THE EFFECT OF ADDED AVAILABLE NITROGEN ON CARBON DIOXIDE EVOLUTION FROM SOIL TREATED WITH SAWDUST AND OTHER ORGANIC ADDITIONS

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HISTORICAL

Of the major nutritional elements carbon and nitrogen are most likely to be limiting under natural conditions. Microorganisms utilize a major portion of available carbon, with varying degrees of efficiency, as energy materials. Accompanying carbon utilization in synthetic processes, there occurs assimilation of appreciable amounts of nitrogen, which are used largely in the formation of protein materials. In culture media for heterotrophic microorganisms, therefore, emphasis is placed upon an adequate supply of compounds containing these elements in available form, carbon being most abundantly supplied.

Addition of organic matter in the form of green or stable manures or plant waste products furnishes the soil microflora with carbon and nitrogen along with other elements. All of these elements are not equally available, since the decomposition of any organic matter rarely results directly in complete breakdown, even under aerobic conditions. The rate of decomposition of organic matter depends upon a number of environmental factors along with the chemical nature of the organic matter itself.

When such organic matter becomes available to the soil microflora, the most readily decomposable materials

are attacked by the active groups of microorganisms. These organisms elaborate various waste materials, some of which favor the development of other types of bacteria. As these processes progress and the available materials disappear from the soil, only such materials as lignin, fats, and waxes and other compounds which are resistant to decomposition remain. At the same time dead bacterial cells, which also are resistant to decomposition, tend to accumulate. This mass of residual material eventually becomes humus. Humus itself undergoes changes which, while extremely slow, are of fundamental importance in soil fertility in that carbon and nitrogen become temporarily unavailable as they are bound in the living and dead protein complexes.

Microbial decomposition of organic matter is dependent upon available nitrogen required for cell synthesis. The amount of nitrogen assimilated is dependent upon the specific organisms and independent of the nitrogen source, whether protein, amino acid, or mineral. In the decomposition of relatively fresh organic matter in the soil, molds will predominate in total mass of active protoplasm, these organisms becoming decreasingly important in the decomposition as the proportion of starches and celluloses in the residual material decreases. The carbon:nitrogen ratio of mold protoplasm is approximately 10:1; in general, molds assimilate about 50 percent of the carbon of the substrate. The remaining carbon is liberated as carbon dioxide from

catabolic processes. For tissue synthesis the molds require one part of nitrogen for every ten parts of carbon assimilated. In bacteria the carbon:nitrogen ratio is 5:1 on the average. Assimilative effeciency of bacteria varies widely, according to species, substrate, and environmental factors; generally it is much lower than for the molds, 10 percent being an inclusive average. As a result 90 percent of the substrate carbon is dissimilated, more or less carbon dioxide being evolved in the process. The proportion of carbon dioxide to organic acids and other waste products becomes greater as aerobiosis increases. Eventually all substrate carbon dissimilated by a mixed microflora in the presence of free oxygen will appear as carbon dioxide. One part of nitrogen is required by bacteria for every five parts of carbon they assimilate. Although bacteria, with a carbon:nitrogen ratio of 5:1, require twice as much nitrogen to synthesize a given mass of cells as do the molds, the latter form much larger volumes of tissue thus utilizing significantly greater amounts of nitrogen.

It is thus apparent that the carbon:nitrogen ratio of organic matter is of outstanding importance in soil fertility, available nitrogen being required for the nutrition of the microorganisms as well as for plant growth. If organic material which is added to soil has an available nitrogen content of 1 percent or less, nitrogen starvation will result. The microorganisms will consume all of the nitrogen

and, in addition, will compete with higher plants for more nitrogen as long as there is readily available carbon present. If the nitrogen content of added organic matter is about 2.5 percent, no starvation will result, and some ammonia may be liberated after decomposition has progressed. If organic matter containing nitrogen in excess of 2.5 percent is added to soil, more nitrogen is added than is required by the soil microflora, and ammonia is liberated throughout the process.

In 1929 Jensen (11, p. 71) noted that cellulose-decomposing fungi consumed nitrogen in a definite ratio to the amount of cellulose decomposed. This ratio was 1 to 30-33. In elaborating these views, Waksman and his coworkers (24, p. 533) observed that during the decomposition of celluloses and hemicelluloses these materials furnished excellent energy sources for the formation of cell substance. Inorganic nitrogen could be used in this synthesis, but if this was not available, organic nitrogen sources could serve. These workers were also able to show a direct correlation between the amount of cellulose decomposed and inorganic nitrogen assimilated.

Hutchings and Martin (10, p. 337) found that when the carbon:nitrogen ratio was lowered by the addition of $NaNO_3$, microbial growth was stimulated, resulting in an increased rate of decomposition as shown by carbon dioxide evolution.

Millar et al (14, pp. 914-919) showed that leguminous

plants with narrow carbon:nitrogen ratios were decomposed much more rapidly than plants of high cellulose content. They stated that during the first period legumes decomposed faster than non-legumes. During the second and third periods the non-legumes were decomposed more rapidly as indicated by carbon dioxide evolution. After about five weeks all rates were observed to level off, and differences noted were only slight, becoming insignificant. More nitrogen apparently resulted in a greater fixation of carbon so that after the first period the amount of carbon evolved as carbon dioxide is significantly less.

When cellulosic material is added to soil, its decomposition is limited by the amount of nitrogen available from other sources. In the presence of sufficient available nitrogen decomposition can occur rapidly, as shown by various pure culture studies (25, p. 118). This is true for a wide variety of cellulose-containing materials. Under such conditions often as much as 30 percent of the carbon utilized is converted to cell substance, thus resulting in the complete assimilation of the available nitrogen. Such action in the soil precludes the liberation of available nitrogen from materials of a wide carbon:nitrogen ratio. On the other hand, materials with a narrow carbon:nitrogen ratio provide more nitrogen than is required for cell synthesis, so microbial activities, unless limited by other factors, provide a maximum supply of carbon dioxide as well

as liberate available nitrogen. Waksman and Starkey (26, p. 96) showed by schematic diagrams that from organic matter containing one thousand pounds of carbon and possessing a carbon:nitrogen ratio of 80:1 as much as 250 pounds of carbon will be evolved as carbon dioxide in a relatively short period of time. Over an extended period of time the carbon:nitrogen ratio narrows to approximately 10:1. This observation has been noted by other experimenters (8, p. 583) working with wheat and barley straw variously treated.

The carbon:nitrogen ratio of various materials cannot always be used as an index of the rate of their decomposition in soil. The chemical structure of these substances often plays a much more determinative role in such processes (24, p. 537), (12, p. 353), (6, pp. 375-377). When plant residues are added to soil, simple carbohydrates, pentosans, and the general group of hemicelluloses as well as soluble protein constituents are readily decomposed. More difficultly soluble protein constituents and lignin are more resistant and tend to accumulate. Fats and waxes which are ether-soluble are decomposed very slowly and may even accumulate. Cellulose is relatively resistant to decomposition under soil conditions. Decomposition by pure cultures of cellulose degrading microorganisms has been observed to occur at comparatively rapid rates (26, pp. 82-87). Martin (12, p. 341) indicated that lignin tends to combine with cellulose forming resistant complexes.

Coinciding with this is the possible presence of tannins which are known to be toxic to many microorganisms. The presence of other toxic substances is also probable. All of these factors play an important role in the decomposition of organic matter.

The formation of humus characterizes the final stages of the decomposition of organic matter. "Unstable humus" (25, p. 256) is still decomposable under favorable conditions; whereas, "stable humus" is characterized by a nearly complete disappearance of cellulose and hemicelluloses. Correspondingly the carbon:nitrogen ratio of "unstable humus" is about 20 or 30 to 1 or even higher, while that of "stable humus", showing a considerable increase in lignin-like complexes, is more nearly 10:1 or less. "Stable humus", which is the more common form, supports a relatively low level of microbial activity due to the resistance of the humus to rapid decomposition (25, p. 354).

Since carbon dioxide is the most constant product of microbial metabolism, a measure of the evolution of this gas has long been used as an index of the activities of microorganisms in the soil. As a justification of this, various investigators have noted that the amount of carbon dioxide liberated from numerous energy sources is quite comparable on the basis of carbon content; duplication of results is thus facilitated. As early as 1880 Wollney showed that the carbon dioxide content of various soils

fluctuates with the amount of organic matter present in those soils. Since that time several other experimenters have shown that the evolution of this gas could be used as an index of decomposition rates, as an index of available organic matter, and as an indication of soil fertility. Stoklasa (20, p. 595) observed that the quantity of carbon dioxide produced in a given time and under specific environmental conditions gives an exact picture of the size and mechanics of physiological combustion. This indicated a very good correlation between carbon dioxide evolution and microbial numbers in the soil. Norman and Newman (16, pp. 44-45) observed that the relative and cumulative rates of carbon dioxide evolution were indices of the efficiency of decomposition by the soil population. They commented further that the period of time elapsing before the maximum rate had been attained might be a measure of the number of active organisms in the soil. Because of observations of this nature (23, pp. 141-144), older beliefs that carbon dioxide evolution in soil was a function of ordinary chemical processes only, gave way to recognition of the importance of microbial action. More recently Dawson (4, pp. 473-475) found that during the decomposition of cellulose, carbon dioxide evolution showed direct correlation with weight losses. It is, therefore, apparent that in many instances the evolution of carbon dioxide from soils can readily furnish a clear picture of microbial activities.

Carbon dioxide measurement is accomplished without disturbing the experimental conditions, and no soil sampling is necessary.

