	15 ml	20 ml	0	5 ml	10 ml	15 ml	20 ml
O_2 Uptake (ml)	1	32.709	41.514	46.842	43.660	35.520	63.862	89.466	89.466	87.172	88.578
	2	56.536	93.536	92.584	95.534	151.108	133.496	143.190	154.068	150.368	141.266
	3	121.452	26.714	144.404	134.458	95.682	150.330	151.182	139.120	146.556	149.554
CO_2 (mg)	1	34.77	53.22	58.44	55.05	42.21	97.26	152.31	128.45	139.46	128.45
	2	106.43	135.79	154.14	141.30	234.88	221.03	227.54	238.55	201.85	207.36
	3	139.46	178.00	192.68	178.00	174.33	258.74	266.08	267.91	284.43	264.24
Formazan (mg)	1	.0029	.0005	.0009	.0010	.0009	.0021	.0013	.0003	.0013	.0012
	2	.0027	.0020	.0012	.0008	.0016	.0008	.0033	.0028	.0015	.0015
	3	.0012	.0008	.0005	.0027	.0004	.0007	.0020	.0008	.0009	.0047
CO_2 (ml)	1	17.733	27.142	29.804	28.076	21.527	49.603	77.678	65.510	71.125	65.510
	2	54.279	69.253	78.611	72.063	119.789	112.725	116.045	121.661	102.944	105.754
	3	71.125	90.780	98.267	90.780	138.408	131.457	135.701	136.634	145.059	134.762
RQ	1	0.542	0.654	0.636	0.643	0.606	0.777	0.868	0.732	0.816	0.740
	2	0.960	0.740	0.849	0.754	0.793	0.844	0.810	0.790	0.685	0.749
	3	0.583	3.398	0.859	0.675	1.452	0.874	0.898	0.982	0.990	0.901

Table 8. Run 17-32, microbial population responses to soil treatments.

Plate Counts	Time (weeks)	Water Minus Hay					Water Plus Hay (0.3 g)				
		0	5 ml	10 ml	15 ml	20 ml	0	5 ml	10 ml	15 ml	20 ml
Total × 10^5	1	225	194	276	236	276	191	167	363	155	213
	2	160	187	214	153	207	157	242	261	204	183
	3	114	196	251	187	206	72	156	143	410	900
Actino × 10^5	1	57	58	51	29	50	30	49	27	77	38
	2	67	64	49	57	46	29	71	61	67	36
	3	24	44	33	82	43	25	45	35	60	40
Fungi × 10^3	1	80	210	150	45	92	340	98	410	96	280
	2	51	200	75	71	45	78	32	250	200	79
	3	51	88	106	63	47	49	210	49	270	190
MPN for nitrosomonas	1	3500	9200	2400	2400	9200	3500	350	3500	170	3500
	2	790	3500	5400	1300	1700	5400	700	490	3500	--
	3	5400	9200	5400	330	3500	3500	3500	3500	790	2400
Percent root biomass per sample	1	0.76	0.63	0.52	0.58	0.62	0.62	1.77	0.56	0.70	0.62
	2	0.60	1.45	1.01	1.03	0.77	1.42	0.59	1.19	1.08	0.88
	3	0.89	1.45	1.01	1.03	0.79	0.98	0.65	0.73	1.02	0.80

Table 9. Run 17-32, statistical analysis.

Source	Parameters							
	CO_2	Formazan	MPN	Total Plate Count	Actino	Fungi	Cumulative O_2	Net O_2
Treatment	NS	NS	NS	***	***	**	***	***
Hay	***	NS	NS	*	**	***	***	***
Water	***	NS	NS	***	**	***	***	***
Treatment + Hay	NS(p)	NS(p)	NS(p)	***	***	**	***	NS
Treatment + Water	NS(p)	NS(p)	NS(p)	***	***	***	***	***
Hay + Water	NS(p)	NS(p)	NS(p)	NS(p)	*	**	***	***
Dry wt	*	NS	NS	NS	*	NS	***	***
Percent soil water	NS	NS	NS	***	***	*	***	***
pH				NS	NS	NS		
Cumulative hours								

NS = nonsignificant for $\alpha = .10$.

NS(p) = nonsignificant for $\alpha = .25$.

* = significant for $\alpha = .10$.

** = significant for $\alpha = .05$.

*** = significant for $\alpha = .01$.

Root biomass readings were available for these soils and indicate that relatively higher levels of belowground material had been developed (Table 8).

Again, the milligrams of formazan and MPN nitrifiers did not indicate a significant relationship for these parameters.

Run 17-42

This run was set up using soils collected at the middle of May 1971, when soil water and microbial activities were still at maximal levels. Treatments used include soil water and varied levels of glucose and hay. Glucose was used here to provide a readily utilizable carbon pulse of known size; incubation was again at 25°C. This run also allowed a comparison of soil responses to 100 mg of either glucose or hay.

Glucose at 100 mg allowed much higher initial response for oxygen, CO_2, and microbial parameters; and the generally higher levels of nitrifiers made it impossible to detect shifts which might have resulted from these treatments (Fig. 4, Tables 10 and 11).

Only with the addition of three times the amount of hay (300 mg) was it possible to achieve a response which began to be equivalent to those observed with 100 mg of glucose. Microbial responses with this or higher levels were still not as rapid as those observed with a 100-mg pulse of glucose.

The stimulation of microbial parameters due to the simple addition of water was minimal with this spring soil. It is of interest to note that by this time, the respiratory quotients have generally increased on the soils in comparison with earlier samples, and also that the RQ values

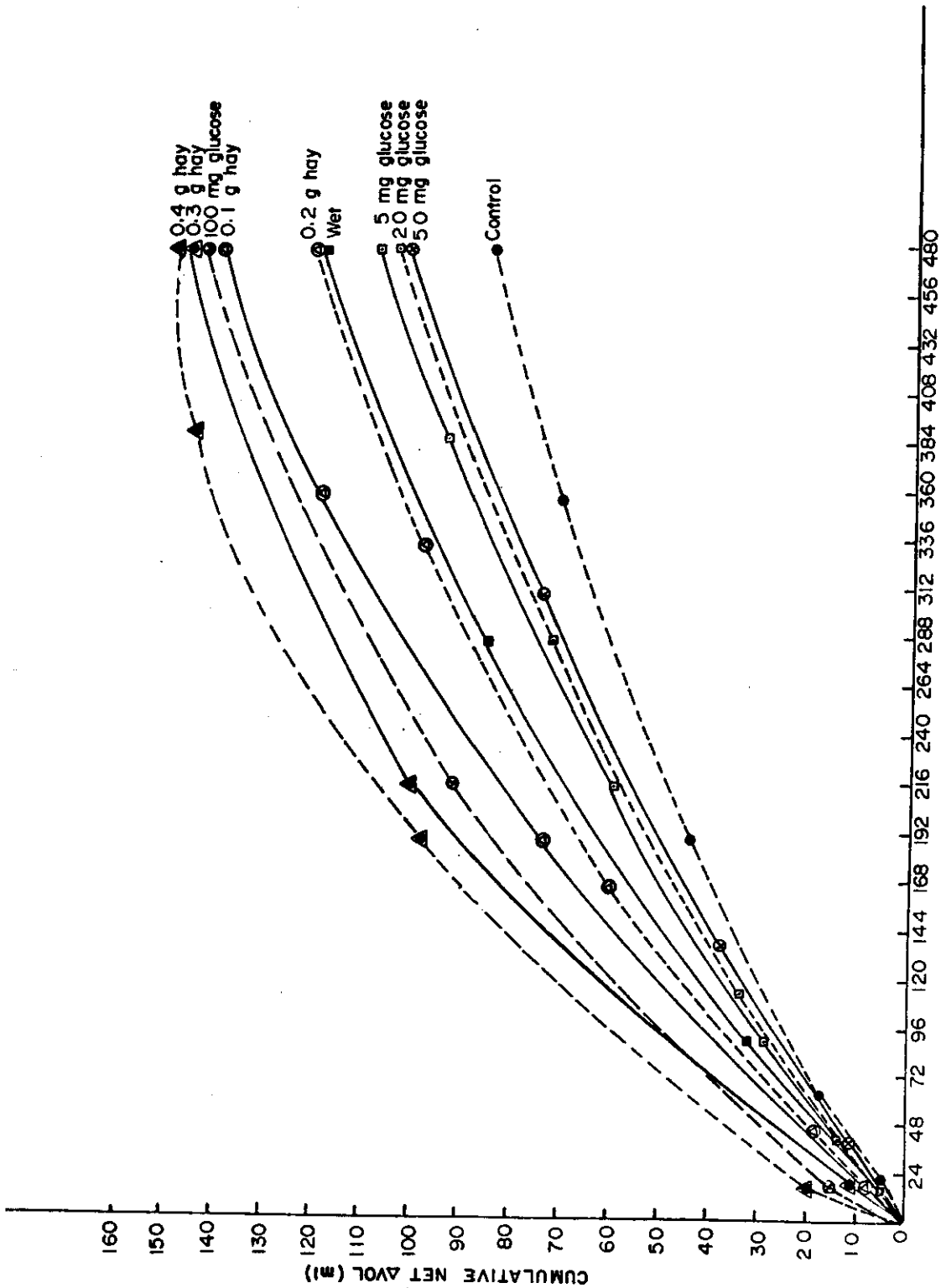

Fig. 4. Run 17-42, oxygen uptake responses.

Table 10. Run 17-42, respiration parameter responses to soil treatments.

Parameter	Time (weeks)	Dry	Wet	Wet + mg Glucose				Wet + g Hay			
				5	20	50	100	0.1	0.2	0.3	0.4
O_2 Uptake (ml)	1	42.180	9.842	37.184	54.982	47.286	89.170	58.164	57.498	81.400	84.804
	2	70.226	100.788	67.858	94.572	107.744	104.562	93.536	121.804	128.982	151.034
	3	84.582	118.992	107.670	103.748	101.824	143.412	140.378	120.768	145.704	146.150
CO_2 (mg)	1	75.24	51.38	77.07	95.42	99.92	178.00	95.42	106.43	154.14	143.13
	2	93.58	130.29	89.92	133.96	159.65	196.35	152.31	172.49	200.02	222.04
	3	95.42	115.61	117.44	128.45	163.32	216.53	174.33	155.98	196.35	236.72
Formazan (mg)	1	.0014	.0009	.0012	.0015	.0009	.0017	.0008	.0006	.0004	.0004
	2	.0008	.0007	.0005	.0006	.0017	.0008	.0007	.0013	.0010	.0012
	3	.0041	.0008	.0008	.0016	.0003	.0014	.0012	.0012	.0028	.0015
CO_2 (ml)	1	38.372	26.204	39.306	48.664	50.959	90.780	48.664	54.279	78.611	72.996
	2	47.726	66.448	45.859	68.320	81.422	100.139	77.678	87.970	102.010	113.240
	3	48.664	58.961	59.894	65.510	83.293	110.430	138.908	79.550	100.139	120.727
RQ	1	0.910	2.662	1.057	0.885	1.078	1.018	0.837	0.944	0.966	0.861
	2	0.680	0.659	0.676	0.722	0.756	0.958	0.830	0.722	0.791	0.750
	3	0.575	0.496	0.556	0.631	0.818	0.770	0.990	0.659	0.687	0.826

Table 11. Run 17-42, microbial population responses to soil treatments.

Plate Counts	Time (weeks)	Dry	Wet	Wet + mg Glucose				Wet + g Hay			
				5	20	50	100	0.1	0.2	0.3	0.4
Total × 10^5	1	165	182	261	162	157	260	270	285	182	141
	2	510	222	221	410	191	640	155	134	330	163
	3	116	107	168	186	480	440	370	520	350	390
Actino × 10^5	1	31	43	59	65	26	60	38	37	45	25
	2	80	50	23	80	86	80	32	44	50	40
	3	29	31	39	40	50	70	70	80	60	40
Fungi × 10^3	1	140	50	52	300	30	240	80	65	84	60
	2	35	66	90	36	300	74	72	210	210	150
	3	180	110	100	170	80	100	120	110	40	30
MPN for nitrosomonas	1	2400	2400	3500	330	2400	2400	9200	9200	2400	2400
	2	3500	2400	2400	9200	220	2400	790	1700	490	2400
	3	2400	490	3500	1700	9200	5400	1300	330	790	2400
Percent root biomass per sample	1	1.56	0.81	0.60	1.01	1.11	2.26	1.09	0.99	0.90	0.81
	2	1.08	0.87	0.59	1.24	1.43	0.76	1.11	0.79	0.95	0.87
	3	0.77	1.01	1.00	0.78[a/]	1.17[a/]	0.98	1.19	0.62	0.56	0.85

[a/] Possible exchanged data.

are beginning to fall to lower levels during the later weeks of the run. A statistical summary for run 17-42 is given in Table 12.

Run 17-52

Run 17-52 was set up to again repeat the parameters tested in run 17-42, using a soil from the first part of June 1971, when conditions in the grassland areas were beginning to be warmer and the early flush of growth had been completed. The soil water content had decreased to 8%. Similar parameters of soil water, glucose, and hay additions were used. For this run, due to a shortage of soil cores, only assays at 2 and 3 weeks were made.

The early higher oxygen uptake responses of these soils to 100 mg of glucose compared with 100 mg of hay were again shown (Fig. 5). In this run, the higher glucose levels (50 and 100 mg) showed decreased oxygen uptake in relation to soils receiving lower levels of glucose. In contrast, hay additions at these higher levels did not cause depression of respiratory activity.

Again, 300 or 400 mg of hay were required to give similar responses. The shift to decreases in respiratory quotients with later incubation times was again shown in these runs (Table 13 and 14). A statistical summary of run 17-52 is given in Table 15.

In this soil response analysis, it is of interest to note that the major microbial responses are shown only with fungal populations.

Run 17-61

Run 17-61 was set up using the parameters tested in the last two runs; however, by this time of year (July 1) the soils had dried drastically,

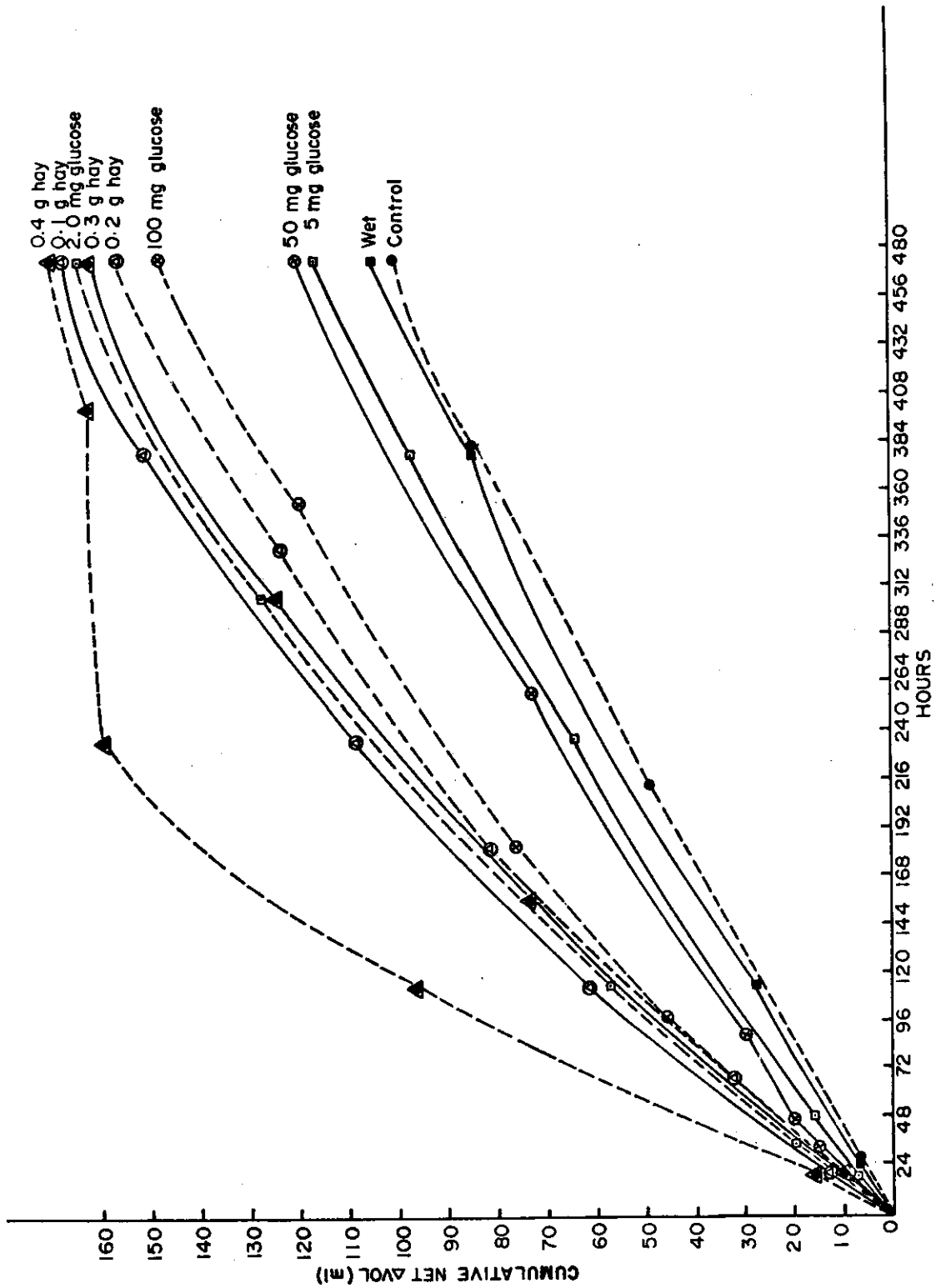

Fig. 5. Run 17-52, oxygen uptake responses.

Table 13. Run 17-52, respiration parameter responses to soil treatments.

Parameter	Time (weeks)	Dry	Wet	Wet + mg Glucose				Wet + g Hay			
				5	20	50	100	0.1	0.2	0.3	0.4
O_2 Uptake (ml)	2	36.334	47.804	74.222	95.164	10.286	106.708	103.156	127.206	154.446	155.104
	3	99.846	103.970	116.476	166.500	120.028	147.260	167.018	156.362	162.726	164.798
CO_2 (mg)	2	53.22	122.95	113.77	137.63	124.78	144.97	128.45	163.32	196.35	222.04
	3	71.57	97.26	104.60	150.47	99.09	146.80	126.62	127.75	187.17	236.72
Formazan (mg)	2	.0042	.0015	.0003	.0023	.0021	.0010	.0017	.0015	.0012	.0008
	3	.0018	.0015	.0014	.0018	.0025	.0018	.0013	.0017	.0017	.0034
CO_2 (ml)	2	27.142	62.705	58.023	70.191	63.638	73.935	65.510	83.293	100.139	113.240
	3	36.501	49.603	53.346	76.740	50.536	74.868	64.576	65.153	95.457	120.727
RQ	2	0.747	1.312	0.782	0.738	6.189	0.693	0.635	0.655	0.648	0.730
	3	0.366	0.477	0.458	0.461	0.421	0.508	0.387	0.417	0.587	0.733

Table 14. Run 17-52, microbial population responses to soil treatments.

Plate Counts	Time (weeks)	Dry	Wet	Wet + mg Glucose				Wet + g Hay			
				5	20	50	100	0.1	0.2	0.3	0.4
Total × 10^6	2	21	37	23	20	32	50	35	33	31	27
	3	14	13	31	21	36	32	29	23	20	104
Actino × 10^6	2	6	8	7	10	8	5	13	7	8	6
	3	3	4	9	4	5	6	5	6	7	7
Fungi × 10^3	2	40	47	32	17	24	210	140	54	42	120
	3	38	43	18	31	34	85	33	37	21	69
MPN for nitrosomonas	2	13	220	540	33	180	9200	33	23	95	33
	3	33	23	490	31	330	46	49	2	33	490
Percent root biomass per sample	2	0.69	0.64	0.61	0.72	0.87	0.80	1.09	0.63	0.61	0.70
	3	0.68	1.06	0.53	0.80	0.49	--[a/]	--	--	--	--

[a/] Data point not available.

Table 15. Run 17-52, statistical analysis.

Source	Parameters							
	CO_2	Formazan	MPN	Total Plate Count	Actino	Fungi	Cumulative O_2	Net O_2
Treatment	NS	NS		NS	NS	NS	NS	NS
Water	NS	NS		*	**	***	NS	***
Replicate (T_1)	NS	NS	NOT	NS	NS	***	***	NS
Replicate (T_2)	**	NS	CARRIED	NS	NS	***	***	***
Replicate (T_3)	***	NS	OUT	***	NS	***	***	**
Treatment + Water	NS(p)	NS(p)		NS(p)	NS(p)	***	***	***
Dry wt	NS	NS		NS	NS	***	***	***
Percent soil water	NS	NS		NS	NS	***	***	*
pH				***	NS	NS		
Cumulative hours							***	

NS = nonsignificant for $\alpha = .10$.

NS(p) = nonsignificant for $\alpha = .25$.

* = significant for $\alpha = .10$.

** = significant for $\alpha = .05$.

*** = significant for $\alpha = .01$.

have a water content of 2%. Under these experimental conditions, the control soil was not capable of oxygen uptake, and the major responses observed were due to the presence of additional soil water (Fig. 6).

In this run, the presence of 5 mg of glucose gave lower levels of responses. This may be due to experimental error.

Addition of glucose and hay at increasing levels allowed development of a family of increasing responses, indicating that the addition of water to 12% w/w allowed excellent respiration in spite of carbon pulses.

The addition of soil water provided such a stimulus to activity that additions of the lower levels of glucose and hay were matched by this basal activity stimulation. This is especially well observed with carbon dioxide evolution (Tables 16 and 17).

Again the respiratory quotients were observed to decrease in the later samples. The extremely low oxygen uptake of the dry cores (2% soil water) caused the RQ to shift to values well above 1.0, normally considered to be an optimal value for this parameter. A statistical summary of run 17-61 is given in Table 18.

Run 17-71

This run was designed to evaluate the response of dry mid-July 1971 soils to soil water and higher levels of carbon pulses than had been used in the previous runs and to determine possible limits to carbon pulses which might be able to be forced through the system. Soil water was added where required to bring the soils from approximately 2 to 12% w/w water, an optimum level for soil respiration.

These samples, taken later in the growing season, actually showed negative oxygen uptake (Fig. 7). The responses to soil water addition

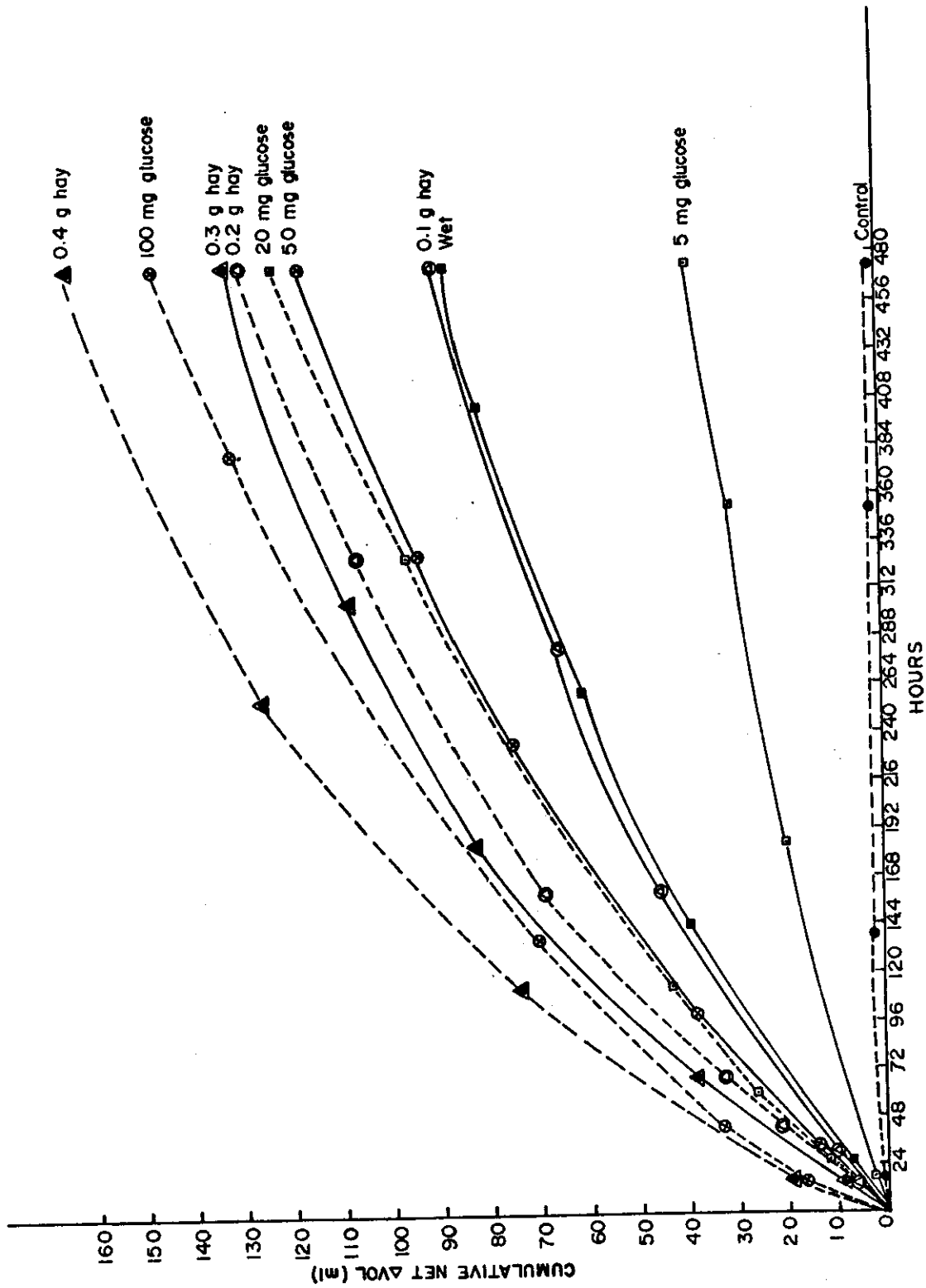

Fig. 6. Run 17-61, oxygen uptake responses.

Table 16. Run 17-61, respiration parameter responses to soil treatments.

Parameter	Time (weeks)	Dry	Wet	Wet + mg Glucose				Wet + g Hay			
				5	20	50	100	0.1	0.2	0.3	0.4
O_2 Uptake (ml)	1	48.10	29.156	27.158	39.220	45.900	45.584	43.290	61.864	86.580	83.768
	2	1.480	89.392	85.100	78.736	79.624	83.472	72.890	80.586	120.324	136.604
	3	1.554	89.540	38.258	121.878	116.920	146.964	87.760	129.426	131.646	164.388
CO_2 (mg)	1	23.86	51.38	66.06	82.58	132.12	100.93	97.26	86.25	139.46	146.80
	2	14.68	124.78	97.26	78.91	113.77	95.42	60.56	77.07	148.64	187.17
	3	12.85	104.60	23.86	176.16	178.00	179.83	104.60	130.29	108.27	225.71
Formazan (mg)	1	.0015	.0053	.0041	.0038	.0025	.0053	.0046	.0073	.0024	.0050
	2	.0013	.0015	.0036	.0050	.0026	.0056	.0054	.0046	.0052	.0037
	3	.0040	.0046	.0035	.0054	.0060	.0073	.0034	.0060	.0057	.0038
CO_2 (ml)	1	12.169	26.204	33.691	41.316	67.381	51.474	49.603	43.988	71.125	74.86
	2	7.487	63.638	49.603	40.244	58.023	48.664	30.891	39.306	75.806	95.45
	3	6.554	53.346	12.169	89.842	90.780	91.713	53.346	66.448	55.218	115.112
RQ	1	2.530	0.899	1.241	1.053	1.468	1.129	1.146	0.711	0.821	0.894
	2	5.059	0.712	0.583	0.511	0.729	0.583	0.424	0.488	0.630	0.699
	3	4.218	0.546	0.318	0.737	0.776	0.624	0.608	0.513	0.419	0.700

Table 17. Run 17-61, microbial population responses to soil treatments.

Plate Counts	Time (weeks)	Dry	Wet	Wet + mg Glucose				Wet + g Hay			
				5	20	50	100	0.1	0.2	0.3	0.4
Total $\times 10^5$	1	119	157	209	218	139	208	255	245	307	250
	2	31	289	179	139	128	229	126	174	220	229
	3	66	530	101	250	176	213	173	169	151	161
Actino $\times 10^5$	1	22	70	77	65	38	60	77	73	93	68
	2	10	48	38	54	33	61	46	44	56	42
	3	28	80	40	60	50	41	55	37	40	52
Fungi $\times 10^3$	1	20	34	31	111	71	31	67	87	57	100
	2	14	220	170	18	21	31	27	80	90	13
	3	16	55	8	30	42	60	53	27	49	58
MPN for nitrosomonas	1	790	4.5	130	220	130	13	33	790	33	170
	2	23	790	790	95	330	33	95	23	49	490
	3	2	13	17	220	7.8	79	79	33	79	11
Percent root biomass per sample	1	0.75	0.31	0.31	0.64	0.42	0.35	0.52	0.59	0.75	0.79
	2	Lost	0.91	0.69	0.51	0.56	0.64	0.74	0.37	0.82	1.01
	3	0.60	0.49	0.43	0.88	0.87	0.38	0.12	0.43	0.24	0.52

Table 18. Run 17-61, statistical analysis.

Source	Parameters							
	CO_2	Formazan	MPN	Total Plate Counts	Actino	Fungi	Cumulative O_2	Net O_2
Treatment	NS	NS	NS	NS	NS	NS	*	NS
Water	NS	NS	NS	NS	***	**	***	***
Replicate (T_1)	NS	NS	NS	***	***	*	***	***
Replicate (T_2)	**	NS	NS	NS	**	NS	***	***
Replicate (T_3)	***	NS	NS	NS	*	NS	***	***
Treatment + Water	NS(p)	NS	NS(p)	***	***	**	***	***
Dry wt	*	*	NS	***	NS	NS	**	NS
Percent soil water	NS	NS	NS	NS	NS	NS	NS	NS
pH				NS	*	NS		
Cumulative hours							***	

NS = nonsignificant for $\alpha = .10$.

NS(p) = nonsignificant for $\alpha = .25$.

* = significant for $\alpha = .10$.

** = significant for $\alpha = .05$.

*** = significant for $\alpha = .01$.

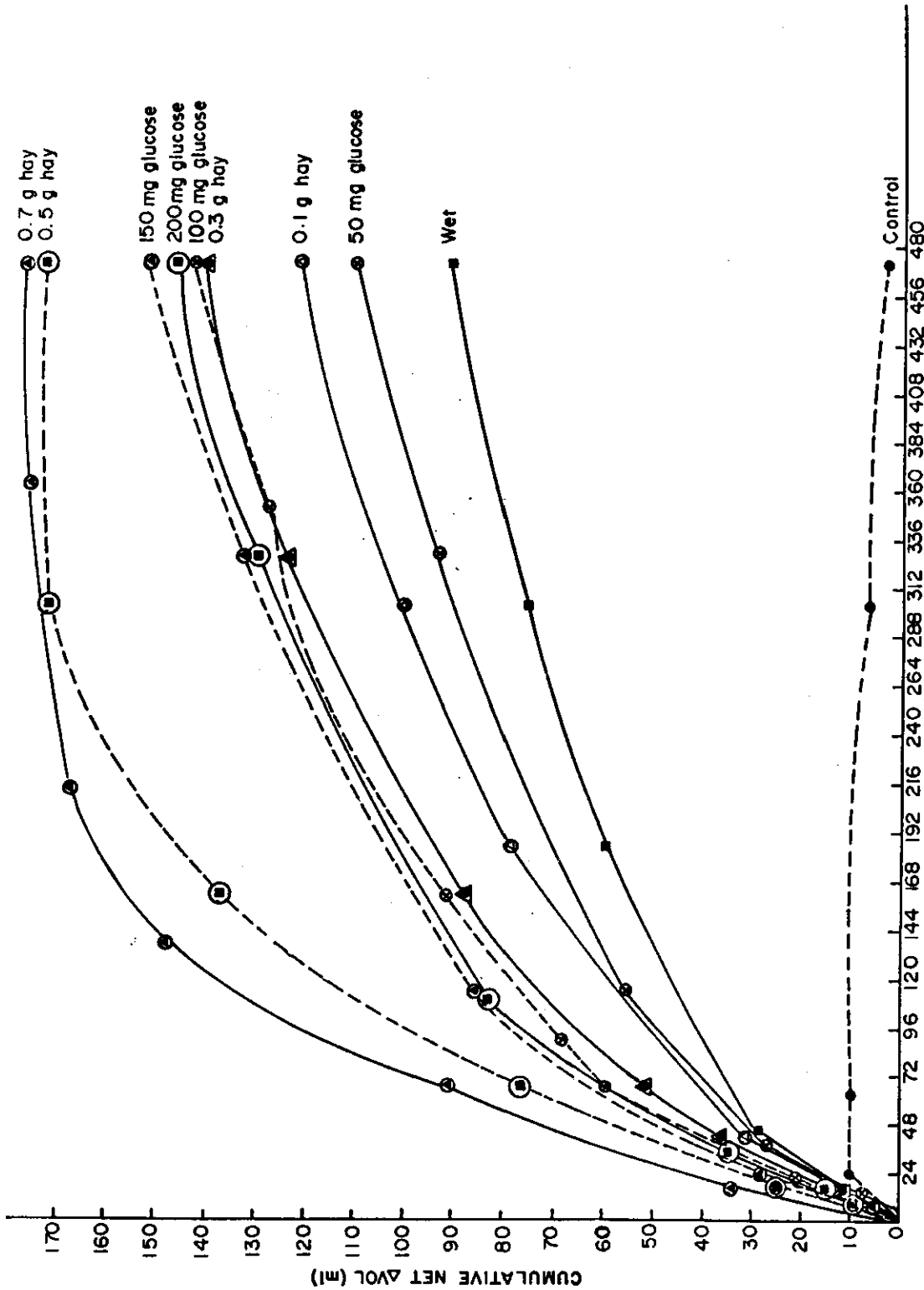

Fig. 7. Run 17-71, oxygen uptake responses.

and the pulsing with increased levels of carbon are clear. In this case, use of 500 or 700 mg of hay exceeded the capacity of the respirometers to allow oxygen uptake, and these data for 2 and 3 weeks should be considered in that context.