EXPERIMENTAL METHODS

Preliminary Investigations

This phase of the work was undertaken to determine the effect of added available nitrogen on the rate of decomposition of various types of organic matter when incorporated with soil under laboratory conditions. Particular attention was given to bark and sawdust of various kinds because of current interest in the use of these materials as mulches and soil conditioners. These materials were all air-dried and ground to pass a 60-mesh screen, except the mechanical fractions of Douglas-fir bark as otherwise noted. Analysis of these materials is given in Table 2. Carbon dioxide evolution was used as an index of decomposition.

The soil used was Chehalis silty clay loam obtained from the Lewis-Brown horticultural farm of Oregon State College. A bulk sample containing about 15 percent moisture was passed through a 20-mesh screen and stored in covered galvanized containers for future use as required. Microbial and chemical analyses of this sample are shown in Table 1.

All organic materials were added in amounts equivalent to 2000 p.p.m. carbon or 2 tons per acre 6 2/3 inches.

This was accomplished by adding an amount of material equivalent to one gram of carbon to 500 grams of soil, on a water-free basis, and mixing thoroughly. Similar additions were made to a second series of soil samples with ammonium nitrate added to adjust the carbon:nitrogen ratio to 20:1. The samples were placed in quart milk bottles, and water was added to 60 percent of saturation capacity. The respiration apparatus and methods employed were essentially the same as those used by Bollen(1, p. 359). Carbon dioxide-free air was passed over the surface of the soil in the bottles and then into test tubes filled to a height of 3 inches with approximately N/1 NaOH. Slight pressure was used so that a slow, even bubbling was maintained. The bottles were incubated at 28°C. At intervals of 2, 5, 7, 12, 19, 27, 35, and 50 days the absorbent was replaced with fresh solution; carbon dioxide absorbed in the alkali removed was titrated with standard H_2SO_4 electrometrically. Results were expressed cumulatively as milligrams of carbon evolved as carbon dioxide per 500 grams of soil, water-free basis. All values are assumed (2, pp. 271-274) to indicate carbon dioxide evolved from the added organic matter since corresponding values for soil only have been subtracted (Table 3, and Figures 1 to 5, inclusive).

Table 1: Analysis of Chehalis Silty Clay Loam Soil*

Chemical Analysis:

Moisture, percent	13.4
Saturation capacity, percent	57.2
pH	6.6
Lime requirement (Truog)	1 ton per acre
Nitrogen:	
Ammonium	trace
Nitrite	trace
Nitrate	11.2 p.p.m.
Kjeldahl, percent	0.132
Total carbon, percent	1.61
Carbon:nitrogen ratio	12.2
Sulfur as sulfate (Water-soluble)	trace
Phosphorus as phosphate (Water-soluble)	2.0 p.p.m.

Biological Analysis:

Molds, per gram	33,500
Penicillia, percent	87
Aspergilli, percent	5
Mucors, percent	5
Bacteria, per gram	18,816,800
Streptomyces, per gram	15,583,200
Azotobacter	present

*Data expressed on water-free basis

Table 2: Analysis of Douglas-fir Bark, Sawdust, and Other Organic Materials Added to Chehalis Silty Clay Loam Soil

Organic Addition	Moisture w-f %	Satn. Cap. %	Total C %	Kjeldahl N %	C:N Ratio
Young bark	5.47	266	51.66	0.169-	306
Young bark, water extd.	5.85	313	50.41	0.256	197
Old bark	2.95	300	58.56	0.119	493
Old bark, water extd.	7.18	296	59.00	0.114	517
Old bark, benzene extd.	12.06	303	65.06	0.138	472
Old bark, benzene and water extd.	4.88	463	48.89	0.140	420
Cork, old bark	5.27	195	59.32	0.125	475
Bast, old bark	7.73	239	54.29	0.111	489
Fines, old bark	7.77	322	54.08	0.115	471
Dust, old bark	8.39	302	57.00	0.180	317
White rot, Douglas-fir	6.86	695	48.68	0.668	73
Red rot, Douglas-fir	7.34	271	59.30	1.208	49
Moss Peat	11.66	814	48.29	0.830	58
Douglas-fir sawdust	5.71	503	49.80	0.050	997
Hemlock sawdust	5.46	602	49.74	0.043	1157
Cedar sawdust	4.88	512	51.05	0.068	752
Weyerhaeuser chute mud	5.12	212	35.50	0.197	180
Ponderosa pine sawdust	8.47	486	53.18	0.052	1023
Corn cobs	5.26	358	46.87	0.448	104
Rice hulls	7.34	178	39.80	0.550	72
Wheat straw	7.04	514	44.70	0.120	37
Dextrose	0.61	-	40.00	0.000	-
Soil	13.4	57.2	1.61	0.132	12.2

Microbial Population Studies

The preliminary investigations indicated that when ammonium nitrate was added to effect a lowering of the carbon:nitrogen ratio of organic additions to 20:1, the overall carbon dioxide evolution was decreased. Since carbon dioxide evolution is due largely to activities of microorganisms, it would seem that their numbers and possibly kinds, would show corresponding response to carbon: nitrogen changes. To investigate this, the previous experiment was repeated and elaborated by running six series of twenty bottles of each treatment for Douglas fir, cedar, hemlock, and pine sawdust, dextrose and control; ten bottles in each case having ammonium nitrate added to adjust the carbon:nitrogen ratio. Carbon dioxide determinations were made at 2, 5, 10, 15, and 20 days, and at the same time duplicate bottles of each treatment were removed for plate counts and pH determinations. Since a general leveling-off in carbon dioxide production took place between fifteen and twenty days in all cases in the preliminary experiment, this more comprehensive study was limited to twenty days. For molds, plates were poured with peptone-glucose-acid agar in triplicate from each bottle, using dilutions of 1:500 and 1:5000. Bacteria and streptomyces counts were made on sodium albuminate agar; dilutions of 1:50,000 and

1:500,000 were used and triplicate plates were poured. Optimum counts for molds were obtained from the 1:500 dilutions, and for bacteria and streptomyces dilutions of 1:500,000 were best throughout the experiment. Results are presented in Tables 4 to 9, and Figures 6 to 18, inclusive.

Warburg Respiration Studies

The general reduction in the soil population observed in the cases with added nitrogen pointed to the possibility of an inhibitive effect of the ammonium nitrate on the microflora of the soil. Warburg respiration studies were therefore undertaken to determine the effect of ammonium nitrate upon oxygen uptake and carbon dioxide evolution of several different groups of microorganisms isolated from soil used in the previous experiments.

a. Isolation and preparation of cultures:

Various molds, bacteria and Streptomyces were picked from soil dilution plates and propagated on slants. Peptone-glucose-acid agar was used for the molds and sodium albuminate agar for the bacteria and Streptomyces. Cultures for the Warburg studies were made by inoculating 75 ml. of sterile nutrient solutions in erlenmeyer flasks with 1 ml. of a heavy suspension of growth from the slant cultures. For the different groups of organisms the procedure was varied as follows:

Molds. Ten isolates, including Penicillia,

Aspergilli, mucors, and Trichoderma were inoculated separately into 500 ml. erlenmeyer flasks containing 75 ml. of a nutrient solution having the following ingredients:

Peptone.....	5 gms.
Beef Extract.....	3 gms.
K_2HPO_4.....	1 gm.
Dextrose.....	20 gms.
Yeast Extract.....	Trace
Distilled Water.....	1000 ml.

The pH, after autoclaving at 15 lbs. pressure for 20 minutes was found to be 6.7. The inoculated flasks were incubated at 28°C. on a reciprocating incubator at 90 excursions per minute until abundant growth was evident. This required from 24 to 48 hours. The cultures were then combined in approximately the proportions observed for the various colonies on the original soil plates and then centrifuged for 15 minutes. After washing twice in sterile M/15 phosphate buffer, pH 6.80, they were macerated for 30 seconds in a sterile micro-blender and then transferred to a 500 ml. erlenmeyer flask containing 75 ml. of the sterile phosphate buffer. This flask of blended mold mycelia was then incubated overnight in the reciprocating incubator. The growth was then centrifuged and washed twice with sterile phosphate buffer and diluted with the same buffer to a moderate turbidity. One ml. of this suspension, subsequently found to be equivalent to 12.25 mg.

dry weight of mycelium, was used in each of 12 Warburg flasks.

Streptomyces. Ten isolates were grown on sodium albuminate slants and then transferred to a liquid medium of the following composition:

Peptone.....	5 gms.
Beef Extract.....	3 gms.
K_2HPO_4.....	1 gm.
Dextrose.....	10 gms.
Yeast Extract.....	Trace
Distilled Water.....1000 ml.	

The organisms were grown and harvested in the same manner as were the molds. All further treatment was the same, including the overnight shaking period to reduce the endogenous respiration which is normally very high in the case of molds and Streptomyces. One ml. of cell suspension equivalent to 12.81 mg. dry weight was used in each of 12 Warburg flasks for the respiration study.

Bacteria. Forty-eight hour-old nutrient broth cultures of 10 isolates were harvested and diluted as were the molds and Streptomyces, except that the starvation period was omitted since endogenous respiration is usually low in the case of the bacteria. One ml. of the cell suspensions was used for each respiration flask in this experiment. The average dry weight of the cells per milliliter of suspension was 13.16 mg.

b. Warburg technique:

Standard Warburg technique (22, pp. 1-20) was followed for determining oxygen uptake and carbon dioxide evolution. In addition to the cell suspensions, the following solutions were employed:

Phosphate buffer. M/15 buffer was made up using K_2HPO_4 and KH_2PO_4 adjusted to pH 6.80. This solution was sterilized at 15 lbs. pressure for 20 minutes.

Dextrose. M/10 C. P. dextrose was made up in the phosphate buffer; 0.5 ml. per flask gave a final concentration of M/60 in the experiment.