As with the previous run, the major responses are due to soil water additions, and additions of either glucose or hay at 100 mg are masked by this basal response of these summer soils.

It is of interest to note that the control soils have even lost the ability to evolve carbon dioxide, and microbial populations have begun to decrease below those observed in soils from the earlier part of the year (Tables 19 and 20).

In general, the RQ values of all soils are lower than observed earlier, and they also decrease with later assays to levels of 0.30 to 0.450.

Run 17-80

Run 17-80 was planned to determine the response of a dry summer soil to the addition of varied nitrogen levels both with and without added soil water. As only minimal responses of nitrifiers had been observed to the present, it was hoped that this run would allow clear responses to be seen.

The clearest characteristic of this soil in the dry state is its inability to respond to the addition of ammonia nitrogen, or to show even positive oxygen uptake characteristics (Fig. 8).

Only with the increase of soil water from 2 to 13% was it possible for increased responses due to nitrogen additions to be observed. These relatively larger pulses also caused varied oxygen uptake, with maximal stimulation seen at 100 ppm. With 150 or 200 ppm of nitrogen, no further stimulation and decreases in oxygen uptake were observed.

Table 19. Run 17-71, respiration parameter responses to soil treatments.

Parameter	Time (weeks)	Dry (1)[a]	Wet (2)	mg Glucose + H_2O				g Hay + H_2O			
				50 (3)	100 (4)	150 (5)	200 (6)	0.1 (7)	0.3 (8)	0.5 (9)	0.7 (10)
O_2 Uptake (ml)	1	-0.741	47.804	65.046	58.238	70.226	85.432	53.132	76.368	116.032	117.882
	2	-2.442	102.638	90.798	128.982	116.882	124.468	122.100	150.886	165.020	163.022
	3a	-5.846	68.154	92.278	157.546	157.138	155.918	166.550	122.036	163.244	164.096
	b[b]	-8.510	93.240	106.560	106.746	125.430	116.550	105.524	151.256	161.172	154.882
CO_2 (mg)	1	0	49.55	100.93	78.91	93.59	135.79	67.90	104.60	161.48	163.32
	2	11.01	144.97	114.61	157.81	146.80	181.67	157.81	161.48	159.65	154.14
	3a	0	36.70	71.57	66.06	66.06	71.57	69.73	124.78	141.30	141.30
	b	0	48.54	75.24	64.23	69.73	71.57	71.57	126.62	144.97	143.13
Formazan (mg)	1	.0039	.0035	.0057	.0030	.0042	.0046	.0022	.0029	.0047	.0036
	2	.0037	.0030	.0036	.0039	.0079	.0080	.0033	.0031	.0040	.0042
	3a	.0036	.0053	.0057	.0051	.0078	.0051	.0058	.0037	.0075	.0062
	b	.0053	.0060	.0051	.0047	.0073	.0044	.0044	.0056	.0077	.0038
CO_2 (ml)	1	0	25.271	51.474	40.244	47.731	69.253	34.629	53.346	82.355	83.293
	2	5.636	73.935	58.451	80.483	74.868	92.652	80.483	82.355	81.422	78.611
	3a	0	18.717	36.501	33.691	33.691	36.501	35.562	63.638	72.063	72.063
	b	0	24.755	38.372	32.757	35.562	36.501	36.501	64.576	73.935	72.996
RQ	1	0	0.52	0.791	0.691	0.680	0.811	0.652	0.699	0.710	0.707
	2	-2.309	0.720	0.644	0.624	0.641	0.744	0.659	0.546	0.493	0.482
	3a	0	0.275	0.396	0.214	0.214	0.234	0.305	0.568	0.441	0.439
	b	0	0.265	0.360	0.307	0.284	0.313	0.346	0.427	0.459	0.471

[a] Sample number, see Appendix Table 1 for soil chemical analysis.

[b] Duplicated series.

Table 20. Run 17-71, microbial population responses to soil treatments.

Plate Counts	Time (weeks)	Dry[a] (1)	Wet (2)	mg Glucose + H_2O 50 (3)	100 (4)	150 (5)	200 (6)	g Hay + H_2O 0.1 (7)	0.3 (8)	0.5 (9)	0.7 (10)
Total × 10^{-5}	1	69	170	230	230	323	490	128	520	176	440
	2	90	219	370	510	350	360	154	490	520	410
	3a	68	234	163	202	190	130	148	127	165	205
	b[b]	42	182	151	219	200	217	330	174	205	208
Actino × 10^{-5}	1	18	50	64	36	57	80	47	190	52	70
	2	23	85	70	90	80	50	63	110	100	110
	3a	29	90	55	54	59	28	41	47	66	58
	b	15	69	47	52	40	34	90	71	63	53
Fungi × 10^{-3}	1	14	61	55	34	39	21	66	68	19	200
	2	9	80	570	430	290	250	89	260	190	100
	3a	15	38	33	160	32	39	68	25	39	95
	b	13	52	52	90	43	55	46	56	35	72
Percentage root biomass per sample	1	1.05	1.13	1.25	0.23	0.51	0.46	0.30	0.78	0.58	0.32
	2	0.56	0.89	0.48	0.57	0.66	0.51	1.09	0.75	0.67	0.95
	3a	0.35	0.31	0.57	2.34	1.05	1.01	0.81	0.23	0.63	0.66
	b	1.01	0.74	0.56	0.61	0.50	0.17	0.46	0.66	1.18	0.94

[a] Sample number, see Appendix Table 1 for soil chemical analysis.

[b] Duplicated series.

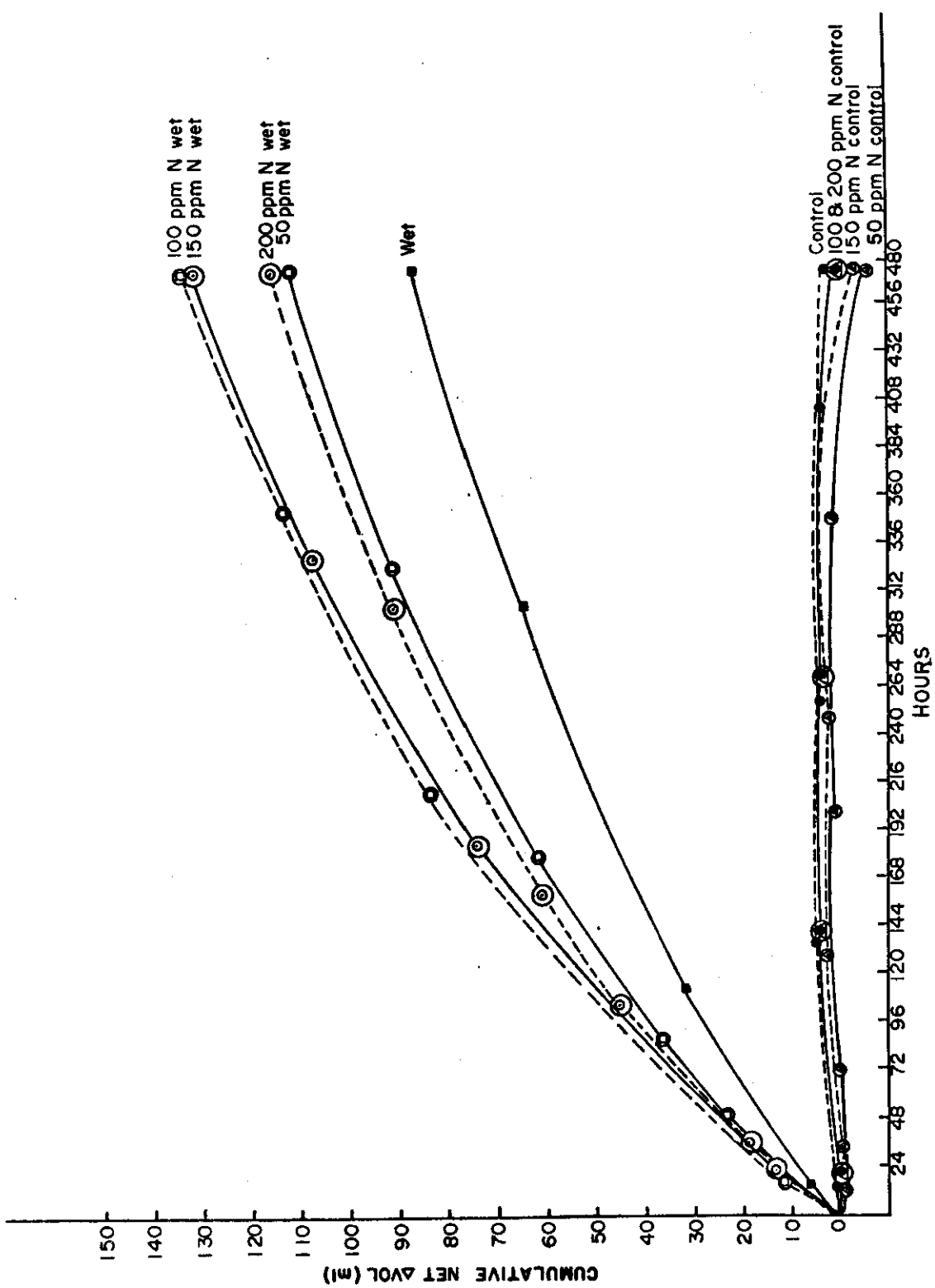

Fig. 8. Run 17-80, oxygen uptake responses.

In spite of their nitrogen stresses, it was not possible to observe shifts in nitrifier populations (Table 21).

Generally, the major microbial responses observed were due to water additions, and the respiratory quotients were at their lowest levels on this run at 1 week, followed by further decreases with continued incubation (Table 22). The presence of added soil water did allow the RQ values to increase above those observed with the drier soils.

Synthesis of Integrated Experiments in Regard to Seasonal Variations

In addition to analysis of possible trends which result from treatments within a soil at a particular time, a synthesis of seasonal effects on decomposer activities and characteristics of these soils was developed.

Results are given in Fig. 9 through 13 which summarize responses at the 2-week assays. This time was chosen to minimize effects of possible limited respiration responses during the third week of incubation and to allow maximum time for the soils to respond to specific perturbations.

Only responses to soil water treatments have been included in this preliminary synthesis. Data for soil responses to nitrogen, carbon, and temperature variations will also provide additional insights into response of soils from this environment.

Fig. 9 shows the oxygen uptake and CO_2 evolution responses of control soils at the seasonal soil water and nutrient status levels and after addition of a further 10% w/w water.

The major point of interest on control soil responses is the point of maximum respiratory activities in the April to May period, with relatively constant carbon dioxide evolution patterns until the June samples. After

Table 21. Run 17-80, respiration parameter responses to soil treatments.

Parameter	Time (weeks)	Dry (1) [a]	Wet (2)	ppm N (dry) 50 (3)	100 (4)	150 (5)	200 (6)	ppm N (wet) 50 (7)	100 (8)	150 (9)	200 (10)
O_2 Uptake (ml)	1	4.144	53.872	1.702	4.144	5.884	5.846	67.710	55.648	67.044	42.180
	2	6.660	82.410	11.544	4.588	1.702	2.072	78.070	80.364	101.232	88.948
	3a	1.332	75.036	2.230	3.852	2.738	2.664	116.180	139.342	137.862	119.362
	b [b]	3.038	97.358	-1.258	-0.370	-2.960	0.360	107.670	124.912	126.466	111.296
CO_2 (mg)	1	0	45.88	--[c]	--	--	--	53.22	55.05	97.26	56.89
	2	3.67	55.05	11.01	1.84	12.85	12.85	49.55	66.06	80.74	88.08
	3a	1.84	44.04	3.67	0	12.48	19.45	60.92	92.48	100.56	95.79
	b	2.94	65.33	4.40	2.20	9.54	11.38	62.39	86.25	105.33	91.38
Formazan (mg)	1	.0061	.0069	.0077	.0058	.0025	.0062	.0070	.0078	.0062	.0055
	2	.0035	.0018	.0047	.0026	.0061	.0043	.0023	.0045	.0035	.0072
	3a	.0033	.0023	.0024	.0047	.0050	.0037	.0018	.0023	.0028	.0030
	b	.0021	.0018	.0043	.0030	.0028	.0049	.0009	.0028	.0021	.0014
CO_2 (ml)	1	0	23.399	--	--	--	--	27.142	28.076	49.603	29.014
	2	1.872	28.076	5.615	0.938	6.554	6.554	25.271	33.691	41.177	44.921
	3a	0.938	22.460	1.872	0	6.365	9.920	31.069	47.165	51.286	48.848
	b	1.499	33.318	2.244	1.122	4.865	5.804	31.819	43.988	53.718	46604
RQ	1	0	0.434	--	--	--	--	0.401	0.505	0.740	0.688
	2	0.281	0.341	0.486	0.204	3.851	3.163	0.324	0.419	0.407	0.505
	3a	0.704	0.299	0.839	0	2.325	3.724	0.267	0.338	0.372	0.409
	b	0.493	0.342	-1.686	-3.032	-2.461	16.122	0.296	1.352	0.425	0.419

[a] Sample number, see Appendix Table 1 for soil chemical analysis.

[b] Duplicated series.

[c] Data point not available.

Table 22. Run 17-80, microbial population responses to soil treatments.

Plate Counts	Time (weeks)	Dry (1) [a]	Wet (2)	ppm N (dry) 50 (3)	100 (4)	150 (5)	200 (6)	ppm N (wet) 50 (7)	100 (8)	150 (9)	200 (10)
Total × 10^{-5}	1	117	540	72	406	40	66	320	590	340	280
	2	77	175	105	91	54	70	166	177	218	233
	3a	41	111	39	43	28	26	65	91	107	116
	b [b]	32	102	50	32	24	32	110	120	113	108
Actino × 10^{-5}	1	4 [b]	50	30	157	17	22	90	70	80	100
	2	22	59	31	13	25	27	28	61	84	72
	3a	19	46	15	18	16	13	29	47	37	52
	b	11	33	10	13	10	5	46	50	26	39
Fungi × 10^{-3}	1	28	370	30	28	12	10	130	47	5	35
	2	22	58	26	17	11	9	18	46	150	36
	3a	31	38	16	17	26	9	35	130	15	29
	b	8	160	17	28	5	5	44	62	37	140
MPN for nitrosomonas	1	790	490	170.0	33	2.0	4.50	49.0	33	23	350
	2	49	22	21.0	490	49.0	.14	4.5	79	79	23
	3a	33	13	4.5	460	7.8	4.50	23.0	130	33	170
	b	13	27	23.0	79	23.0	3.30	49.0	17	49	33
Percentage root biomass per sample	1	0.84	0.71	0.96	0.53	0.63	0.77	0.83	0.47	0.72	0.55
	2	0.74	0.57	0.45	0.71	0.64	0.78	0.81	0.51	0.94	0.80
	3a	0.64	0.59	0.73	1.07	0.96	0.62	0.77	0.59	0.98	0.48
	b	0.56	0.91	1.14	0.73	0.64	1.23	0.92	0.72	0.81	0.49

[a] Sample number, see Appendix Table 1 for soil chemical analysis.

[b] Duplicated series.

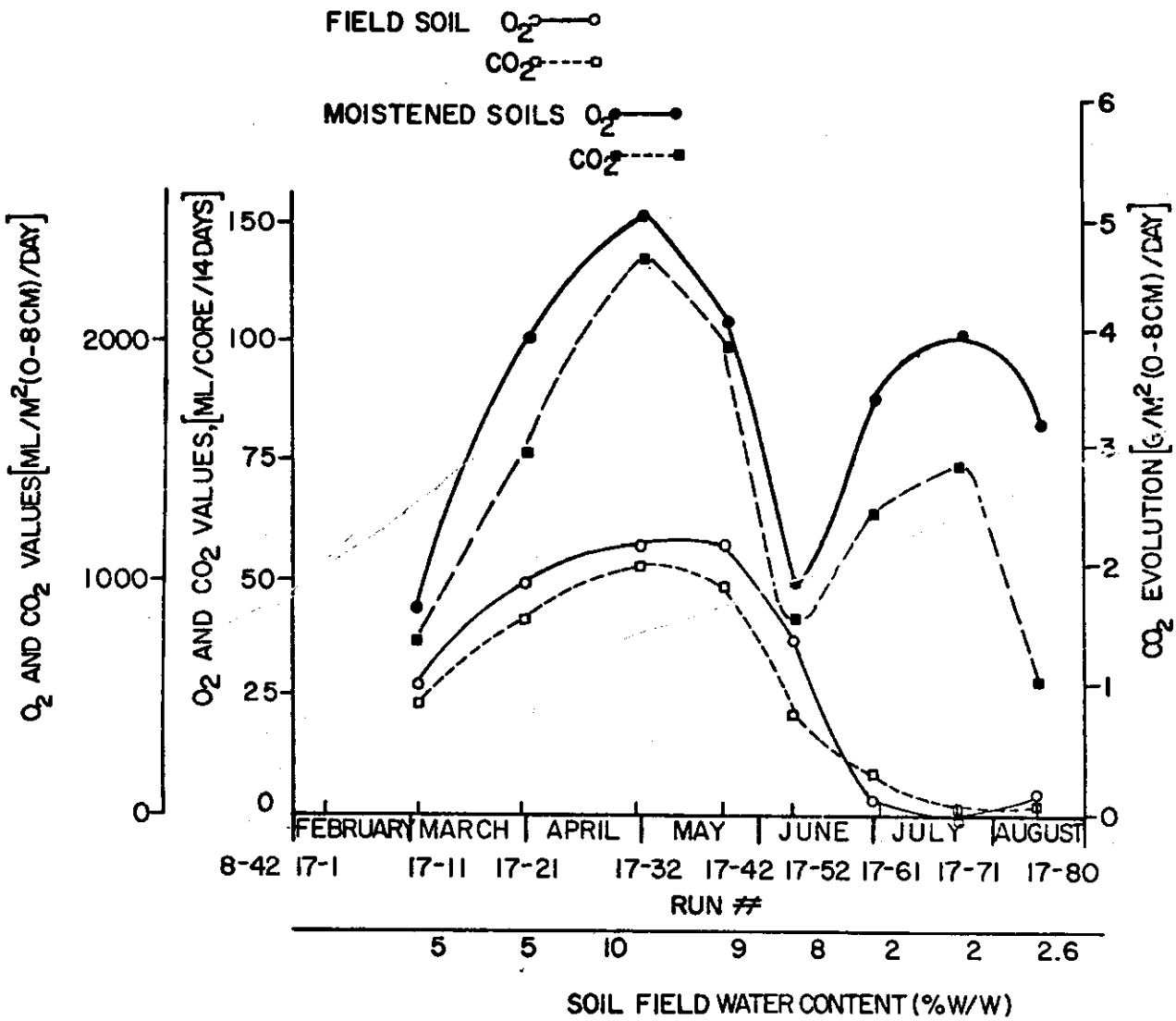

Fig. 9. Oxygen uptake and carbon dioxide evolution of Pawnee grassland soils as related to season, soil water levels, and water additions.

initiation of drier summer conditions, the oxygen uptake is observed to drop rapidly, and in July the carbon dioxide evolution exceeds the oxygen uptake, which could indicate the level of abiotic carbon loss occurring during this period.