Ammonium nitrate. A concentration equivalent to 0.51 mg. nitrogen per ml. was made up in the buffer. The final concentration in the flask gave a carbon:nitrogen ratio of 20:1, exclusive of the nitrogen in the cells, to correspond to preceding experiments with the soil.

The pH of all solutions was adjusted to 6.80.

Each experiment included dextrose without added nitrogen and dextrose plus ammonium nitrate for the carbon: nitrogen ratio of 20:1. Duplicate flasks were run in all cases (Tables 10 to 12, and Figure 19).

Reciprocating Incubator Studies

Results from the Warburg respiration studies indicated that added ammonium nitrate stimulated rather than

inhibited the activity of the various groups of soil microorganisms. This tends to confirm the idea that, since available nitrogen is required for cell growth and utilization of energy materials, depletion of carbon by microbial synthesis may be considered the cause of observed depressive effects noted with plate counts and carbon dioxide evolutions. To further corroborate this, respiration studies on soil suspensions were made. Quart milk bottles containing 100 ml. of a modified[1] Winogradsky's solution, with and without added nitrogen[2], were inoculated with 10 ml. 1:5 soil suspension[3] and fitted with absorption units identical with those used in the soil respiration studies. The bottles were placed on the reciprocating incubator and connected to a supply of carbon dioxide-free air. The carbon dioxide evolved was collected in N/1 NaOH. At selected

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[1]a. Winogradsky's solution.....10 ml.
(10 ml. contained:)
NaCl.....0.02 gms.
$MnSO_4$.....0.02 gms.
$FeSO_4$.....0.02 gms.
$(NH_4)_2Mo_7$).....0.001 gms.

b. $MgSO_4$.....0.02 gms.
c. M/15 PO_4 buffer.....100.0 ml.
d. Dextrose, C.P. ..... 3.0 gms.
e. Soil suspension (1:5 dilution).....100.0 ml.
f. Distilled water to make.....1000.0 ml.

[2]Ammonium nitrate added to give a carbon:nitrogen ratio of 5:1

[3]This soil suspension contained no ammonia and less than 0.002 mg. nitrate nitrogen per ml.

intervals one bottle of each treatment was removed and determinations made for residual glucose, ammonia, and carbon dioxide evolved. The results are given in Tables 13 and 14, and Figures 20 and 21.

RESULTS

Preliminary Investigations

In the preliminary soil respiration study to determine the relative decomposability of bark, sawdust, and other plant residues, ammonium nitrate added with the various organic materials lowered the total carbon dioxide evolved during 50 days incubation. The only exception was shown by the Douglas-fir white rot. Results obtained for old bark, with and without added nitrogen, are presented graphically in Figures 1 and 2. Figure 1 shows total carbon dioxide from soil plus additives, while in Figure 2 the values for the soil control have been subtracted. These are typical respiration curves representing cumulative carbon dioxide production. For the other organic materials the curves differ mainly in slope, representing rate of evolution, and in final values indicating total carbon dioxide evolved. The latter with control subtracted is an approximate (2, p. 271) measure of the extent of decomposition. This disregards any effect the added organic material may have on the native organic matter or humus, but the

main interest generally is in the increase in carbon dioxide of biological origin, from whatever source. For similar reasons the soil only, and not soil plus nitrogen, is used as the control for indicating increases or decreases due to nitrogen added with organic matter. It is assumed that the nitrogen will exert more, if not all, of its influence on the organic addition rather than on the soil's own organic matter. This is true as long as the latter has a "normal" carbon:nitrogen ratio near 12/1. The Chehalis silty clay loam soil used in all our experiments had a carbon:nitrogen ratio of 12.2:1.

To avoid the multiplicity of graphs required to show the many similar and overlapping curves, data for all the additives studied are presented as bar graphs in Figures 3, 4, and 5. Bar divisions show total carbon dioxide production and also indicate relative rates of decomposition during three intervals chosen to represent approximately 1/4, 1/2 and the entire incubation period. Dextrose was used in each experiment as a readily available substrate with which to compare the other organic additions.

Microbial Population Studies

Results of this more comprehensive study to determine any correlation of microbial numbers and kinds with carbon dioxide evolution and pH are shown in Tables 4 to

Table 3: Carbon Dioxide Evolution During Decomposition of Douglas-fir, Bark, Sawdust and Other Organic Additions* in the Chehalis Silty Clay Loam Soil**

No.	Treatment	Milligrams of carbon equivalent to carbon dioxide evolved, cumulative basis at time indicated							
		44 hrs.	120 hrs.	166 hrs.	282 hrs.	456 hrs.	640 hrs.	836 hrs.	1191 hrs.
1	D-fir Bark - Young	14.4	26.2	41.8	57.6	98.2	151.3	206.8	262.2
1N	" " " plus N***	6.7	14.3	21.7	24.9	32.0	44.8	57.4	86.5
2	D-fir Bark - Young, water ext.	14.2	23.6	34.1	46.3	80.8	128.6	183.6	235.8
2N	" " Young, water ext. plus N	7.1	17.9	26.6	31.1	46.3	67.7	91.8	129.5
3	D-fir Bark - Old	15.9	23.8	32.1	39.2	61.3	93.2	130.5	182.2
3N	" " " plus N	17.6	24.3	30.4	31.9	35.5	39.4	42.8	48.8
4	D-fir Bark - Old, water ext.	10.5	16.8	24.6	35.5	65.2	157.4	152.0	199.0
4N	" " " water ext. plus N	8.0	11.5	13.7	12.8	12.5	17.0	19.0	18.3
5	D-fir Bark - Old, benz. ext.	17.3	24.3	31.6	38.1	55.9	76.1	99.4	140.2
5N	" " " benz. ext. plus N	14.1	18.7	21.6	21.4	22.2	24.3	25.2	25.8
6	D-fir Bark - Old, benz. & water ext.	6.90	11.3	19.8	22.6	37.5	58.2	78.7	118.4
6N	" " Old, benz. & water ext. plus N	5.8	8.5	9.5	7.0	4.2	2.4	-2.9	-10.6
7	D-fir Cork	31.7	59.9	82.4	99.9	141.9	201.7	261.6	318.7
7N	" " plus N	32.3	52.9	71.7	81.4	94.2	106.8	118.0	134.4
8	D-fir Bast	16.4	27.1	31.9	43.2	65.3	96.2	128.8	175.2
8N	" " plus N	13.9	21.1	31.2	34.1	36.1	41.7	50.3	57.7
9	Dextrose	121.1	245.1	346.8	393.6	440.2	492.5	537.7	580.5
9N	" plus N	128.4	250.1	329.1	361.2	393.9	429.1	460.7	496.5
10	Soil only	8.4	17.8	37.8	59.6	90.3	117.5	147.6	198.8
10N	" plus N	-0.8	-2.4	-4.1	-8.0	-14.1	-20.7	-30.9	-50.0

		47 hrs.	88 hrs.	135 hrs.	183 hrs.	284 hrs.	455 hrs.	647 hrs.	838 hrs.	1176 hrs.
11	D-Fir Bark Fines	13.6	17.5	23.2	26.8	38.6	47.4	65.9	89.0	109.6
11N	" " " plus N	11.6	14.6	17.4	18.8	25.2	21.1	22.9	26.4	38.8
12	D-Fir Bark Dust	18.2	24.2	29.8	33.8	47.1	59.3	77.6	90.8	113.2
12N	" " " plus N	16.1	21.4	25.8	27.3	28.8	31.8	31.3	24.6	8.7
13	D-Fir White Rot	14.4	51.4	72.6	84.8	111.8	158.2	211.8	269.4	296.9
13N	" " " plus N	13.6	47.0	104.9	139.7	180.5	214.9	253.4	289.0	318.6
14	D-Fir Red Rot	8.4	12.8	16.8	19.2	20.8	30.3	34.9	39.3	47.8
14N	" " " plus N	7.2	11.1	13.2	15.8	15.8	23.2	25.1	27.2	13.6
15	Moss Peat	5.8	13.1	16.0	19.8	23.6	30.8	39.4	42.2	35.8
15N	" " plus N	8.2	7.9	10.5	13.7	19.3	19.5	20.8	19.0	10.4
16	Dextrose	112.4	215.4	306.0	357.4	393.8	439.9	481.8	514.7	530.8
16N	"	118.3	228.8	300.5	333.1	373.6	401.6	425.9	440.4	456.1
17	Soil only	9.1	17.2	25.4	33.9	47.3	85.3	111.2	141.6	199.9
17N	" plus N	-1.1	-3.5	-5.1	-7.4	-7.9	-15.6	-25.4	-35.6	-50.9

		44 hrs.	116 hrs.	164 hrs.	284 hrs.	452 hrs.	716 hrs.	884 hrs.	1220 hrs.
18	D-fir Sawdust	17.0	28.5	35.3	53.5	105.9	175.2	234.4	302.8
18N	" " plus N	14.6	23.0	20.9	34.1	53.9	86.3	106.1	138.2
19	Hemlock Sawdust	4.3	6.0	9.1	27.9	89.9	153.2	201.5	264.6
19N	" " plus N	4.4	5.0	0.1	15.5	37.0	79.0	105.5	146.9
20	Cedar Sawdust	6.5	10.8	15.8	40.7	108.7	190.8	252.5	324.6
20N	" " plus N	10.0	17.2	20.1	22.9	26.0	28.7	33.0	42.2
21	Pine Sawdust	20.7	40.3	48.1	71.3	138.8	206.2	261.4	329.2
21N	" " plus N	23.4	39.4	44.0	57.7	90.2	133.8	156.9	193.2
22	Weyerhaeuser Chute Mud	9.8	19.0	23.6	36.2	61.5	106.1	140.8	195.6
22N	" " " plus N	8.4	12.3	13.8	17.1	21.2	75.8	82.6	95.1
23	Corn Cobs	60.1	146.0	185.2	252.0	323.5	389.1	442.1	495.7
23N	" " plus N	67.9	165.1	209.4	266.2	320.1	381.5	416.2	465.5
24	Rice Hulls	44.5	75.1	89.8	121.1	169.7	231.8	272.8	335.2
24N	" " plus N	47.0	75.5	85.5	106.0	138.5	180.1	202.9	246.6
25	Wheat Straw	44.6	123.8	162.6	214.6	279.7	347.1	410.5	483.0
25N	" " plus N	40.5	121.4	177.8	259.3	323.6	381.9	420.0	469.3
26	Dextrose	119.9	232.8	316.6	379.3	431.9	489.0	527.3	572.6
26N	" plus N	119.7	239.6	272.9	319.4	353.1	384.2	405.4	430.5
27	Soil only	13.1	27.9	36.1	45.6	70.7	104.8	126.0	146.6
27N	" plus N	-1.5	-4.4	-5.6	-8.6	-12.6	-19.8	-24.9	-36.8

*Organic additions equivalent to 2000 p.p.m. C.