The soil water response curves indicate a clear biphasic oxygen uptake and carbon dioxide evolution response. During the maximum spring productivity period, oxygen uptake with soil water additions peaks at the April-May period with carbon dioxide evolution, indicating a respiratory quotient of approximately 0.8 through 0.9. After the soils have passed their period of maximal productivity, a second activity peak is observed with soil water additions. This second response peaked in the middle of July. The carbon dioxide evolution patterns during this period indicate a major shift in the physiology of the soil microorganisms, with a RQ value of between 0.6 and 0.7 and decreasing to 0.3 during the last analysis period in August. This pulse in carbon dioxide evolution and oxygen uptake reflects the time-related increase and decrease in the availability of amino acids from native organic matter available for microbial utilization upon continual drying of the soil.

The lower RQ value reflects the fact that such amino acid products will be directed into central metabolic pathways without use of the pyruvate decarboxylase step which is used more in carbohydrate utilization which occurs in the earlier period of activity (April to May) of the year. Thus, the RQ value will give a measure of the type of carbon which is flowing through the decomposer component of the ecosystem in relation to time and seasonal variations.

Fig. 10, 11, and 12 show the responses of soil microbial populations to seasonal changes in the soils and responses to soil water additions. For total bacteria (actinomycetes and fungi), decreases in populations

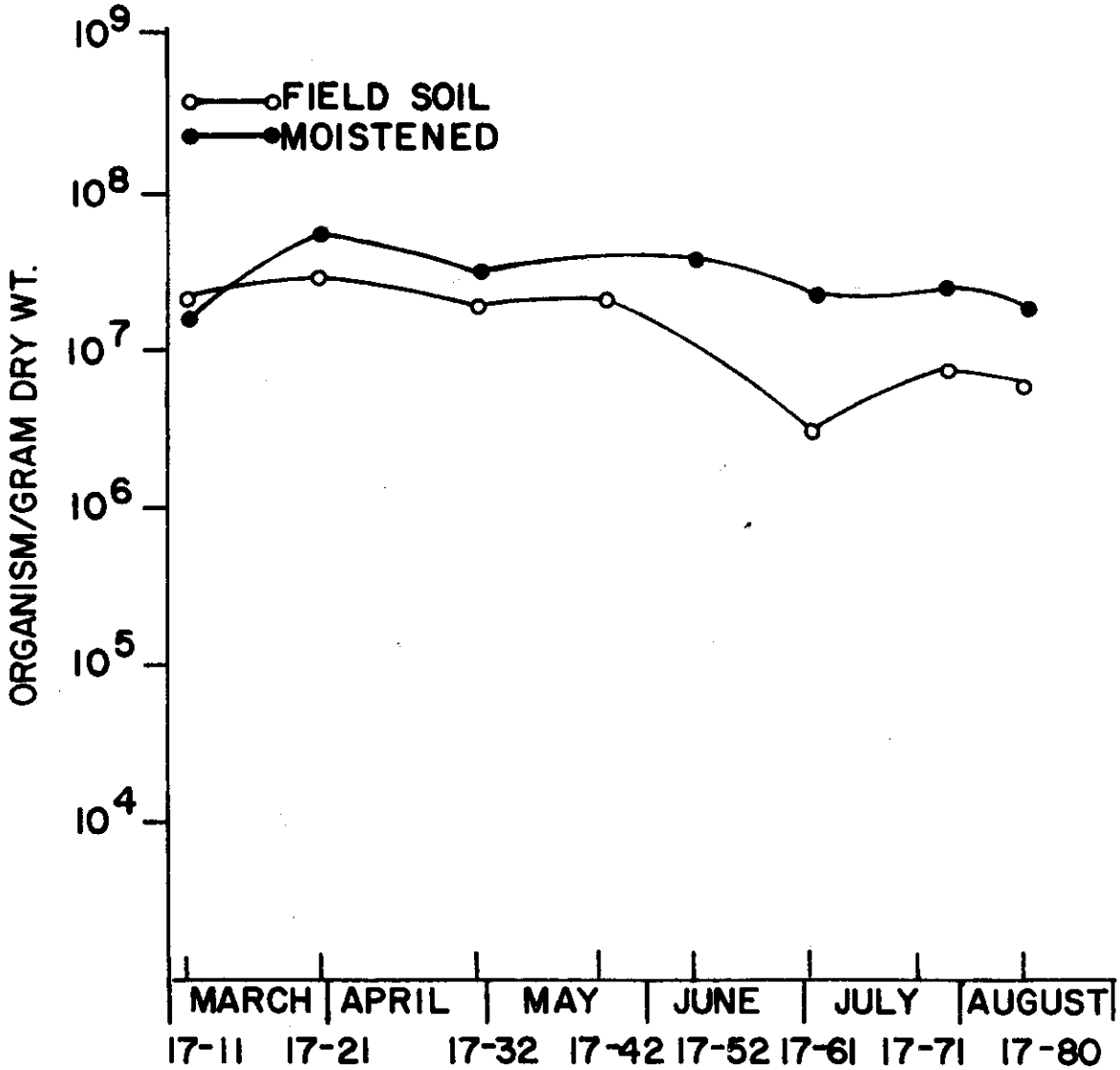

Fig. 10. Seasonal responses of total bacteria in Pawnee grassland soils at field soil water levels and with water additions.

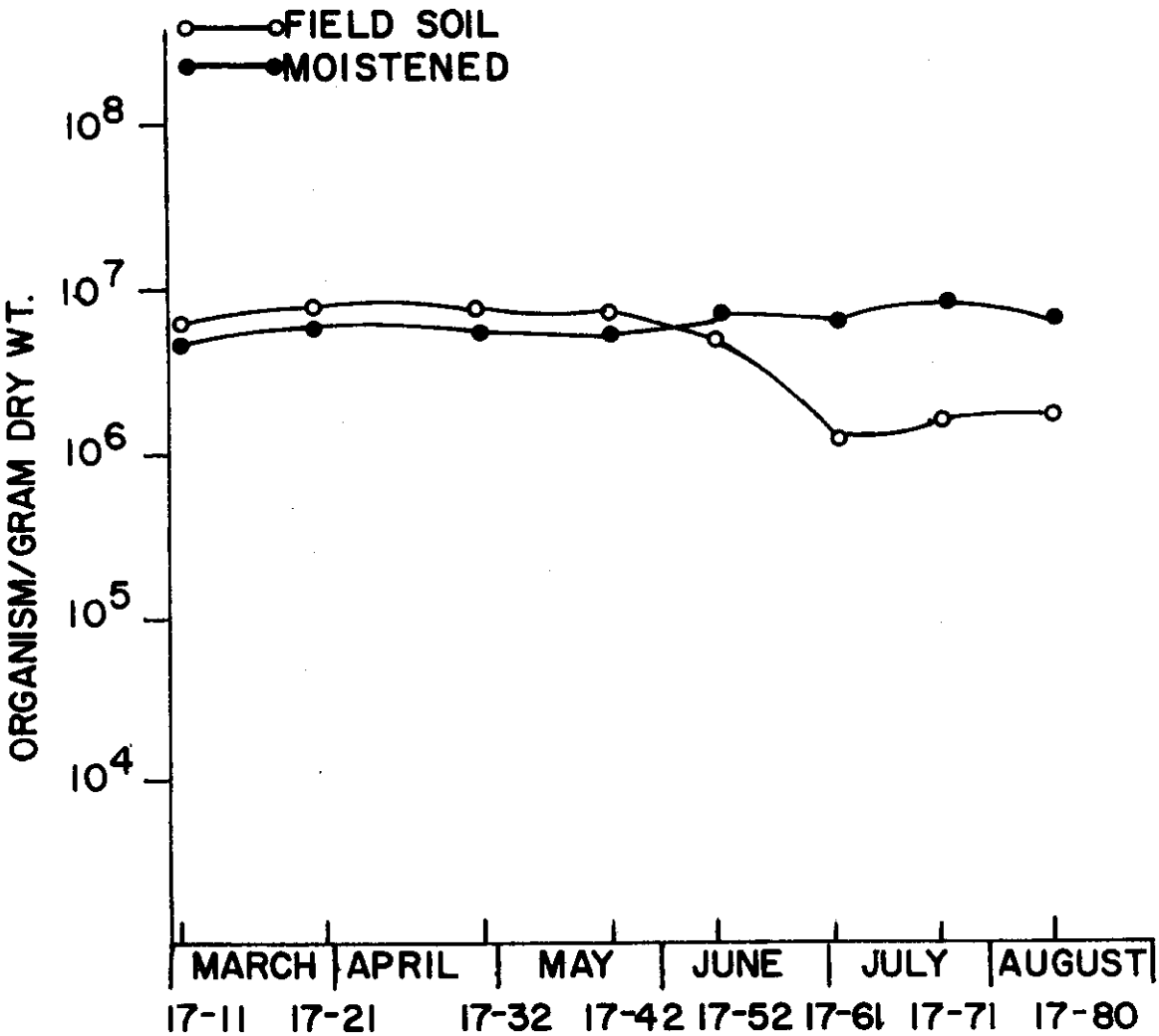

Fig. 11. Seasonal responses of total bacteria in Pawnee grassland soils at field soil water levels and with water additions.

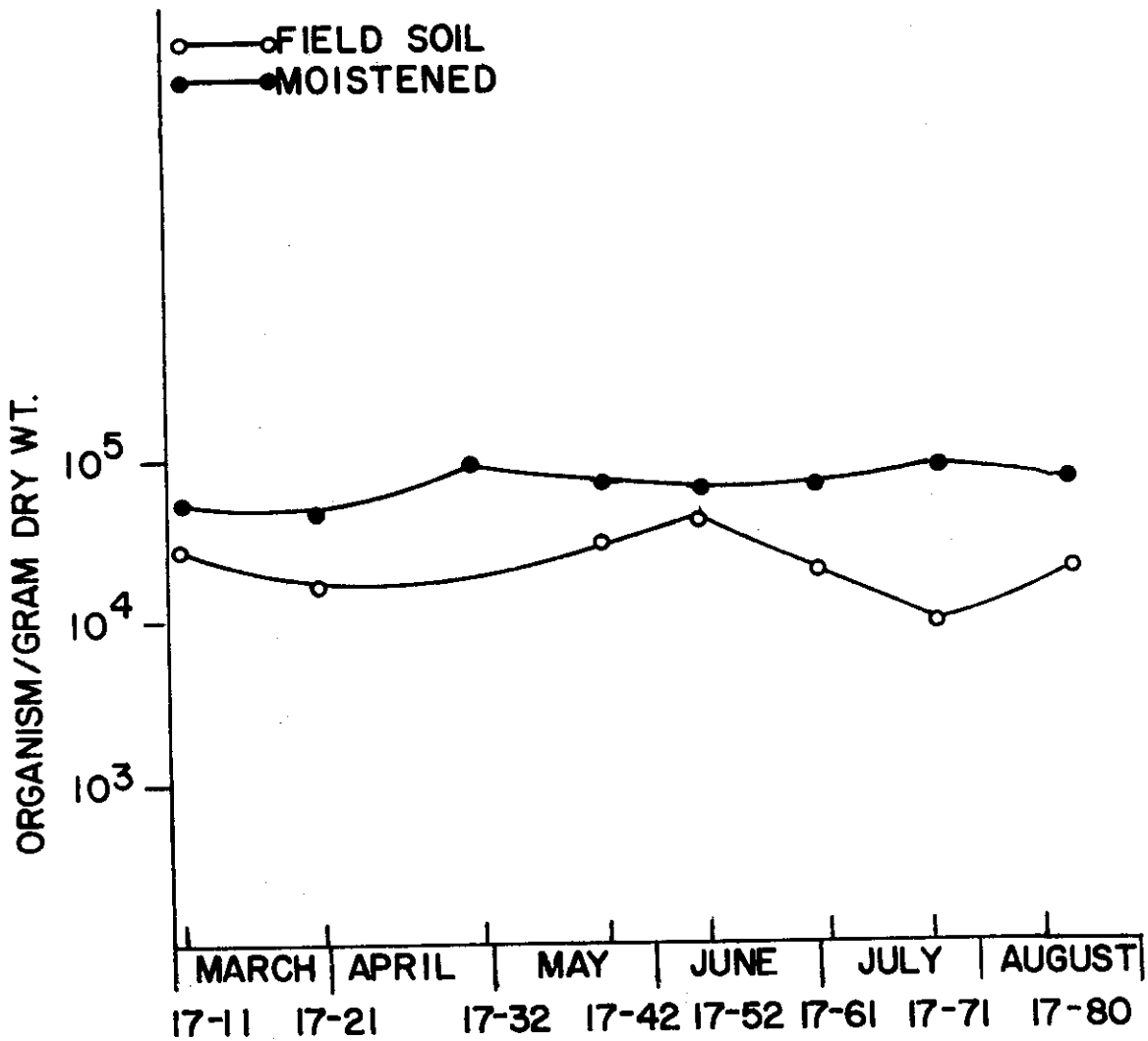

Fig. 12. Seasonal responses of fungi in Pawnee grassland soils at field soil water levels and with water additions.

are only observed after the beginning of the dry summer period. These microbial types appear to respond to soil water additions, reaching levels which approach or are equal to those found in the earlier season, high activity soils.

This synthesis of microbiological population data indicate that microbial populations do change with seasonal variations, but that their numbers will not provide as good an index of decomposer activity as oxygen uptake and carbon dioxide evolution.

Fig. 13 shows the respiratory quotient values for weekly intervals after initiation of analysis in the laboratory of moistened soils. During the spring period of maximal microbial activity, the RQ values are observed to be generally higher, in the range of 0.75 to 0.95. This would reflect the use of carbohydrates derived from plant leachates and root exudates as the primary source of carbon and energy, as these compounds are predominantly metabolized through the pyruvate decarboxylase step which results in a higher rate of carbon dioxide evolution in relation to oxygen utilization.

Through the June to August period, a shift in the RQ values to levels of 0.35 to 0.55 is observed, together with the development of a shift towards decreasing RQ values with increasing incubation time within each sample set. These data indicate that the soil microbes shift towards an amino acid metabolism as the supply of plant-derived carbohydrates in the soil is exhausted, both on a seasonal basis and in any of these later soils where the microbes will have to utilize more native soil organic matter with increasing incubation time.