**Values for soil only subtracted from all other data.

***All nitrogen added as ammonium nitrate to give a C:N ratio of 20:1.

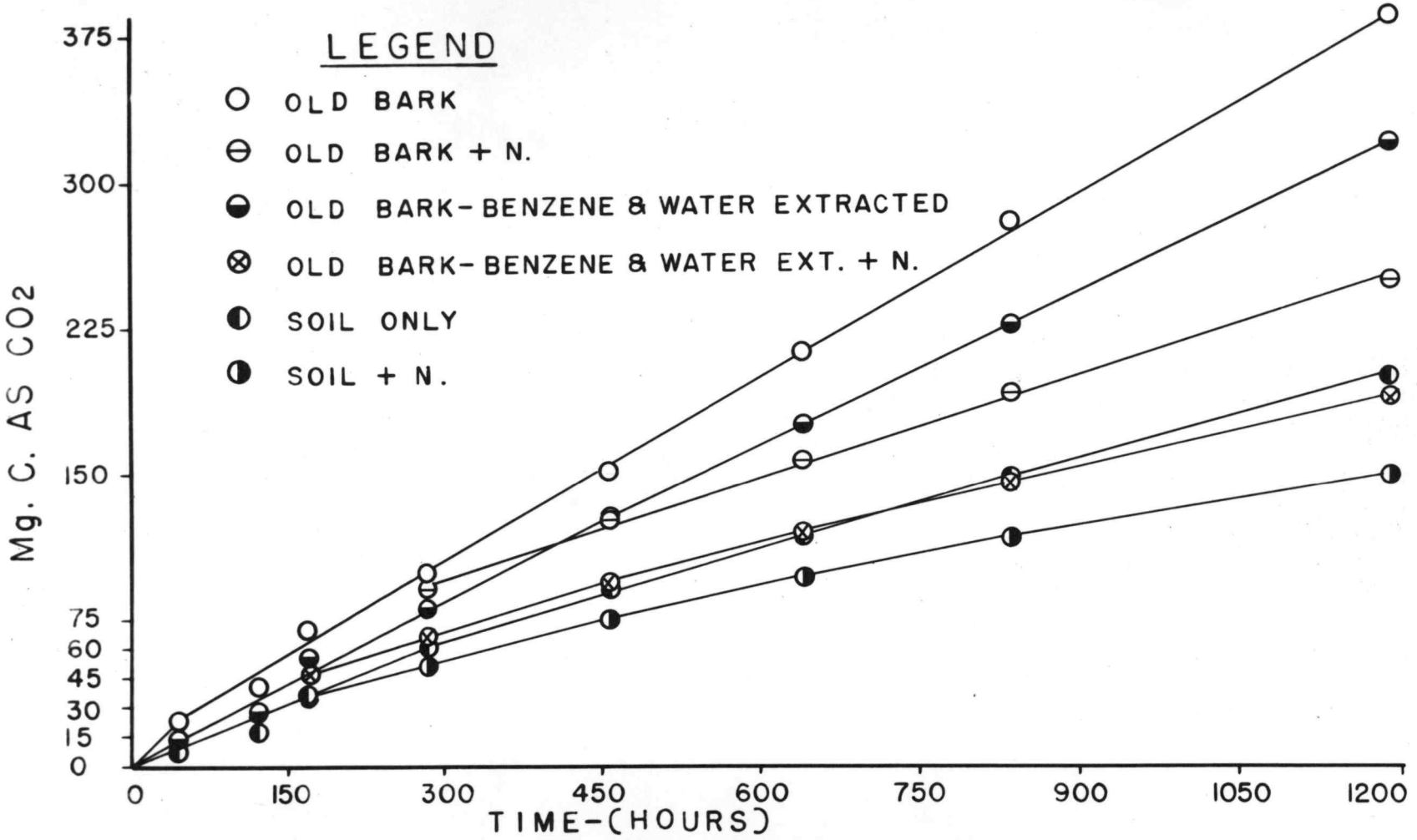

FIGURE 1: CARBON DIOXIDE EVOLUTION, CUMULATIVE BASIS, FROM SOIL TREATED WITH DOUGLAS-FIR BARK, WITH & WITHOUT ADDED AMMONIUM NITRATE