Table 23 indicates the averaged root biomass levels in the soil samples over the experimental period used in this study. Maximal biomass

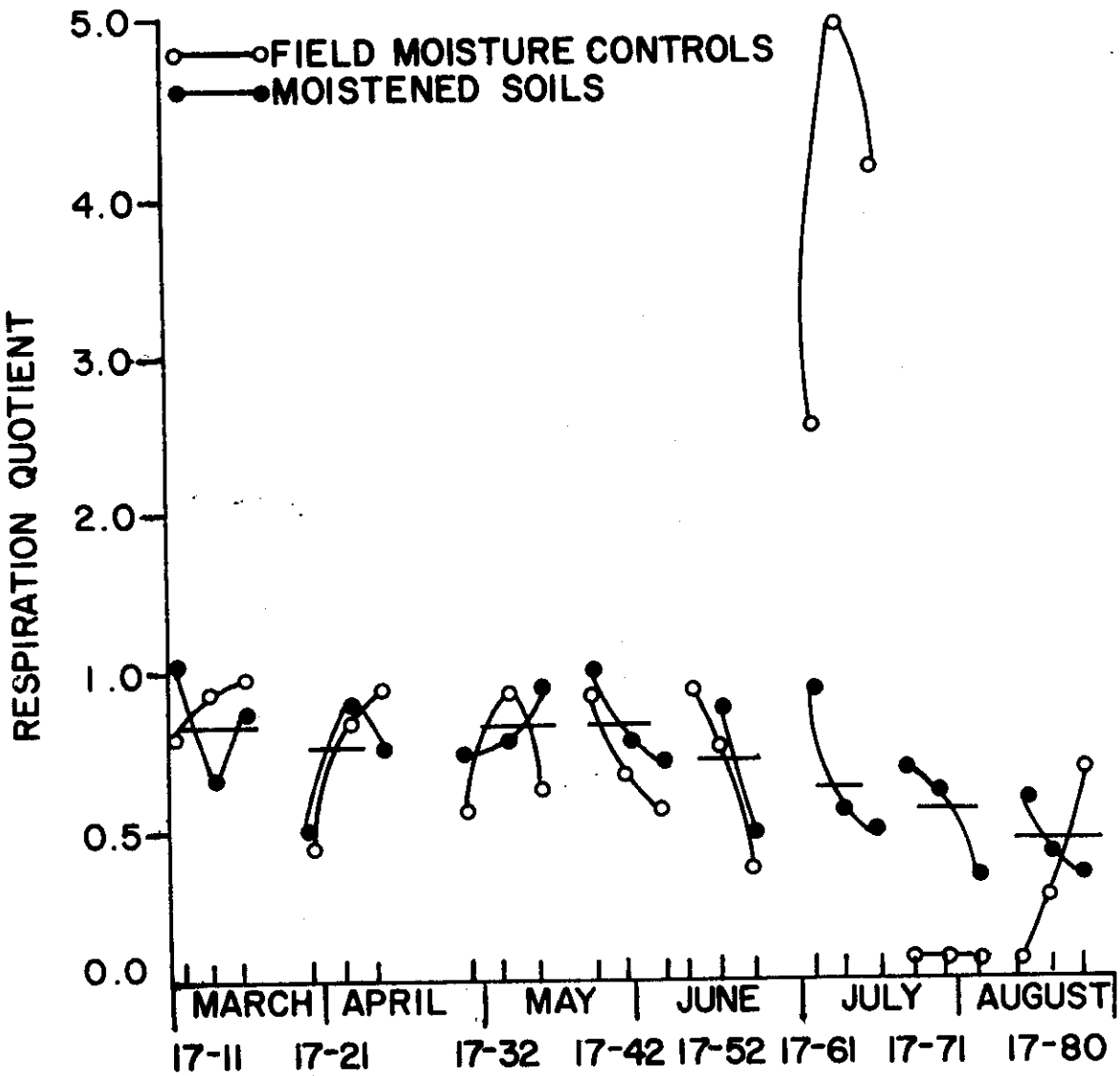

Fig. 13. Seasonal responses of Pawnee soil respiration quotients over 3-week laboratory incubation periods.

Table 23. Root biomass statistics.

Runs	Average Root Biomass (%)	Standard Deviation
17-11	.664	0.155
17-21	.932	0.344
17-32	.890 } .938	
17-42	.992	
17-52	.683	0.183
17-61	.5738	0.221
17-71	.7132	0.387
17-80	.7388	0.188

was observed during the spring period, with a decrease in biomass observed in the May to June period and a gradual increase in biomass observed in the latter part of July, during the period in which lesser rainfall events had occurred.

Changes in root biomass which were observed appear, of themselves, not to reflect the major changes in microbial respiration observed over the active growing season. It would appear that the carbon flow and related photosynthesis fixation rates of plant biomass would provide a more meaningful relationship with the observed changes in microbial activity.

Amoebal Responses to Soil Perturbation

Results of bacterial, actinomycete, fungal, and amoebal response to the varied treatments of Pawnee soil test cores over 1, 2, and 3 weeks of incubation are given in Table 24. The amoebal responses to the soil modifications are also expressed graphically in Fig. 14 which summarizes results from three experiments (Menapace, 1971).

Control Soil Responses

The control soil apparently reached an equilibrium and maintained approximately the same amoebal numbers throughout the 3-week test period.

Glucose Plus Water Soil Responses

At the end of the first week, the glucose plus water treatment had the highest amoebal numbers. Glucose provides a readily available carbon source for the bacteria and possibly for some of the protozoans. Therefore, one would expect an immediate stimulation of activity from the bacteria, followed by the bacterial feeding amoebae. This appears to be the case, as the numbers were highest at this point for the glucose plus water treatment.

Table 24. Comparison of treatment effects on microorganism responses.[a/]

Organism and Treatment	1 Week	2 Weeks	3 Weeks
Amoebae			
Control	4.7×10^3	5.0×10^3	4.9×10^3
Water	6.3×10^3[b/]	8.1×10^3	6.8×10^3
Hay + Water	4.9×10^3	7.6×10^3	8.0×10^3
Glucose + Water	6.8×10^3	5.1×10^3	4.7×10^3
Total Bacteria			
Control	2.7×10^7	2.4×10^7	2.0×10^7
Water	2.8×10^7	2.2×10^7	3.0×10^7
Hay + Water	2.3×10^7	2.5×10^7	3.0×10^7
Glucose + Water	2.8×10^7	2.7×10^7	2.7×10^7
Actinomycetes			
Control	6.5×10^6	5.5×10^6	6.3×10^6
Water	5.4×10^6	5.1×10^6	6.0×10^6
Hay + Water	6.0×10^6	6.2×10^6	5.4×10^6
Glucose + Water	7.1×10^6	6.9×10^6	7.8×10^6
Fungi			
Control	1.9×10^4	5.1×10^4	3.8×10^4
Water	3.8×10^4	9.1×10^4	2.2×10^4
Hay + Water	1.0×10^4	4.4×10^4	3.6×10^4
Glucose + Water	3.3×10^4	5.6×10^4	2.1×10^4

[a/] Zero time amoebal count = 3.2×10^3.

[b/] Expressed on a dry weight basis.

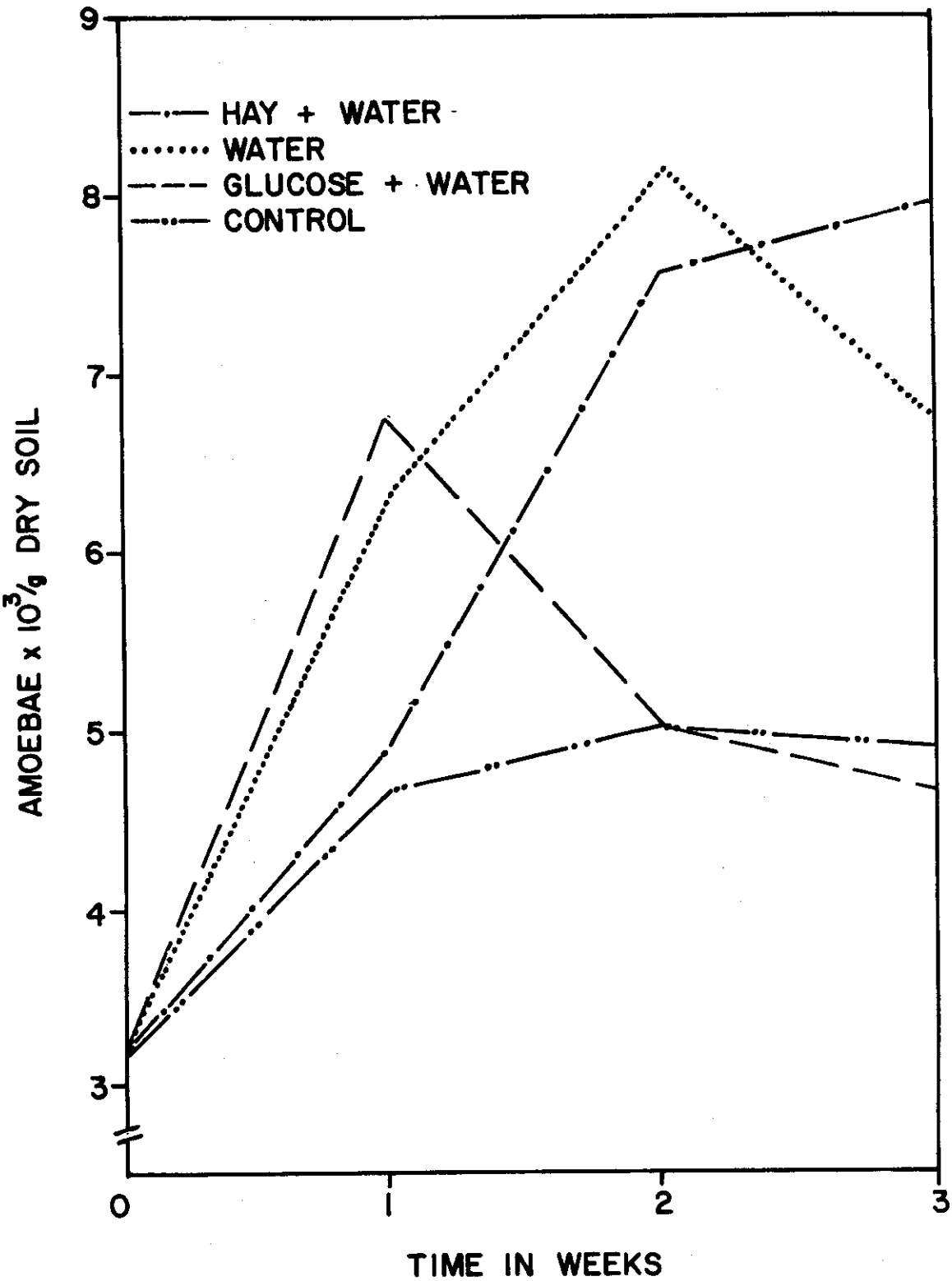

Fig. 14. Amoebal responses to soil modifications.

Due to the rapid initial respiration and metabolism in the glucose plus water core, the available nutrients apparently had been exhausted, and wastes had accumulated by the second week. This is indicated by the large observed decrease in amoebal numbers (from 6.8×10^3 to 5.13×10^3 per gram of soil) in this treatment. Although the bacterial numbers did not markedly decrease (2.82×10^7 to 2.70×10^7 per gram of soil), this does not mean there was not a decrease in the edible bacteria. There could easily have been a shift of the bacterial population from edible to inedible types.

By the third week, the glucose plus water treatment numbers fell below those of controls which may further indicate that the entire system had a decreased microbial activity.

Water Addition Responses

Water itself is very important as a stimulant for amoebal growth. This effect is noticed as the water treatment produced the second highest numbers of amoebae the first week. Addition of water would cause excystment and utilization of the available nutrients. Reproduction would occur followed by encystment as the nutrients were depleted and the water evaporated.

In the second week, the water treatment had the highest numbers. By this time the water stimulation had taken full effect; bacterial numbers had increased and the amoebae had been feeding heavily and increasing rapidly. Since this count includes both active and encysted forms, many of the amoebae in the water treatment were probably encysted at this time. The available nutrients were probably depleted and encystment had possibly taken place.

By the third week the water treatment numbers were decreasing. This is further evidence of the possible depletion of nutrients and accumulation of wastes, thus causing the observed shifts in bacterial populations and amoebal feeding.

Hay Plus Water Soil Responses

Although water was added with the hay, hay does not appear to be a source of readily available nutrients. Therefore, until the nutrients are made more available by decomposition, the bacterial and amoebal growth is somewhat retarded. The number of amoebae was higher for the hay plus water than for the control the first week. However, the water treatment amoebal count was much higher than that of the hay plus water.

From the first to the second week, the amoebal count was observed to change from 4.9×10^3 to 7.6×10^3 per gram of soil in the hay plus water treatment. This seems to indicate that the nutrients from the hay were becoming more available to the amoebae and the bacteria they feed upon.

A two-way analysis of variance was computed to determine if significant differences existed between the various treatment means. The results indicated that (i) the interaction between time and nutrient treatments was not significant, (ii) the time effect was highly significant ($\alpha = 0.05$), and (iii) the nutrient effect was very highly significant ($\alpha = 0.005$). Duncan's multiple range test was performed to detect possible significant differences ($\alpha = 0.05$) between the pairs of means for each week. For the first week, no significant differences between any sets of means were observed. However, for the next week the four pairs (water compared to control, water compared to glucose plus water, hay plus water compared to control, and hay plus water

compared to glucose plus water) showed statistically significant differences. Finally, for the third week interval, the means between two pairs (hay plus water compared to glucose plus water, and hay plus water compared to control) were significantly different. The other pairs examined in the second and third weeks showed no significant statistical differences, as would be anticipated (Snedecor and Cochran, 1968).

Comparison of Amoebal Responses to Total Bacterial Responses

In comparing the amoebal with the total bacterial counts, several observations are of interest. While the control amoebal counts remained essentially constant, the bacterial counts exhibited a downward trend. This could indicate some amoebal grazing, in addition to the normal depletion of nutrients.

The hay plus water treatment maintained a steady upward trend for both bacteria and amoebae. Amoebal grazing is indicated by the rapid increase in numbers the second week and the small increase the third week. Since amoebal grazing is indicated, the increase in bacterial numbers was probably due to the responses of inedible forms.

The water treatment appeared to be an ideal bacterial-amoebal interaction system, i.e., an inverse relationship between bacterial and amoebal counts was observed. This was probably due to the fact that soil water provides an ideal environment for the growth of amoebae. A possible reason for the decrease in amoebal numbers and the increase in the bacterial numbers in the third week with water addition is amoebal grazing during the prior interaction period. By the second week, the amoebae could have consumed most of the edible bacteria.

Comparison of Actinomycete Responses to Amoebal Responses

When comparing the amoebal counts with those of actinomycetes, it appears that the actinomycetes may have been taking advantage of the decrease in the bacterial, fungal, and amoebal populations. All treatments gave a definite upward trend in actinomycete numbers by the third week, except the hay plus water, and the hay plus water treated soil is the only one where the amoebal count increased the third week.

Comparison of Fungal Responses to Amoebal Responses

A fungal and amoebal count comparison indicated that in the glucose plus water and water treatments when the protozoan numbers increased, the fungal count decreased, an inverse relationship.

Estimation of the Number of Amoebae per Gram of Grassland Soil

To estimate the numbers of the two amoebae per gram of grassland soil, a 1-week run was done on cores that had not been subjected to respirometer test conditions. After averaging the results of the replications and correcting for dry weight, the number of amoebae was estimated to be 3.2×10^3 per gram of grassland soil. Singh (1946), using his "ring" method, estimated from 3,600 to 116,440 amoebae per gram of soil, depending upon the soil and the bacterial strain used as the host organism. The observation that the Pawnee soil amoebal population is in the lower range of Singh's estimations would perhaps reflect on the restricted nutrient status, low soil water, and low annual biomass production of the Pawnee grassland.

Enumeration Dynamics

Although the dynamics of amoebal appearance on enumeration plates were not of prime importance, some of the observations should be considered.

Fig. 15 shows the rate of plaque formation by the amoebae on enumeration plates. Plaques rarely appeared before the second day after plating.

It seems more reasonable to assume that the vegetative forms produced the first plaques to appear. The encysted forms produced the later appearing plaques, depending upon the stage of encystment.

With further work, it may be possible to interpret the differences in plaque appearance rate with the ratio of vegetative to encysted forms in any particular soil sample. The growth rate curves generally showed an initial rapid growth followed by a leveling off, indicating a rate decrease. The initial growth could be the vegetative forms followed on days 4 and 5 (Fig. 15) by the encysting cystic forms. To confirm this postulation, HCl techniques or their equivalent should be used to determine the total significance of the two-part curve.

Plaque formation usually was complete in 6 to 8 days for the first and second week test runs, and in 5 to 7 days for the third week analyses. Lower dilution plates usually were completely cleared of *A. aerogenes* by this time. Few, if any, new plaques appeared on the higher dilution plates.

Comparison of Amoebal Responses to Oxygen Uptake and Dehydrogenase Activity

Results for comparison of amoebal responses with oxygen uptake and dehydrogenase activity are presented in Table 25. Soil modification definitely influenced the dyhydrogenase activity. The control showed .0049 mg/ml, and the other treatments were all lower at the end of one week. By the second week all the treatments increased (with the glucose plus water measurement increasing from .0021 to .0051 mg/ml) and the control decreased to .0032 mg/ml, indicating that in the first week possible

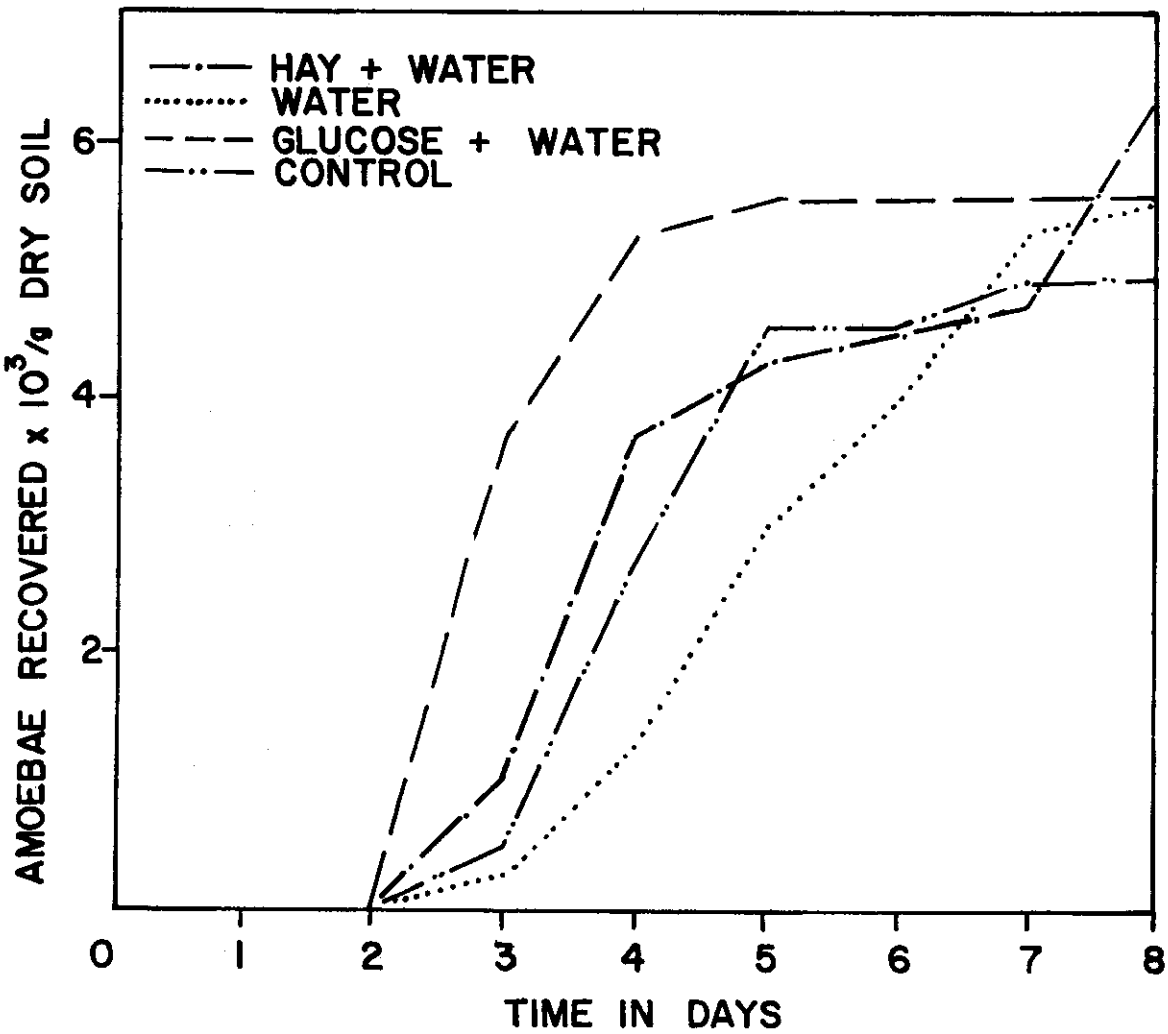

Fig. 15. Amoebal plaque appearance rate on overlay plates.

Table 25. Comparison of amoebal responses to oxygen uptake and dehydrogenase activity.[a/]

Week	Control			Water			Hay + Water			Glucose + Water		
	Amoebae	Dehydrogenase (mg/ml)	Oxygen Uptake (ml)[b/]	Amoebae	Dehydrogenase (mg/ml)	Oxygen Uptake (ml)[b/]	Amoebae	Dehydrogenase (mg/ml)	Oxygen Uptake (ml)[b/]	Amoebae	Dehydrogenase (mg/ml)	Oxygen Uptake (ml)[b/]
1	4.7×10^3	.0049	16	6.3×10^3	.0018	28	4.9×10^3	.0032	68[c/]	6.8×10^3	.0021	32[c/]
2	5.0×10^3	.0032	32	8.1×10^3	.0027	55	7.6×10^3	.0039	127[c/]	5.1×10^3	.0051	65[c/]
3	4.9×10^3	.0046	44	6.8×10^3	.0031	84	8.0×10^3	.0040	145[c/]	4.7×10^3	.0031	92[c/]

[a/] Zero time amoebal counts = 3.2×10^3/g dry soil.

[b/] Measured cumulatively 0-7, 7-14, and 14-21 days, respectively.

[c/] Hay is present in these test systems at 0.3 g compared with 20-mg glucose addition, thus possibly explaining the increased O_2 uptake in the hay plus water treatment.

repression of decomposer functions was being overcome. However, by the third week the control was at .0046 mg/ml, with all treatments lower than the control but higher than their first week readings.

Oxygen usage reflects the effects of water and carbon source additions on microbial activities, with the oxygen uptake observed related to water addition. There was increased response to the addition of 0.3 g of hay and 20 mg of glucose. Thus, oxygen uptake could possibly be considered as an index which would correlate with possible amoebal responses. It can be assumed that the small soil amoebae that were studied contributed only fractionally to the respiration of the system. A system of soil amoebae alone or the entire system minus the amoebae would have to be developed to determine the particular amoebal respiration in this environment.

Soil Water Effects on Amoebal Numbers and Activity

The effect of soil water levels on amoebal numbers was tested using 5, 10, 15, and 20% soil water to determine which levels would allow maximal amoebal recovery (Fig. 16). In the first week 5, 15, and 20% water levels gave high counts with 20% soil water giving 9.3×10^3. In the second week the 5% soil water addition gave best results. The 5% soil water level also gave the best counts on the third week test run, with the 15% soil water level giving the lowest count.

Amoebal Identification

The identification of the two amoebal forms recovered as being predominant in the test soil was based on microscopic characteristics, with the genus determined by the cystic and trophic form characteristics. The species designation was tentatively established by the size of the cyst and

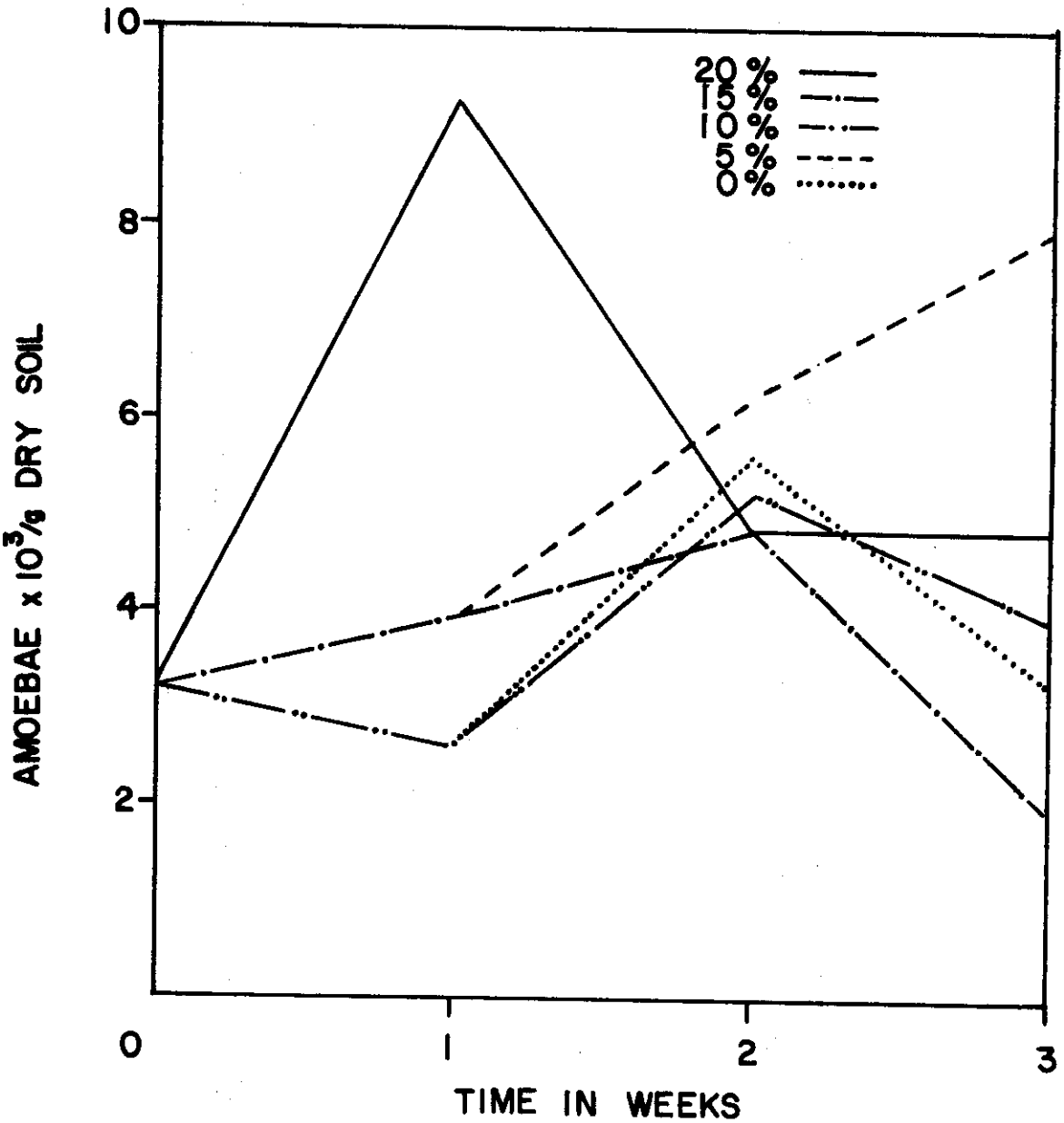

Fig. 16. Soil water effects on amoebal numbers in soil.

trophic forms. Identification of these organisms on a species level should be considered tentative, as chemical, excysting, and nuclear division tests have not been carried out.

Cultures 15-32h and 15-32i were tentatively identified as *Hartmannella exundans* (Page, 1967*a*). The cyst was smooth, ovoid to circular, and had a single wall made up of well-defined outer and inner layers. The single nucleus, usually difficult to discern, had an average diameter of 4.2 µm. The trophic organism was monopodial with a rounded to tapering posterior end. The posterior end appeared to be sticky with adherent debris but had no uroidal filaments. There was a single vesiculate nucleus with a nocleolus that appeared as a dark granule. The length of the trophic organism averaged 11.5 µm. These amoebae produced a very distinct plaque with uneven edges. Approximately 75% of the plaques recovered were of this type. The amoebae also produced a plaque with even edges. However, these amoebal plaques tended to appear as an indentation in the agar which made it possible to also tentatively identify these as *Hartmannella* despite their variation from the characteristic plaque formation observed for this species.

Cultures 15-31b and 15-31g were tentatively identified as *Acanthamoeba polyphaga* Puschkarew as redefined by Page (1967*a*). The cyst consisted of an ectocyst and an endocyst. The ectocyst had folds and ripples and was rarely circular. The endocyst was irregularly polyhedral, rarely approaching stellate. There was a single nucleus with an average diameter of 3.3 µm and a single layer of granules at the periphery of the cytoplasm. The average diameter of the cyst was 16 µm with a very mammillated appearance. The trophic organism had a broad hyaline pseudopod and acanthopodia. The posterior end was rounded and sticky with adherent bacteria; no uroidal filaments were observed. These amoebae produced plaques which were fairly

faint with an even outline. The plaque outline may also be uneven, and the plaques are not as indented as those of the *Hartmannella*.

Amoebal Feeding Characteristics

Bacterial feeding studies were carried out using the amoebal isolates derived from the Pawnee grassland samples, and the results are summarized in Tables 26 through 29. The potential bacterial hosts used in this portion of the study were selected to give a range of morphology, Gram reaction, and pigmentation which would allow evaluation of possible effects of these bacterial properties on availability as amoebal food sources. Times to complete feeding (in days) on the bacterial isolates are summarized in Table 26.

The *Acanthamoeba* were wide-range feeders. Although some bacteria were more readily eaten than others, all the known cultures were eventually consumed. On the other hand, the *Hartmannella* were more selective feeders. *Staphylococcus aureus*, *Streptomyces albus*, *Pseudomonas flourescens*, *Neisseria perflava*, *Bacillus subtilis*, *Bacillus cereus*, and *Micrococcus luteus* were not observed to be consumed by the *Hartmannella*. *Sarcina lutea* was partially consumed at the end of 7 days, and the other bacteria were fully consumed. The *Hartmannella* were also slower in consuming edible bacteria than were the *Acanthamoeba*.

Gram Reaction Effects on Amoebal Feeding Patterns

Although both the Gram-positive and Gram-negative bacteria were consumed, the Gram-negative isolates were consumed faster (on the average) than the Gram-positive bacteria (Table 27). Coler and Gunner (1969), using *Colopoda*, found a definite difference in the consumption of Gram-negative

Table 26. Amoebal feeding on bacterial isolates.

Bacteria	*Acanthamoeba*		*Hartmannella*	
	Culture Number		Culture Number	
	15-31b	15-31g	15-32h	15-32i
Agrobacterium tumefaciens	4[a]	4	6	5
Arthrobacter globiformis	2	2	7	7[b]
Bacillus cereus	3	4	--[c]	--
Bacillus subtilis	4	4	--	--
Erwinia carotovora	2	5	7	5
Escherichia coli	4	6	4	5
Micrococcus luteus	2	2	--	--
Neisseria perflava	5	4	--	--
Proteus vulgaris	4	4	6	5
Pseudomonas aeruginosa	3	3	3	3
Pseudomonas fluorescens	4	4	--	--
Sarcina lutea	7	7	7	7
Serratia marcescens	3	4	7	6
Staphylococcus aureus	4	4	--	--
Streptomyces albus	2	3	--	--
Streptomyces lactis	3	3	4	3

[a] Time in days needed to complete 5.5-cm bacterial streak.

[b] Feeding begun but not completed.

[c] No feeding observed.

Table 27. Effect of bacterial Gram reaction on amoebal feeding patterns.

Bacteria	*Acanthamoeba*		*Hartmannella*	
	Culture Number		Culture Number	
	15-31b	15-31g	15-32h	15-32i
	Gram-negative			
Agrobacterium tumefaciens	4[a]	4	6	5
Escherichia coli	4	6	4	5
Erwinia carotovora	2	5	7	5
Neisseria perflava	5	4	--[b]	--
Proteus vulgaris	4	4	6	5
Pseudomonas aeruginosa	3	3	3	3
Pseudomonas fluorescens	4	4	--	--
Serratia marcescens	3	4	7	6
Streptomyces albus	2	3	--	--
Streptomyces lactis	3	3	4	3
	Gram-positive			
Bacillus cereus	3	4	--	--
Bacillus subtilis	4	4	--	--
Micrococcus luteus	2	2	--	--
Sarcina lutea	7	7	7[c]	7
Staphylococcus aureus	4	4	--	--
	Gram-variable			
Arthrobacter globiformis	2	2	7	7[c]

[a] Time in days needed to complete a 5.5-cm streak of bacteria.

[b] No feeding observed.

[c] Feeding begun but not completed.

bacteria (preferred) and Gram-positive (not as readily eaten). On the other hand, Singh (1941) found little or no differences in the feeding on Gram-negative and Gram-positive bacteria in his amoebal feeding studies.

Laboratory Culture Pigmentation Effects on Amoebal Feeding Patterns

On the average, the non-pigmented laboratory bacteria were consumed faster than the pigmented types (Table 28). However, the *Hartmannella* species in comparison with the *Acanthamoeba* were more selective in both groups, consuming some and not others. The two bacteria with the most pigment (*Sarcina lutea* and *Serratia marcescens*) were consumed by all the amoebae. Therefore, the type of pigment may be the limiting factor in amoebal utilization with these particular isolates, and not pigmentation itself.

Amoebal Feeding on Bacteria Recovered from Grassland Soil

When compared to the *Acanthamoeba*, *Hartmannella* species were the slower feeders in utilization of bacteria recovered from grassland soils (Table 29). Except for culture D, all bacterial cultures were partially or completely consumed. This would indicate that the soil bacteria, based on the isolates examined, do indeed serve as a better food source for these amoebae than the previously examined laboratory isolates. All the grassland isolates, other than culture I were Gram-negative rods.

Pigmentation Effects on Soil Bacteria Utilization by Soil Amoebae

It would appear that the pigmented bacteria were preferred over the non-pigmented bacteria in this case (Table 29). Culture D was non-pigmented, and no amoebal utilization was observed. Non-pigmented bacterial consumption was slower, with some not completely consumed within the 7 days. All the

Table 28. Effect of bacterial pigmentation on amoebal feeding patterns.

Bacteria	*Acanthamoeba*		*Hartmannella*	
	Culture Number		Culture Number	
	15-31b	15-31g	15-32h	15-32i
	Pigmented			
Erwinia carotovora	2[a]	5	7	5
Pseudomonas aeruginosa	3	3	3	3
Pseudomonas fluorescens	4	4	--[b]	--
Sarcina lutea	7	7	7[c]	7
Serratia marcescens	3	4	7	6
Staphylococcus aureus	4	4	--	--
	Non-pigmented			
Agrobacterium tumefaciens	4	4	6	5
Arthrobacter globiformis	2	2	7	7[c]
Bacillus cereus	3	4	--	--
Bacillus subtilis	4	4	--	--
Escherichia coli	4	6	4	5
Micrococcus luteus	2	2	--	--
Neisseria perflava	5	4	--	--
Proteus vulgaris	4	4	6	5
Streptomyces albus	2	3	--	--
Streptomyces lactis	3	3	4	3

[a] Time in days needed to complete a 5.5-cm streak of bacteria.