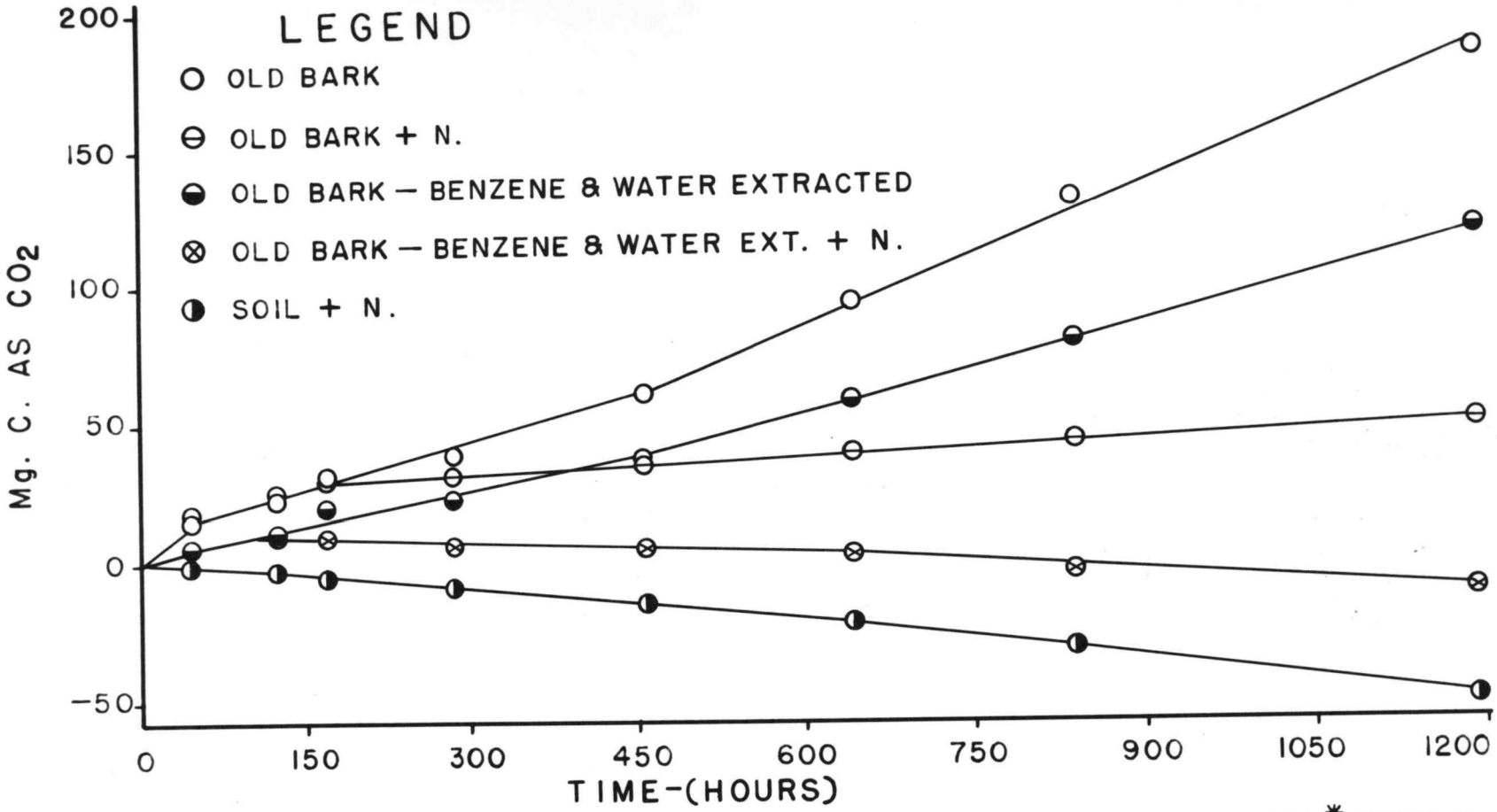

FIGURE 2: CARBON DIOXIDE EVOLUTION, CUMULATIVE BASIS, FROM DOUGLAS-FIR BARK* WITH & WITHOUT ADDED AMMONIUM NITRATE**

*VALUES FOR SOIL ONLY SUBTRACTED

**AMMONIUM NITRATE ADDED TO GIVE A CARBON : NITROGEN RATIO OF 20 : 1

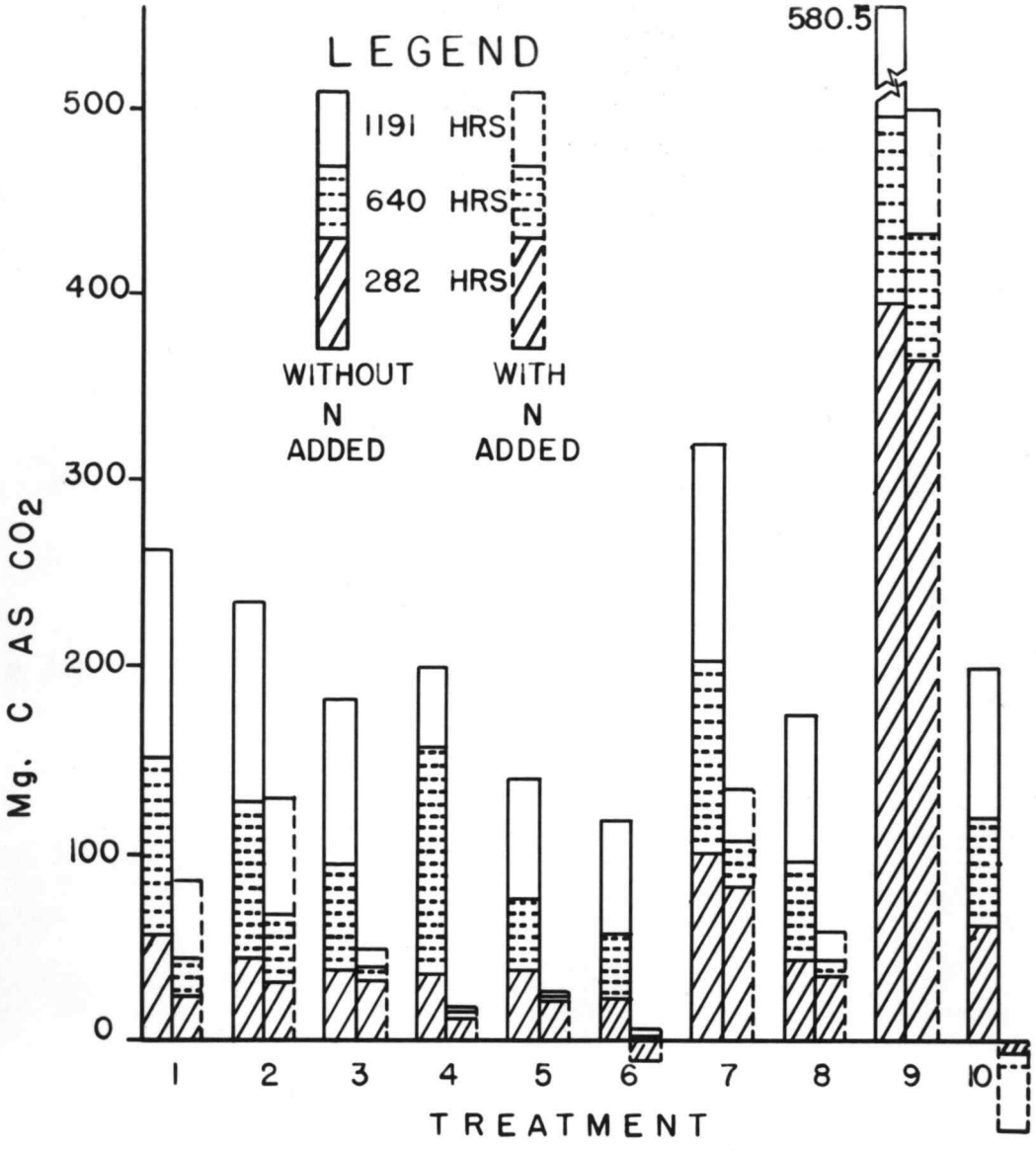

FIGURE 3: CUMULATIVE CARBON DIOXIDE EVOLUTION DURING DECOMPOSITION OF VARIOUS ORGANIC ADDITIONS* IN CHEHALIS SILTY CLAY LOAM SOIL**

* ORGANIC ADDITIONS EQUIVALENT TO 2000 p.p.m. C.

** VALUES FOR SOIL ONLY SUBTRACTED

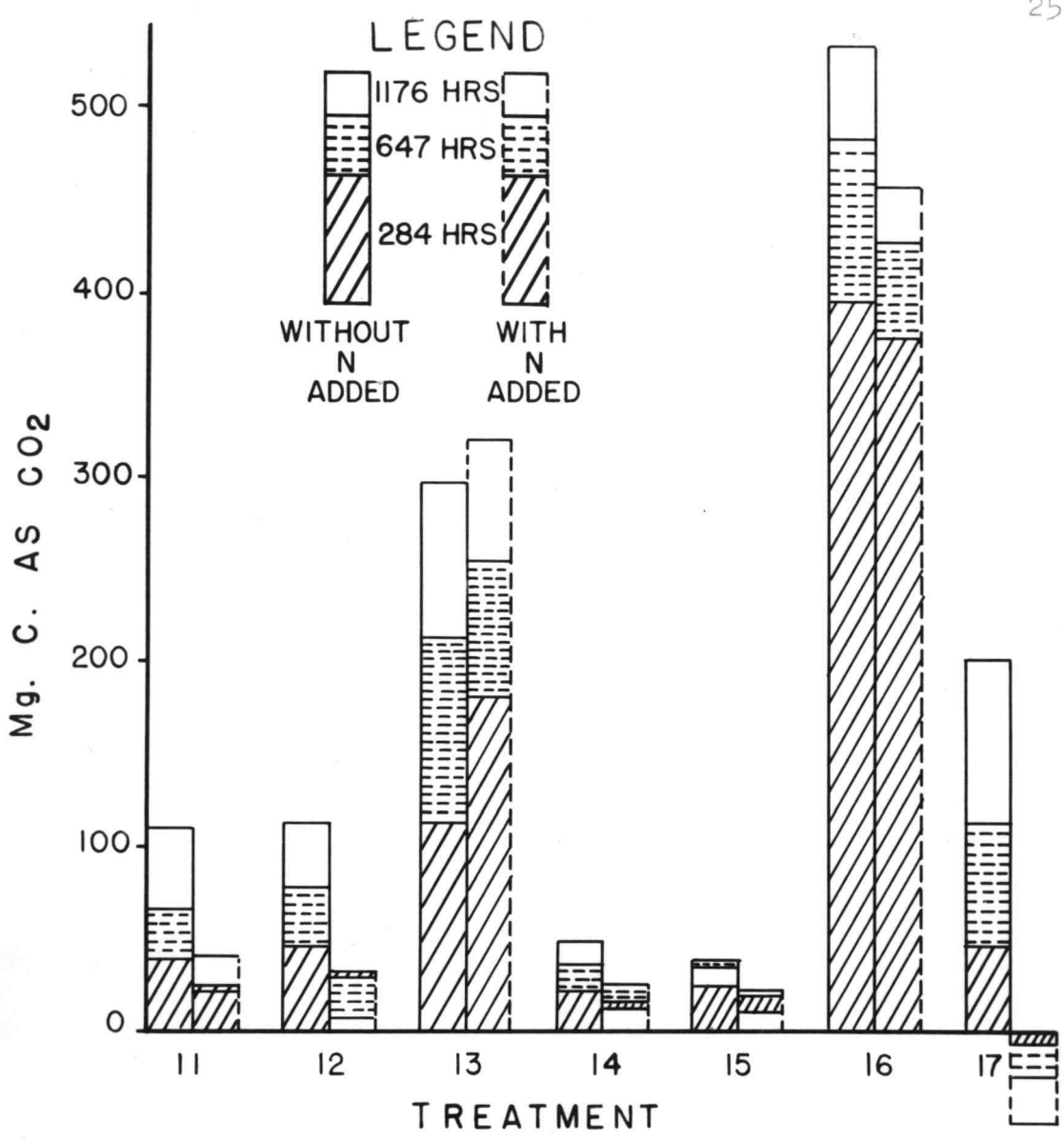

FIGURE 4: CUMULATIVE CARBON DIOXIDE EVOLUTION FROM DOUGLAS-FIR FRACTIONS, WOOD ROT, & OTHER ORGANIC ADDITIONS * IN CHEHALIS SILTY CLAY LOAM SOIL * *

* ORGANIC ADDITIONS EQUIVALENT TO 2000 p.p.m. C.