[b] No feeding observed.

[c] Feeding begun but not completed.

Table 29. Effect of bacterial pigmentation on soil bacteria utilization by soil amoebae.

Culture	*Acanthamoeba*		*Hartmannella*	
	Culture Number		Culture Number	
	15-31b	15-31g	15-32h	15-32i
	Pigmented			
A	2[a]	3	6	6
B	2	3	7	7
C	2	2	4	4
E	2	3	5	5
G	4	4	5	5
	Non-pigmented			
D	[b]	--	--	--
F	3	3	5	7[c]
H	7[c]	7[c]	7[c]	7[c]
I	3	3	5	6
J	3	3	5	7[c]

[a] Time in days to complete a 5.5-cm streak of bacteria.

[b] No feeding observed.

[c] Feeding begun but not completed.

pigmented bacteria were completely consumed in this time period. This is of interest as pigmentation is generally considered inhibitory to heavy amoebal feeding. This observation would indicate that the use of laboratory cultures in amoebal feeding studies should be questioned.

Soil Responses to Plant Leachate Additions

Plant leachates have been found to cause significant shifts in microbial populations and carbon flow characteristics of Pawnee grassland soils (Seaman, 1971). The sequential leaching of blue grama hay resulted in the successive removal of decreasing amounts of materials. These results would indicate that even a 15-min precipitation event (heavy rain) can remove substantial amounts of material from a dry straw. With the test hay used, approximately 15% of the original dry weight could be removed after removal of the five leachate fractions.

Oxygen Plate and Carbon Dioxide Evolution Responses

Table 30 shows the amounts of carbon dioxide evolved from a Pawnee soil at temperatures of 15, 25, and 37°C, with addition of equal volumes of fractions 1 through 5 or of water as a control. The final soil water level approached 20% in these experiments.

At 15°C it is possible to detect an approximately linear response of the soil to the decreasing amounts of carbon in the successive fractions. The stimulation of adding a control level of soil water to the cores was found to be minimal. With incubation of the soils at 25°C, it was now possible to note that additions of any carbon levels up to 45 mg, as contained in fraction 2, could not be clearly observed over the response of fraction 5 which only contained 18 mg of carbon. This result would indicate that the

Table 30. Effects of leachate fraction and soil incubation temperature on carbon dioxide evolution.

Treatment	Carbon (mg)	CO_2 Evolved (mg)		
		15°C	25°C	37°C
F-1	70.8	103.5	143.8	211.5
F-2	45.2	62.5	115.0	202.5
F-3	22.4	51.2	95.0	200.5
F-4	23.4	49.2	99.8	215.0
F-5	18.0	49.0	107.8	216.5
Water		37.5	66.2	184.0
Dry		34.5	67.8	106.0

carbon added is causing a priming effect or stimulation of microbial populations and/or activity, which then allows utilization of native organic matter.

Finally, at 37°C a priming effect is still observed; however, at this temperature level the carbon dioxide evolution responses are similar regardless of the size or nature of the carbon pulse used.

Results from a second run carried out using the same protocol of varied leachate fractions and temperatures, with oxygen uptake and carbon dioxide evolution expressed in milliliters to allow calculation of the respiratory quotient (RQ), are given in Table 31. Generally, the RQ values of carbon dioxide evolution over oxygen uptake were found to increase with temperature, independent of the size or nature of the carbon pulse. This result was not shown clearly on the water treatment controls, however, or in soils using varied leachate fractions and soil water levels (Table 32). The shift in RQ value with temperature would indicate that the soil microbes would be shifting the pattern of metabolic pathways used for carbon flow in relation to temperature.

Responses of Total Plate Counts

At the end of each respiration experiment, the cores were diluted and plated onto sodium caseinate agar (Table 33). The 5-day total plate counts showed variation with the amount of carbon added, the soil water concentration, and the temperature at which the cores had been incubated in the respiration experiment.

Generally, the cores to which leachate fraction 1 was added had higher total plate counts than those to which fraction 2 through 4 or water were added.

Table 31. Temperature and leachate fraction effects on the Pawnee soil respiratory quotient.

	Treatment	CO_2 (ml)	O_2 (ml)	RQ	RQ Average
15°C	F-1	56.5	79.0	0.72	
	F-2	40.6	62.8	0.65	
	F-3	34.1	64.7	0.53	
	F-4	31.3	50.0	0.63	x = 0.70
	F-5	30.1	34.5	0.87	
	Water	21.5	25.5	0.84	
	Dry	14.0	0.0	--	
25°C	F-1	79.8	81.9	0.97	
	F-2	55.5	63.3	0.88	
	F-3	30.4	35.4	0.86	
	F-4	34.8	44.8	0.78	x = 0.73
	F-5	52.5	71.3	0.73	
	Water	61.5	54.4	1.13	
	Dry	22.8	28.7	0.80	
37°C	F-1	108.8	116.3	0.94	
	F-2	109.9	122.6	0.89	
	F-3	95.0	128.4	0.74	
	F-4	123.8	97.9	1.26	x = 0.77
	F-5	124.9	149.7	0.84	
	Water	122.4	119.6	1.03	
	Dry	52.2	71.4	0.73	

Table 32. Effects of varied leachate fractions and soil water levels on Pawnee soil respiratory quotients at 25°C.

	Treatment (%)	CO_2 (ml)	O_2 (ml)	RQ
F1	5	31.3	47.2	0.66
	10	47.6	74.0	0.64
	20	52.4	87.1	0.60
	30	59.8	101.4	0.59
F3	5	36.4	53.1	0.68
	10	44.0	73.0	0.60
	20	45.4	73.9	0.61
	30	37.4	49.4	0.76
F5	5	22.4	40.7	0.55
	10	39.8	63.3	0.63
	20	47.6	86.5	0.55
	30	47.6	87.8	0.54
Water	5	28.1	50.8	0.55
	10	28.1	52.9	0.53
	20	43.1	75.3	0.57
	30	37.0	64.5	0.57
	Dry	0	5.0	--

Table 33. Effects of leachate fraction and temperature on microbial populations in a Pawnee grassland soil.

		Plate Counts/g Dry Soil		
	Treatment	Total ($\times 10^7$)	Actino ($\times 10^6$)	Fungi ($\times 10^4$)
15°C	F-1	3.60	2.99	3.41
	F-2	2.13	4.53	2.98
	F-3	1.89	3.32	3.22
	F-4	1.59	3.66	1.98
	F-5	2.13	3.71	3.95
	Water	1.46	3.00	1.99
	Dry	0.94	2.79	2.67
25°C	F-1	2.35	2.19	2.88
	F-2	2.25	4.51	4.82
	F-3	1.76	3.57	2.47
	F-4	2.53	4.23	2.60
	F-5	1.67	4.33	5.33
	Water	1.80	3.43	2.87
	Dry	1.18	2.89	1.10
37°C	F-1	1.15	2.43	8.58
	F-2	0.83	1.61	8.71
	F-3	0.98	1.69	1.76
	F-4	1.30	1.90	2.05
	F-5	0.77	1.92	1.34
	Water	0.58	1.34	1.46
	Dry	0.64	1.78	6.65

The effect of temperature on the total plate counts was marked. All plates were incubated at 25°C, but the cores were incubated at 15, 25, and 37°C before plating. It was consistently shown that the total counts for the 15°C cores were higher than those for the 25°C or the 37°C cores. This observation may be related to the mean soil temperatures in the Pawnee grassland area.

Responses of Actinomycete Counts

The actinomycete counts showed no consistent variation with temperature, carbon pulse, or soil water concentration.

Responses of Fungal Counts

Fungal counts were not dependent on temperature or carbon pulse, but the counts of the dry cores were usually lower than those of moist cores.

Responses of Counts to Soil Water Variation

No consistent trends were seen in the effect of soil water on any microbial counts.

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APPENDIX I

SOIL CHEMICAL ANALYSIS RESULTS FOR RUNS

17-11, 17-21, 17-71, and 17-80

Appendix Table 1. Soil chemical analysis results for runs 7-11, 17-21, 17-71, and 17-80.

Lab No.	Run No.	Organic Matter (%)	NO_3-N (ppm)	P (ppm)	K (ppm)	Zn (ppm)	Fe (ppm)	Total N (%)
596	17-11-1[a/]	1.5	1.6	12.5	213	0.67	14.2	0.07
597	2	2.4	5.5	19.0	249	0.60	26.0	0.08
598	3	1.7	16.0	16.3	182	0.45	20.8	0.10
599	4	1.3	18.5	15.8	187	0.47	19.8	0.08
600	5	1.5	14.0	16.8	233	0.53	26.7	0.08[d/] 0.09
601	6	1.7	18.8	17.5	223	0.59	24.5	0.08
602	7	2.1	26.3	19.8	244	1.38	38.6	0.12
603	8	1.7	14.5	17.0	223	0.75	26.0	0.09
604	9	1.3	10.5	15.0	182	0.63	33.6	0.08
605	0	1.2	6.0	15.5	203	0.54	17.9	0.08
606	17-71-1[b/]	1.7	1.0	14.0	187	1.13	20.7	0.09
607	2	1.7	5.0	13.8	244	1.25	21.5	0.11
608	3	1.5	2.6	14.3	238	1.06	20.7	0.09
609	4	1.4	1.6	17.0	233	0.32	20.0	0.09
610	5	1.5	1.0	15.0	288	0.57	21.2	0.09
611	6	1.2	0.8	12.0	223	0.26	23.5	0.07
612	7	1.4	3.3	12.5	268	0.75	22.4	0.09
613	8	1.9	3.3	15.0	228	0.76	22.8	0.10
614	9	1.8	0.8	15.5	253	1.19	28.6	0.11
615	0	1.9	1.4	18.3	312	1.81	41.25	0.12
616	17-21-1[c/]	1.9	5.4	13.0	213	2.32	34.1	0.10
617	2	2.1	7.5	18.8	203	1.19	39.6	0.12
618	3	1.2	6.0	17.0	208	0.76	24.8	0.07
619	4	1.7	8.3	19.8	231	0.75	26.9	0.10
620	5	1.6	11.8	20.8	253	1.11	45.5	0.10[d/] 0.11
621	6	1.5	3.3	17.5	244	0.96	31.3	0.10
622	7	1.7	5.4	23.0	211	1.15	34.7	0.10
623	8	2.0	5.4	22.3	223	1.24	44.0	0.12
624	9	1.4	6.8	18.3	182	0.46	29.7	0.08
625	0	1.2	2.6	13.0	344	0.48	21.2	0.07

Appendix Table 1 (continued).

Lab No.	Run No.	Organic Matter (%)	NO_3-N (ppm)	P (ppm)	K (ppm)	Zn (ppm)	Fe (ppm)	Total N (%)
626	17-80-1[e/]	1.3	0.8	14.5	203	0.69	27.1	0.08
627	2	1.4	8.6	17.5	197	0.87	33.6	0.09
628	3	1.7	1.0	20.3	197	0.94	30.4	0.09
629	4	1.1	0.8	17.0	167	0.40	25.2	0.08
630	5	1.3	1.0	17.0	192	1.07	13.9	0.10 0.10[f/]
631	6	1.7	0.8	28.3	218	1.46	17.9	0.13
632	7	1.7	39.6	30.0	203	0.62	53.5	0.09
633	8	1.5	+50.0	23.0	274	1.20	30.3	0.10
634	9	1.2	33.6	15.3	203	0.47	17.7	0.09
635	0	1.3	31.8	17.0	253	0.79	15.6	0.09

[a/] See Tables 2 and 3 for biological data.

[b/] See Tables 19 and 20 for biological data.

[c/] See Tables 4 and 5 for biological data.

[d/] Duplicate sample.

[e/] See Tables 21 and 22 for biological data.

[f/] Duplicate data set.

APPENDIX II

FIELD DATA

Soil chemical data collected at the Pawnee site were recorded on forms NREL-46, NREL-47, and NREL-48. These data are stored as Grassland Biome data set A2U405B. Sample data forms and examples of the data follow. Header cards explaining the treatment codes preceed each data set.

IBP

GRASSLAND BIOME

U.S. INTERNATIONAL BIOLOGICAL PROGRAM

LAB DATA SHEET--MICROBIOLOGY - PLATE COUNTS

Data Type	Initials	Starting Date			Location	Run Number	Vessel Number	Dry Weight of Core	Percent Moisture in Core	Treatment 1	Moisture	Treatment 2	Week	pH	Dilution	Total Count NaC	Dilution	Actino NaC	Dilution	Fungi RB
		Day	Month	Year																
1-2	3-5	6-7	8-9	10-11	12	13-17	19-20	22-25	26-27	28	29	30	31	32-34	35-40	41-44	45-50	51-54	55-60	61-64
46																				

Treatment 1 (19-29) (31-32) (34-80)

1
2
3
4
5
6
7

Treatment 2

1
2
3
4

Location

1 Lab
2 Field

Moisture

1 Dry
2 Moist

+++ EXAMPLE OF DATA +++

```
         1         2         3         4         5         6         7
1234567890123456789012345678901234567890123456789012345678901234567890


46 PM310371117-21 TREATMENT 1 01 NO WATER
46 PM310371117-21 TREATMENT 1 02 5 ML
46 PM310371117-21 TREATMENT 1 03 10 ML
46 PM310371117-21 TREATMENT 1 04 15 ML
46 PM310371117-21 TREATMENT 1 05 20 ML
46 PM310371117-21 TREATMENT 2 01 NO HAY
46 PM310371117-21 TREATMENT 2 02 PLUS HAY
46 PM310371117-21 01 274206101167 51.0E-5 1821.0E-5  621.0E-3  13
46 PM310371117-21 01 274206101167 51.0E-5 1741.0E-5  631.0E-3  16
46 PM310371117-21 01 274206101167 51.0E-5    1.0E-5    1.0E-3  16
46 PM310371117-21 02 278907201164 01.0E-5 1531.0E-5  541.0E-3  16
46 PM310371117-21 02 278907201164 01.0E-5 1341.0E-5  541.0E-3  22
46 PM310371117-21 02 278907201164 01.0E-5 1081.0E-5  411.0E-3   3
46 PM310371117-21 03 280308301165 51.0E-5 1431.0E-5  451.0E-3  17
46 PM310371117-21 03 280308301165 51.0E-5 1261.0E-5  501.0E-3  13
46 PM310371117-21 03 280308301165 51.0E-5 1161.0E-5  441.0E-3  25
46 PM310371117-21 04 286010401172 51.0E-5 1401.0E-5  551.0E-3   7
46 PM310371117-21 04 286010401172 51.0E-5 1671.0E-5  371.0E-3  16
46 PM310371117-21 04 286010401172 51.0E-5 1481.0E-5  461.0E-3   7
46 PM310371117-21 05 258412501185 51.0E-5 4801.0E-5  311.0E-3  42
46 PM310371117-21 05 258412501185 51.0E-5 5461.0E-5  321.0E-3  37
46 PM310371117-21 05 258412501185 51.0E-5 5351.0E-5  341.0E-3  36
46 PM310371117-21 06 253206102164 51.0E-5 1691.0E-5  371.0E-4  28
46 PM310371117-21 06 253206102164 51.0E-5 1871.0E-5  271.0E-4  34
46 PM310371117-21 06 253206102164 51.0E-5 1881.0E-5  361.0E-4  28
46 PM310371117-21 07 276512202163 01.0E-5 2481.0E-5  701.0E-3  83
46 PM310371117-21 07 276512202163 01.0E-5 1861.0E-5  511.0E-3  62
46 PM310371117-21 07 276512202163 01.0E-5 2471.0E-5  711.0E-3   0
46 PM310371117-21 08 253410302167 01.0E-5 2511.0E-5  631.0E-3  22
46 PM310371117-21 08 253410302167 01.0E-5 2061.0E-5  671.0E-3  28
46 PM310371117-21 08 253410302167 01.0E-5 2091.0E-5  661.0E-3   0
46 PM310371117-21 09 268811402170 01.0E-5 2081.0E-5  671.0E-4  31
46 PM310371117-21 09 268811402170 01.0E-5 2881.0E-5  971.0E-4  15
46 PM310371117-21 09 268811402170 01.0E-5 2881.0E-5  741.0E-4  10
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46 PM310371117-21 10 281609502169 51.0E-5 2361.0E-5  601.0E-4  31
46 PM310371117-21 10 281609502169 51.0E-5 2081.0E-5  451.0E-4  22
46 PM310371117-21 11 250005101266 51.0E-5 2051.0E-5  691.0E-3  47
46 PM310371117-21 11 250005101266 51.0E-5 2221.0E-5  801.0E-3  49
46 PM310371117-21 11 250005101266 51.0E-5 2541.0E-5 1081.0E-3  47
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46 PM310371117-21 12 275707201269 01.0E-5 1861.0E-5  751.0E-3  26
46 PM310371117-21 12 275707201269 01.0E-5 1951.0E-5  731.0E-3  11
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46 PM310371117-21 13 26120830126351.0E-5 1101.0E-5  471.0E-4  28
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46 PM310371117-21 14 28010940126751.0E-5 1821.0E-5  541.0E-3  62
46 PM310371117-21 15 26741550126751.0E-5 3931.0E-5  611.0E-3  74
46 PM310371117-21 15 26741550126751.0E-5 2911.0E-5  541.0E-3  74
46 PM310371117-21 15 26741550126751.0E-5 2931.0E-5  601.0E-3  84
46 PM310371117-21 16 26650210227101.0E-510341.0E-5 2021.0E-3  32
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46 PM310371117-21 17 27390620226901.0E-5 2011.0E-5  601.0E-3  46
46 PM310371117-21 17 27390620226901.0E-5 2051.0E-5  451.0E-3  32
46 PM310371117-21 17 27390620226901.0E-5 1851.0E-5  581.0E-3  38
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46 PM310371117-21 18 27660730226901.0E-5 1871.0E-5  341.0E-3  57
46 PM310371117-21 18 27660730226901.0E-5 1891.0E-5  511.0E-3  54
46 PM310371117-21 19 27580940227001.0E-5 1261.0E-5  461.0E-3  24
46 PM310371117-21 19 27580940227001.0E-5 1541.0E-5  461.0E-3  36
46 PM310371117-21 19 27580940227001.0E-5 1781.0E-5  411.0E-3  37
46 PM310371117-21 20 24241250226501.0E-5 1621.0E-5  191.0E-4  13
46 PM310371117-21 20 24241250226501.0E-5 1571.0E-5  261.0E-4  17
46 PM310371117-21 20 24241250226501.0E-5 2111.0E-5  371.0E-4  12
46 PM310371117-21 21 25890210136351.0E-5 1151.0E-5  461.0E-3  24
46 PM310371117-21 21 25890210136351.0E-5 1061.0E-5  421.0E-3  33
46 PM310371117-21 21 25890210136351.0E-5 1201.0E-5  431.0E-3  36
46 PM310371117-21 22 26520520136851.0E-5 1681.0E-5  631.0E-4  21
46 PM310371117-21 22 26520520136851.0E-5 1871.0E-5  771.0E-4  23
46 PM310371117-21 22 26520520136851.0E-5 2271.0E-5  861.0E-4  28
46 PM310371117-21 23 26430530136901.0E-5 2031.0E-5  621.0E-3  23
46 PM310371117-21 23 26430530136901.0E-5 2021.0E-5  471.0E-3  22
46 PM310371117-21 23 26430530136901.0E-5 1881.0E-5  531.0E-3  31
46 PM310371117-21 24 26670840136851.0E-5  341.0E-5  221.0E-3  53
46 PM310371117-21 24 26670840136851.0E-5  401.0E-5  161.0E-3  32
46 PM310371117-21 24 26670840136851.0E-5  771.0E-5  311.0E-3  40
46 PM310371117-21 25 27180950136901.0E-5 1341.0E-5  411.0E-3  79
46 PM310371117-21 25 27180950136901.0E-5  901.0E-5  301.0E-3  60
46 PM310371117-21 25 27180950136901.0E-5  901.0E-5  351.0E-3  73
46 PM310371117-21 26 24920210237001.0E-5 1841.0E-5  381.0E-4  13
46 PM310371117-21 26 24920210237001.0E-5 2021.0E-5  451.0E-4  26
46 PM310371117-21 26 24920210237001.0E-5 2031.0E-5  541.0E-4  16
46 PM310371117-21 27 25660220236851.0E-5 2071.0E-5  691.0E-4  24
46 PM310371117-21 27 25660220236851.0E-5 1491.0E-5  501.0E-4  26
46 PM310371117-21 27 25660220236851.0E-5 1601.0E-5  371.0E-4  28
46 PM310371117-21 28 26290530237001.0E-5 3981.0E-5  711.0E-4  29
46 PM310371117-21 28 26290530237001.0E-5 4111.0E-5  841.0E-4  25
46 PM310371117-21 28 26290530237001.0E-5 3991.0E-5  771.0E-4  22
46 PM310371117-21 29 25060640236851.0E-5 1851.0E-5  601.0E-3  32
46 PM310371117-21 29 25060640236851.0E-5 1821.0E-5  521.0E-3  24
46 PM310371117-21 29 25060640236851.0E-5 1841.0E-5  451.0E-3  39
46 PM310371117-21 30 23890850237551.0E-5  651.0E-5  361.0E-3  25
46 PM310371117-21 30 23890850237551.0E-5  671.0E-5  261.0E-3  27
46 PM310371117-21 30 23890850237551.0E-5 1251.0E-5  511.0E-3  30
```

IBP

GRASSLAND BIOME

U.S. INTERNATIONAL BIOLOGICAL PROGRAM

LAB DATA SHEET--MICROBIOLOGY - CO_2, FORMAZAN, MPN

Data Type	Initials	Starting Date			Location	Run Number	Vessel Number	Dry Weight of Core	Percent Moisture in Core	Treatment 1	Moisture	Treatment 2	Week	Total CO_2 Evolved	Total Formazan	MPN Nitrifiers
		Day	Month	Year												
1-2	3-5	6-7	8-9	10-11	12	13-17	19-20	22-25	26-27	28	29	30	31	32-36	37-40	41-45
47																

Treatment 1 (19-29) (31-32) (34-80)

1
2
3
4
5
6
7

Treatment 2

1
2
3
4
5

Location

1 Lab
2 Field

Moisture

1 Dry
2 Moist

+++ EXAMPLE OF DATA +++

```
         1         2         3         4         5
12345678901234567890123456789012345678901234567890

47 PM310371117-21 TREATMENT 1 01 NO WATER
47 PM310371117-21 TREATMENT 1 02 5 ML
47 PM310371117-21 TREATMENT 1 03 10 ML
47 PM310371117-21 TREATMENT 1 04 15 ML
47 PM310371117-21 TREATMENT 1 05 20 ML
47 PM310371117-21 TREATMENT 2 01 NO HAY
47 PM310371117-21 TREATMENT 2 02 PLUS HAY
47 PM310371117-21  1 2742 61011 30380051  130
47 PM310371117-21  2 2789 72011 38900037  350
47 PM310371117-21  3 2803 83011 33030030   70
47 PM310371117-21  4 2860104011    00045   46
47 PM310371117-21  5 2584125011 47710031  240
47 PM310371117-21  6 2532 61021 62390031  540
47 PM310371117-21  7 27651220211123000415400
47 PM310371117-21  8 2534103021 79270033 9200
47 PM310371117-21  9 2688114021    00024 1700
47 PM310371117-21 10 2816 95021108630029    9
47 PM310371117-21 11 2500 51012 80740002 2800
47 PM310371117-21 12 2757 72012108630006  130
47 PM310371117-21 13 2612 83012154140003   33
47 PM310371117-21 14 2801 94012102760021   79
47 PM310371117-21 15 267415501215047001 9200
47 PM310371117-21 16 2665 21022163680017  130
47 PM310371117-21 17 2739 62022215060002 5400
47 PM310371117-21 18 2766 73022182030004  350
47 PM310371117-21 19 2758 94022209920034 1100
47 PM310371117-21 20 2424125022220200018   23
47 PM310371117-21 21 2589 21013 86610036   23
47 PM310371117-21 22 2652 52013132120040  230
47 PM310371117-21 23 2643 53013128350042  790
47 PM310371117-21 24 2667 84013157810036   79
47 PM310371117-21 25 2718 95013168820022  130
47 PM310371117-21 26 2492 21023194510022   22
47 PM310371117-21 27 2566 22023212860041   79
47 PM310371117-21 28 2629 53023260570031 3500
47 PM310371117-21 29 2506 64023209190035   23
47 PM310371117-21 30 2389 85023187700024  220
```

IBP

GRASSLAND BIOME

U.S. INTERNATIONAL BIOLOGICAL PROGRAM

LAB DATA SHEET--MICROBIOLOGY - CO_2, FORMAZAN, MPN

Data Type	Initials	Starting Date			Location	Run Number	Vessel Number	Dry Weight of Core	Percent Moisture in Core	Treatment 1	Moisture	Treatment 2	Week	Day Number	Cumulative Hours	Net dv x .074	Cumulative Net dv
		Day	Month	Year													
1-2	3-5	6-7	8-9	10-11	12	13-17	19-20	22-25	26-27	28	29	30	31	33-34	36-39	40-44	45-50
48																	

Treatment 1 (19-29) (31-32) (34-80)

1
2
3
4
5
6
7

Treatment 2

1
2
3
4
5

Location

1 Lab
2 Field

Moisture

1 Dry
2 Moist

+++ EXAMPLE OF DATA +++

```
         1         2         3         4         5         6
12345678901234567890123456789012345678901234567890123456789
48 PM310371117-21 TREATMENT 1 01 NO WATER
48 PM310371117-21 TREATMENT 1 02 5 ML
48 PM310371117-21 TREATMENT 1 03 10 ML
48 PM310371117-21 TREATMENT 1 04 15 ML
48 PM310371117-21 TREATMENT 1 05 20 ML
48 PM310371117-21 TREATMENT 2 01 NO HAY
48 PM310371117-21 TREATMENT 2 02 PLUS HAY
48 PM310371117-21 01 2742061 11  1   18 4958  4958
48 PM310371117-21 01 2742061 11  2   42 5476 10434
48 PM310371117-21 01 2742061 11  3   67 6660 17094
48 PM310371117-21 01 2742061 11  4   93 5772 22866
48 PM310371117-21 01 2742061 11  5  114 4662 27528
48 PM310371117-21 01 2742061 11  6  138 4884 32412
48 PM310371117-21 01 2742061 11  7  162 4662 37074
48 PM310371117-21 02 2789072 11  1   18 5032  5032
48 PM310371117-21 02 2789072 11  2   42 4366  9398
48 PM310371117-21 02 2789072 11  3   67 5254 14652
48 PM310371117-21 02 2789072 11  4   93 4884 19536
48 PM310371117-21 02 2789072 11  5  114 3774 23310
48 PM310371117-21 02 2789072 11  6  138 4144 27454
48 PM310371117-21 02 2789072 11  7  162 3922 31376
48 PM310371117-21 03 2803083 11  1   18 2812  2812
48 PM310371117-21 03 2803083 11  2   42 5032  7844
48 PM310371117-21 03 2803083 11  3   67 7178 15022
48 PM310371117-21 03 2803083 11  4   93 5106 20128
48 PM310371117-21 03 2803083 11  5  114 5032 25160
48 PM310371117-21 03 2803083 11  6  138 5402 30562
48 PM310371117-21 03 2803083 11  7  162 6216 36778
48 PM310371117-21 04 2860104 11  1   18 4292  4292
48 PM310371117-21 04 2860104 11  2   42 5550  9842
48 PM310371117-21 04 2860104 11  3   67 6512 16354
48 PM310371117-21 04 2860104 11  4   93 6364 22718
48 PM310371117-21 04 2860104 11  5  114 5032 27750
48 PM310371117-21 04 2860104 11  6  138 5254 33004
48 PM310371117-21 04 2860104 11  7  162 5254 38258
48 PM310371117-21 05 2584125 11  1   18 6512  6512
48 PM310371117-21 05 2584125 11  2   4216946 23458
48 PM310371117-21 05 2584125 11  3   6719018 42476
48 PM310371117-21 05 2584125 11  4   9321830 64306
48 PM310371117-21 05 2584125 11  5  11420350 84656
48 PM310371117-21 05 2584125 11  6  138243461090O2
48 PM310371117-21 05 2584125 11  7  16226714135716
48 PM310371117-21 06 2532061 21  1   18 5624  5624
48 PM310371117-21 06 2532061 21  2   4210730 16354
48 PM310371117-21 06 2532061 21  3   6714504 30858
48 PM310371117-21 06 2532061 21  4   9311692 42550
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48 PM310371117-21 06 2532061 21  5  114 7992 50542
48 PM310371117-21 06 2532061 21  6  138 7992 58534
48 PM310371117-21 06 2532061 21  7  162 7696 66230
48 PM310371117-21 07 2765122 21  1   18 9472  9472
48 PM310371117-21 07 2765122 21  2  4213024 22496
48 PM310371117-21 07 2765122 21  3  6715910 38406
48 PM310371117-21 07 2765122 21  4  9315392 53798
48 PM310371117-21 07 2765122 21  5 11411174 64972
48 PM310371117-21 07 2765122 21  6 13811322 76294
48 PM310371117-21 07 2765122 21  7 16210804 87098
48 PM310371117-21 08 2534103 21  1   18 7622  7622
48 PM310371117-21 08 2534103 21  2  4211470 19092
48 PM310371117-21 08 2534103 21  3  6714578 33670
48 PM310371117-21 08 2534103 21  4  9313394 47064
48 PM310371117-21 08 2534103 21  5  114 9546 56610
48 PM310371117-21 08 2534103 21  6  138 9472 66082
48 PM310371117-21 08 2534103 21  7  162 8954 75036
48 PM310371117-21 09 2688114 21  1   18 8732  8732
48 PM310371117-21 09 2688114 21  2  4212876 21508
48 PM310371117-21 09 2688114 21  3  6711692 33200
48 PM310371117-21 09 2688114 21  4  9314504 47704
48 PM310371117-21 09 2688114 21  5 11410360 58064
48 PM310371117-21 09 2688114 21  6 13810804 68868
48 PM310371117-21 09 2688114 21  7 16210138 79006
48 PM310371117-21 10 2816095 21  1   18 7844  7844
48 PM310371117-21 10 2816095 21  2  4212210 20054
48 PM310371117-21 10 2816095 21  3  6713912 33966
48 PM310371117-21 10 2816095 21  4  9313912 47878
48 PM310371117-21 10 2816095 21  5 11411100 58978
48 PM310371117-21 10 2816095 21  6 13811322 70300
48 PM310371117-21 10 2816095 21  7  162 9398 79698
48 PM310371117-21 11 2500051 11  1   18 4218  4218
48 PM310371117-21 11 2500051 11  2   42 3774  7992
48 PM310371117-21 11 2500051 11  3   67 4810 12802
48 PM310371117-21 11 2500051 11  4   93 4144 16946
48 PM310371117-21 11 2500051 11  5  114 3034 19980
48 PM310371117-21 11 2500051 11  6  138 3700 23680
48 PM310371117-21 11 2500051 11  7  162 3552 27232
48 PM310371117-21 12 2757072 11  1   18 4810  4810
48 PM310371117-21 12 2757072 11  2   42 4218  9028
48 PM310371117-21 12 2757072 11  3   67 6216 15244
48 PM310371117-21 12 2757072 11  4   93 4292 19536
48 PM310371117-21 12 2757072 11  5  114 3848 23384
48 PM310371117-21 12 2757072 11  6  138 4070 27454
48 PM310371117-21 12 2757072 11  7  162 4440 31894
48 PM310371117-21 13 2612083 11  1   18 6438  6438
48 PM310371117-21 13 2612083 11  2   42 6216 12654
48 PM310371117-21 13 2612083 11  3   67 6808 19462
48 PM310371117-21 13 2612083 11  4   93 6882 26344
48 PM310371117-21 13 2612083 11  5  114 5328 31672
48 PM310371117-21 13 2612083 11  6  138 5920 37592
48 PM310371117-21 13 2612083 11  7  162 5698 43290
48 PM310371117-21 14 2801094 11  1   18 4588  4588
48 PM310371117-21 14 2801094 11  2   42 4958  9546
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48	PM310371117-21	14	2801094	11	6	138	5106	30932
48	PM310371117-21	14	2801094	11	7	162	4884	35816
48	PM310371117-21	15	2674155	11	1	18	6142	6142
48	PM310371117-21	15	2674155	11	2	42	7400	13542
48	PM310371117-21	15	2674155	11	3	67	8510	22052
48	PM310371117-21	15	2674155	11	4	93	9436	31488
48	PM310371117-21	15	2674155	11	5	114	6290	37778
48	PM310371117-21	15	2674155	11	6	138	7030	44808
48	PM310371117-21	15	2674155	11	7	162	7252	52060
48	PM310371117-21	16	2665021	21	1	18	5032	5032
48	PM310371117-21	16	2665021	21	2	42	8880	13912
48	PM310371117-21	16	2665021	21	3	6714282		28194
48	PM310371117-21	16	2665021	21	4	9312136		40330
48	PM310371117-21	16	2665021	21	5	114	7548	47878
48	PM310371117-21	16	2665021	21	6	138	7622	55500
48	PM310371117-21	16	2665021	21	7	162	7104	62604
48	PM310371117-21	17	2739062	21	1	18	9472	9472
48	PM310371117-21	17	2739062	21	2	4212432		21904
48	PM310371117-21	17	2739062	21	3	6715688		37592
48	PM310371117-21	17	2739062	21	4	9315170		52762
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48	PM310371117-21	17	2739062	21	7	16210286		84360
48	PM310371117-21	18	2766073	21	1	18	6882	6882
48	PM310371117-21	18	2766073	21	2	42	9842	16724
48	PM310371117-21	18	2766073	21	3	6713172		29896
48	PM310371117-21	18	2766073	21	4	9313172		43068
48	PM310371117-21	18	2766073	21	5	114	9250	52318
48	PM310371117-21	18	2766073	21	6	138	9324	61642
48	PM310371117-21	18	2766073	21	7	162	8288	69930
48	PM310371117-21	19	2758094	21	1	18	8362	8362
48	PM310371117-21	19	2758094	21	2	4211914		20276
48	PM310371117-21	19	2758094	21	3	6714578		34854
48	PM310371117-21	19	2758094	21	4	9314504		49358
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