* * VALUES FOR SOIL ONLY SUBTRACTED

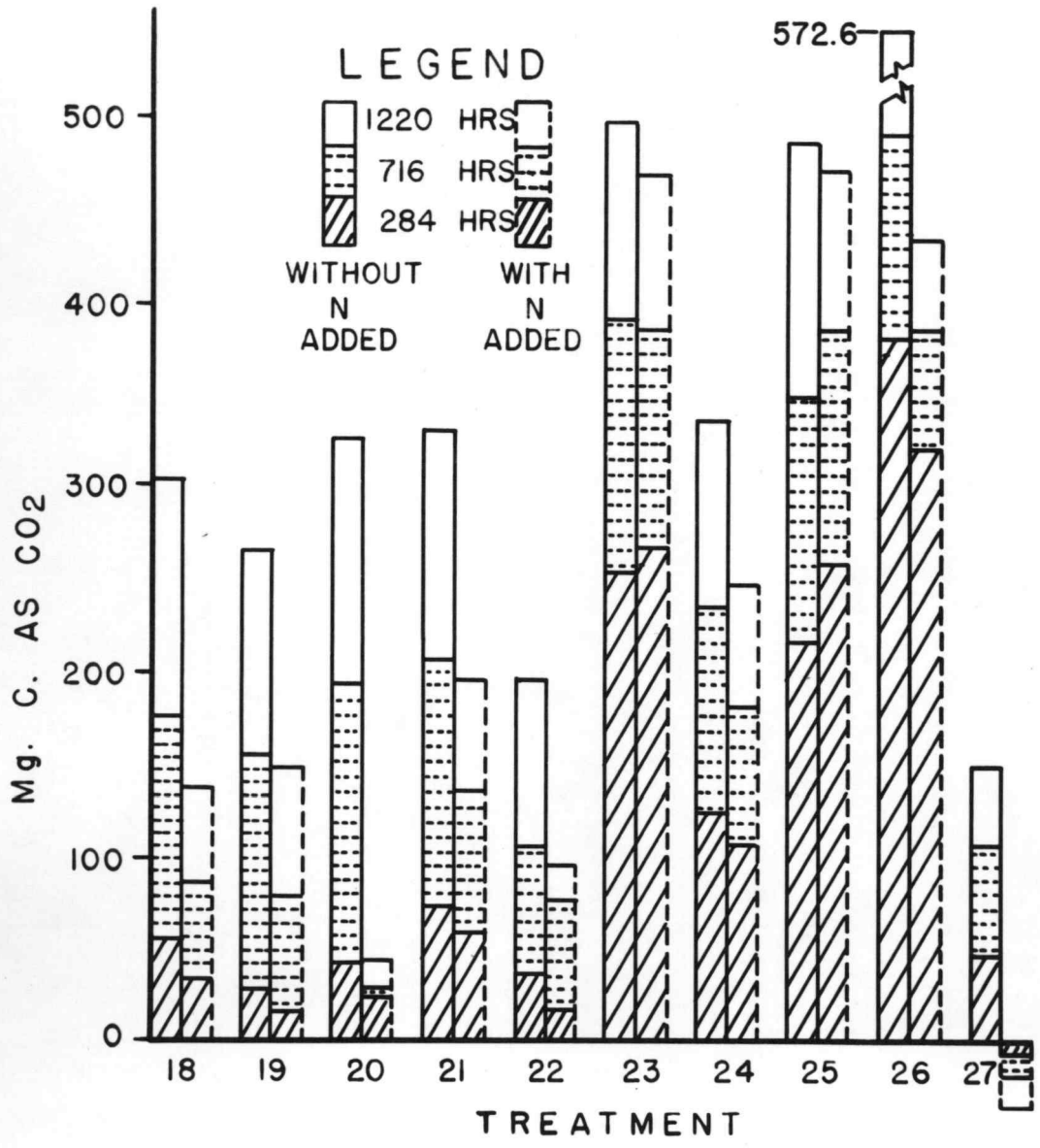

FIGURE 5: CUMULATIVE CARBON DIOXIDE EVOLUTION FROM SAWDUST & OTHER ORGANIC ADDITIONS* IN CHEHALIS SILTY CLAY LOAM SOIL**

* ORGANIC ADDITIONS EQUIVALENT TO 2000 p.p.m. C.

** VALUES FOR SOIL ONLY SUBTRACTED

8 and Figures 6 to 18, inclusive. The tabulated data also include calculated values for the net carbon dioxide evolved from the sawdust and from dextrose by intervals as well as cumulatively. Figures 6 and 7 show the course of total carbon dioxide production from the soil with different sawdusts and dextrose, compared with the soil control.

For all sawdust additions the carbon dioxide evolved was always less where available nitrogen was added. However, with dextrose the addition of nitrogen resulted in an increased evolution within the first 48 hours, followed by a leveling off and a final fall below the curve for dextrose without nitrogen at the close of the experiment. Differences were not marked at any time but were distinct at the beginning and at the end. The depression increases with time, as in the preliminary experiments. In the three different 50-day runs, early sections of the curves showing carbon dioxide evolution from dextrose and dextrose plus nitrogen correspond closely with the 20-days run, thereafter showing increasing divergence to an average decrease of approximately 15 percent due to the nitrogen.

In calculating decomposition from the difference in carbon dioxide evolution between control and treated soils the values may be only close approximations. If the organic addition stimulates decomposition of the native organic matter, the latter will give more carbon dioxide in this case than in the control.

Table 4: Cumulative Carbon Dioxide Evolution and pH Changes During the Decomposition of Douglas-fir Sawdust in Chehalis Silty Clay Loam Soil

Treatment	Time (hrs.)	pH	Carbon as CO_2 from soil + sawdust by interval p.p.m.	Carbon as CO_2 from soil + sawdust cumulative p.p.m.	Carbon as CO_2 from soil only by interval p.p.m.	Carbon as CO_2 from soil only cumulative p.p.m.	Carbon as CO_2 from sawdust by interval p.p.m.	Carbon as CO_2 from sawdust cumulative p.p.m.
Sawdust°	47	7.0	31.2	31.2	26.0	26.0	5.2	5.2
	114	6.8	92.0	124	26.5	52.5	66.3	71.5
	229.5	6.8	65.2	189.2	37.5	90.0	27.7	99.2
	357	6.8	91.6	280.8	32.0	122.0	59.6	158.8
	474	6.8	109.8	390.6	39.5	161.5	70.3	229.1
Sawdust plus N*	47	6.6	20.6	20.6	22.0	22.0	-1.4	-1.4
	114	6.4	83.6	104.2	27.0	49.0	56.6	55.2
	229.5	6.4	55.6	159.8	38.5	87.5	17.1	72.3
	357	6.5	65.4	225.2	28.5	116.0	36.9	109.2
	474	6.4	65.6	290.8	27.5	143.5	38.1	147.3

°Sawdust addition equivalent to 2000 p.p.m. carbon
*Nitrogen added as ammonium nitrate to give a carbon:nitrogen ratio of 20:1

Table 5: Cumulative Carbon Dioxide Evolution and pH Changes During the Decomposition of Hemlock Sawdust in Chehalis Silty Clay Loam Soil

Treatment	(hrs.)	pH	Carbon as CO_2 from soil + sawdust by interval p.p.m.	Carbon as CO_2 from soil + sawdust cumulative p.p.m.	Carbon as CO_2 from soil only by interval p.p.m.	Carbon as CO_2 from soil only cumulative p.p.m.	Carbon as CO_2 from sawdust by interval p.p.m.	Carbon as CO_2 from sawdust cumulative p.p.m.
Sawdust°	43	6.8	44.4	44.4	26.0	26.0	18.4	18.4
	118	6.8	51.4	95.8	26.5	52.5	24.9	43.3
	237	6.7	69.8	165.6	38.5	91.0	31.3	74.6
	360	6.8	92.4	258.0	32.5	123.5	59.9	134.5
	478	6.8	125.6	383.6	40.0	163.5	85.6	220.1
Sawdust plus N*	43	6.6	39.2	39.2	21.5	21.5	17.7	17.7
	118	6.6	44.0	83.2	27.0	48.5	17.0	34.7
	237	6.5	55.0	138.2	39.0	87.5	16.0	50.7
	360	6.5	55.6	193.8	29.0	116.5	26.6	77.3
	478	6.4	64.6	258.4	27.5	114.0	37.1	114.0

°Sawdust addition equivalent to 2000 p.p.m. carbon
*Nitrogen added as ammonium nitrate to give a carbon:nitrogen ratio of 20:1

Table 6: Cumulative Carbon Dioxide Evolution and pH Changes During the Decomposition of Cedar Sawdust in Chehalis Silty Clay Loam Soil

Treatment	(hrs.)	pH	Carbon as CO_2 from soil + sawdust by interval	Carbon as CO_2 from soil + sawdust cumulative	Carbon as CO_2 from soil only by interval	Carbon as CO_2 from soil only cumulative	Carbon as CO_2 from sawdust by interval	Carbon as CO_2 from sawdust cumulative
			p.p.m.		p.p.m.		p.p.m.	
Sawdust°	45.5	6.8	43.8	43.8	26.0	26.0	17.8	17.8
	117.5	6.8	48.2	92.0	26.5	52.5	21.7	39.5
	240.5	6.7	59.8	151.8	28.5	81.0	31.3	70.8
	358	6.8	46.6	198.4	32.0	113.0	14.6	85.4
	477	6.6	29.4	227.8	39.5	152.5	-10.1	75.3
Sawdust plus N*	45.5	6.7	39.2	39.2	22.0	22.0	17.2	17.2
	117.5	6.6	43.6	82.8	27.0	49.0	13.6	30.8
	240.5	6.6	49.2	132.0	39.0	88.0	10.0	40.8
	358	6.7	39.4	171.4	29.0	117.0	10.4	51.2
	477	6.4	20.0	191.4	27.5	144.5	-7.5	43.7

°Sawdust addition equivalent to 2000 p.p.m. carbon
*Nitrogen added as ammonium nitrate to give a carbon:nitrogen ratio of 20:1

Table 7: Cumulative Carbon Dioxide Evolution and pH Changes During the Decomposition of Pine Sawdust in Chehalis Silty Clay Loam Soil

Treatment	Time (hrs.)	pH	Carbon as CO_2 from soil + sawdust by interval	Carbon as CO_2 from soil + sawdust cumulative	Carbon as CO_2 from soil only by interval	Carbon as CO_2 from soil only cumulative	Carbon as CO_2 from sawdust by interval	Carbon as CO_2 from sawdust cumulative
			p.p.m.		p.p.m.		p.p.m.	
Sawdust°	44	6.8	44.0	44.0	26.0	26.0	18.0	18.0
	117	6.6	46.6	90.4	26.5	52.5	19.9	37.9
	238	6.7	55.8	146.2	38.5	91.0	17.3	55.2
	356	6.8	79.8	226.0	32.0	123.0	47.8	103.0
	480	6.8	106.0	332.0	40.0	163.0	66.0	169.0
Sawdust plus N*	44	6.6	41.2	41.2	21.5	21.5	19.7	19.7
	117	6.4	40.8	82.0	27.0	48.5	13.8	33.5
	238	6.5	47.2	129.2	39.0	87.5	8.2	41.7
	356	6.4	60.4	189.6	28.5	116.0	31.9	73.6
	480	6.2	68.0	257.6	27.5	143.5	40.5	114.1

°Sawdust addition equivalent to 2000 p.p.m. carbon
*Nitrogen added as ammonium nitrate to give a carbon:nitrogen ratio of 20:1

Table 8: Cumulative Carbon Dioxide Evolution and pH Changes During the Decomposition of Dextrose in Chehalis Silty Clay Loam Soil

Treatment	Time (hrs.)	pH	Carbon as CO_2 from soil + dextrose by interval p.p.m.	Carbon as CO_2 from soil + dextrose cumulative p.p.m.	Carbon as CO_2 from soil only by interval p.p.m.	Carbon as CO_2 from soil only cumulative p.p.m.	Carbon as CO_2 from dextrose by interval p.p.m.	Carbon as CO_2 from dextrose cumulative p.p.m.
Dextrose°	44	6.8	219.8	219.8	26.0	26.0	193.8	193.8
	120	6.6	210.8	430.6	26.5	52.5	184.3	378.1
	237	6.8	194.2	624.8	38.5	91.0	155.7	533.8
	355	6.9	113.2	738.0	32.0	123.0	81.2	615.0
	474	6.8	100.8	838.8	39.5	162.5	61.3	676.3
Dextrose plus N*	44	6.9	259.2	259.2	22.0	22.0	237.2	237.2
	120	6.8	238.8	498.0	27.0	49.0	211.8	449.0
	237	6.8	166.6	664.6	39.0	88.0	127.6	576.6
	355	6.7	89.0	753.6	28.5	116.5	60.5	637.1
	474	6.5	67.8	821.4	27.5	144.0	40.3	677.4

°Dextrose addition equivalent to 2000 p.p.m. carbon
*Nitrogen added as ammonium nitrate to give a carbon:nitrogen ratio of 20:1

Subtracting the control value, therefore, gives a result that may be higher than actual. While this is of theoretical interest, and may lead to more or less erroneous percentage decomposition values, the practical significance is not great. Since carbon dioxide evolution is an acceptable index of general microbial action and organic decomposition, a change in carbon dioxide production indicates a change in these over-all activities. Whether or not some small part of the amount of carbon dioxide involved in calculated differences has originated from the addition or from the soil's own organic matter is less important.

Allowing for these mutual influences, the calculated decomposition in Table 9 show a definite depressive effect of added available nitrogen. Except for dextrose, the effect was exerted on the different materials in the order of their decomposability without added nitrogen, but there was no correlation with total carbon, Kjeldahl nitrogen or carbon:nitrogen ratio. Douglas-fir and hemlock sawdusts were decomposed most, while cedar was comparatively much more resistant. The pine sawdust was intermediate in decomposability. Ammonium nitrate lowered carbon dioxide evolution from sawdusts throughout the experiment, and, based on the first observation at 48 hours, apparently from the beginning.

Table 8 presents data showing more clearly the initial stimulating influence and the final depressive effect of nitrogen on the rate of net carbon dioxide evolution from dextrose. The total carbon dioxide from the soil plus dextrose shows essentially the same effect, but the final difference is slightly greater. This indicates that dextrose with added nitrogen increased to some extent the decomposition of the native soil organic matter. In the absence of dextrose the added nitrogen depressed carbon dioxide evolution from the soil's own organic matter throughout the respiration period. Such effects were previously described by Bollen (1, p. 369). In this connection, the results of Broadbent and Bartholomew (2, pp. 271-274) are of interest; they observed that when sudan grass residues enriched with C^{13} were added to a soil, a larger proportion of carbon dioxide originated from the soil organic matter than in the control soil. It is therefore apparent that organic additions, by promoting activity of the soil population, also may cause the native organic matter to be more vigorously attacked.

Figure 8 represents pH changes during the course of decomposition of the various materials with and without added available nitrogen. Differences were relatively small but increased at the end of the experiment. The addition of ammonium nitrate with the sawdust samples held the pH consistently lower. Reasons for this were not

Table 9: Calculated Decomposition* of Sawdust and of Dextrose in Chehalis Silty Clay Loam Soil

Treatment	Decomposition*** percent
Douglas-fir sawdust	11.5
Douglas-fir sawdust plus N**	7.3
Hemlock sawdust	11.0
Hemlock sawdust plus N	5.7
Cedar sawdust	3.8
Cedar sawdust plus N	2.2
Pine sawdust	8.5
Pine sawdust plus N	5.7
Dextrose	33.9
Dextrose plus N	33.9

*Based on assumption that all carbon dioxide in excess of values for control (soil only) originated from added 2000 p.p.m., 60-mesh organic substance

**Nitrogen added as ammonium nitrate to give a carbon: nitrogen ratio of 20:1

***Decomposition at 28°C with optimum moisture for 20 days

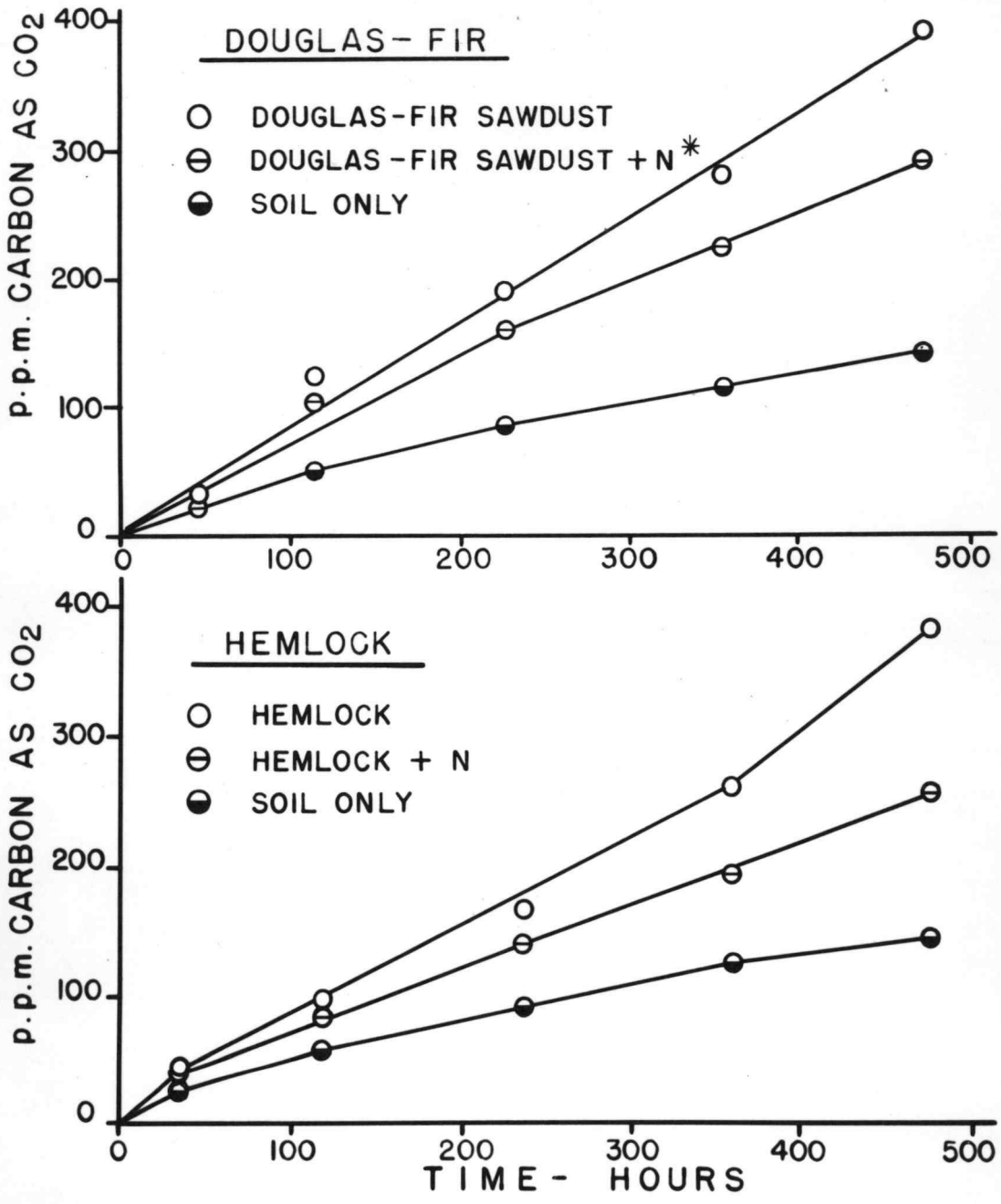

FIGURE 6: CUMULATIVE CARBON DIOXIDE EVOLUTION DURING DECOMPOSITION OF DOUGLAS-FIR & HEMLOCK SAWDUST WITH & WITHOUT ADDED NITROGEN* IN CHEHALIS SILTY CLAY LOAM SOIL

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF 20 : 1

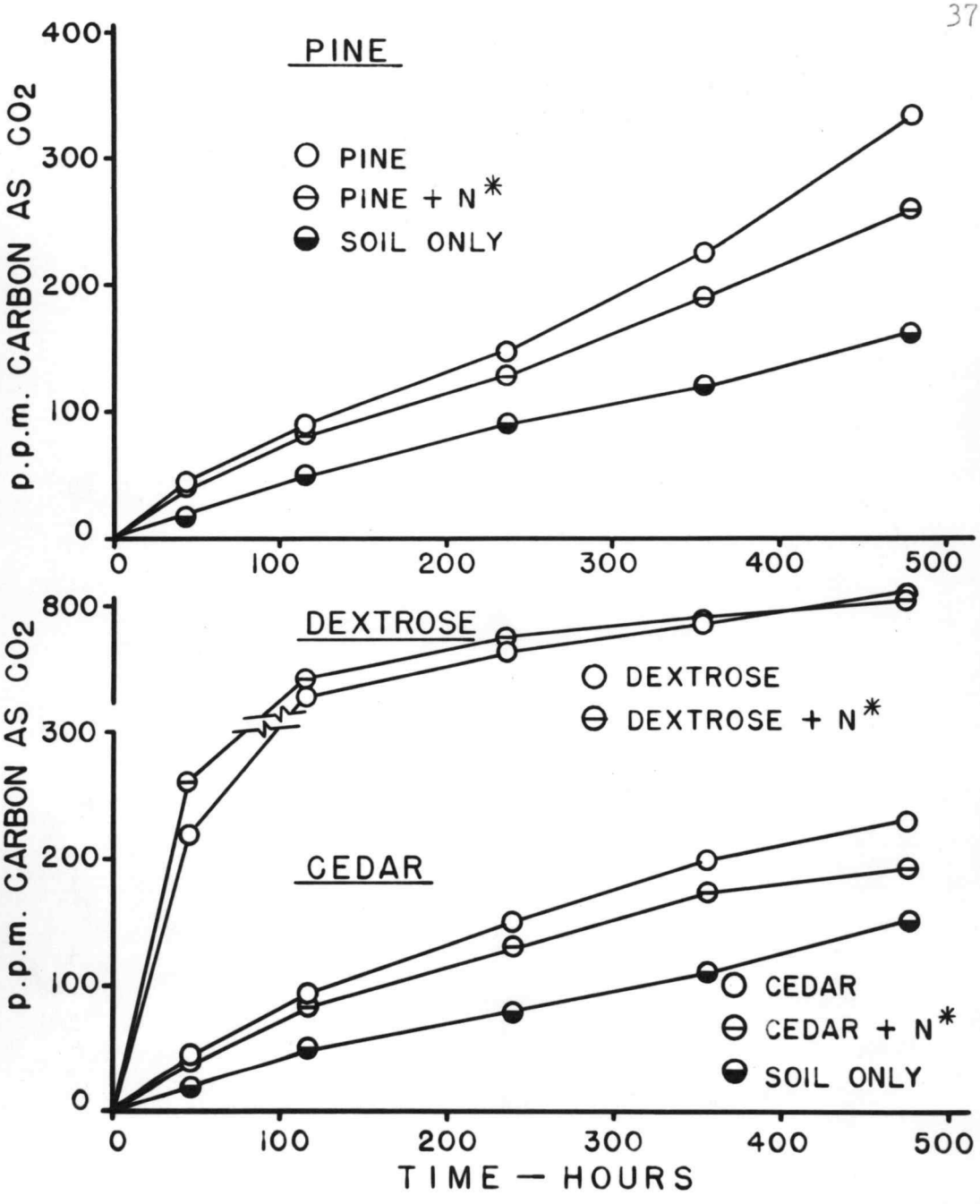

FIGURE 7: CUMULATIVE CARBON DIOXIDE EVOLUTION FROM PINE & CEDAR SAWDUST & DEXTROSE WITH & WITHOUT ADDED NITROGEN IN CHEHALIS SILTY CLAY LOAM SOIL; VALUES FOR SOIL CONTROL NOT SUBTRACTED

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON: NITROGEN RATIO OF 20:1

established, but could involve preferential assimilation of the ammonium ion from ammonium nitrate and the accumulation of organic acids.

Data from plate counts is presented in Figures 9 to 18. Values for zero time, shown only for the soil control, are from results obtained by plating the original bulk sample at the time it was brought to the laboratory for use. These first determinations were used as a basis of comparison for all experiments reported in the thesis. Subsequent platings were made from time to time, but, inasmuch as the bulk sample was kept in a closed container stored in a cool place, the numbers and proportions of the several groups of microorganisms changed insignificantly.

Changes in numbers of bacteria and percentage of Streptomyces resulting from the different treatments are shown in Figures 9 to 13 inclusive. The Streptomyces percentage remained essentially constant throughout the experiment for all organic additions, and it was not markedly altered by the addition of available nitrogen. These organisms therefore showed responses similar to those of the true bacteria. The curves for total bacteria including Streptomyces follow a general pattern. There is an initial rise in numbers during the first few days after which a sharp decline is evident. For added available nitrogen the curves follow this general trend after the initial decrease. The graphs for cedar and pine sawdusts

and for dextrose are typical. Curves for Douglas-fir and hemlock sawdusts did not level off but continued to drop at the last plating. This could have been due to more rapid depletion of available carbon or to persistance of toxic substances, such as tannins or other extractives.

Figures 14 to 18 show changes in numbers of molds during the decomposition of the sawdusts and dextrose. Trends similar to those found for the bacteria and Streptomyces are indicated but are more variable. In nearly every case the total numbers were lower at first where available nitrogen was added. The effect on mucors and penicillia were not consistent; percentage occurence for these two groups showed a roughly inverse relationship.

Warburg Respiration Studies

The addition of nitrogen as ammonium nitrate increased respiration of the various groups of soil microorganisms as indicated by the data in Figure 19 and Tables 10 to 13 inclusively. Carbon dioxide evolution and oxygen uptake both were influenced in a similar manner. Data for the respiratory quotient (R.Q.), given in the tables, are not significantly different for the various treatments, but the trends during the course of the experiment are different, indicating occurence of growth during later stages.

Figure 19 indicates the influence of the available

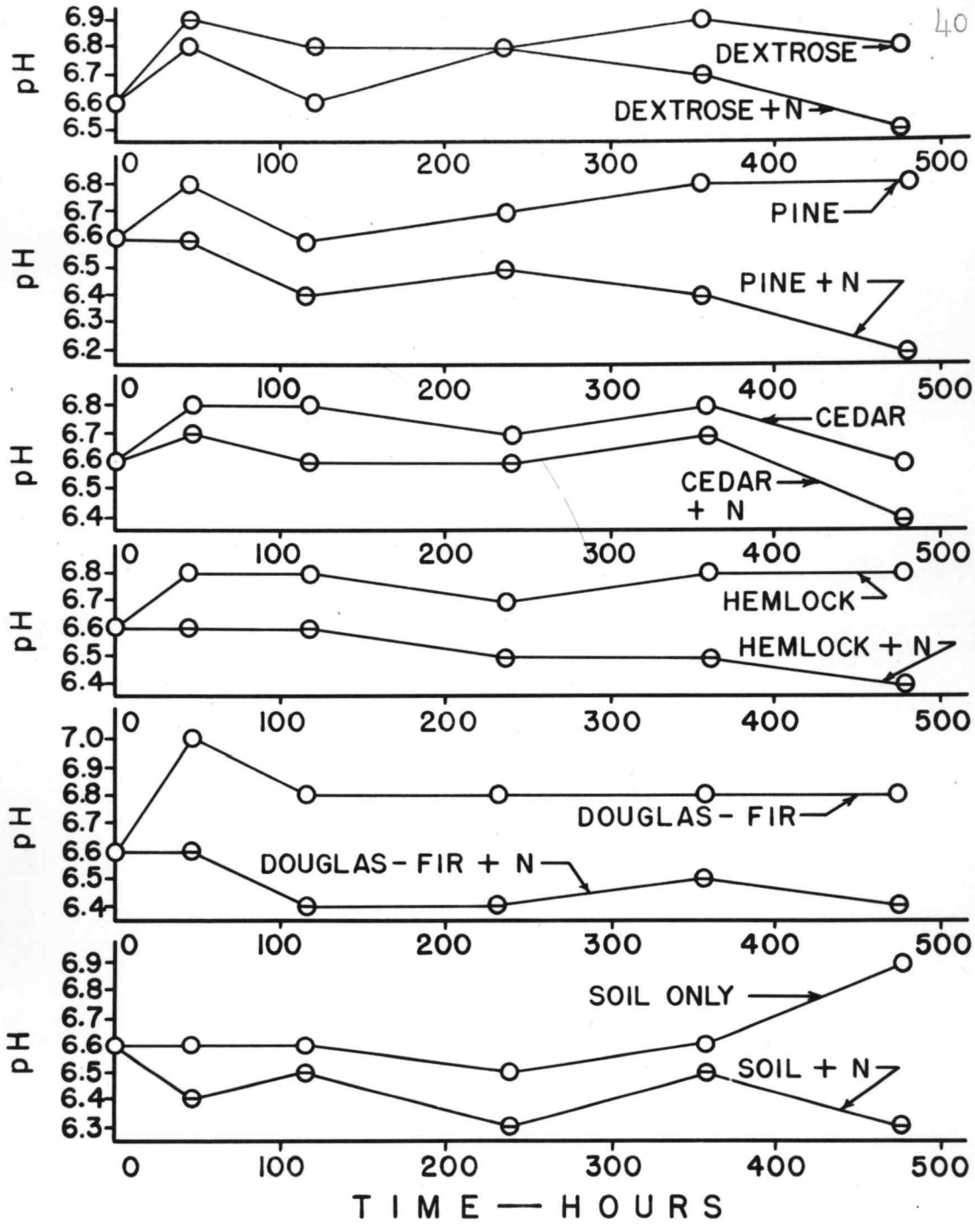

FIGURE 8: pH CHANGES IN CHEHALIS SILTY CLAY LOAM SOIL WITH SAWDUST & OTHER ORGANIC ADDITIONS, WITH & WITHOUT ADDED NITROGEN*

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON:NITROGEN RATIO OF 20:1

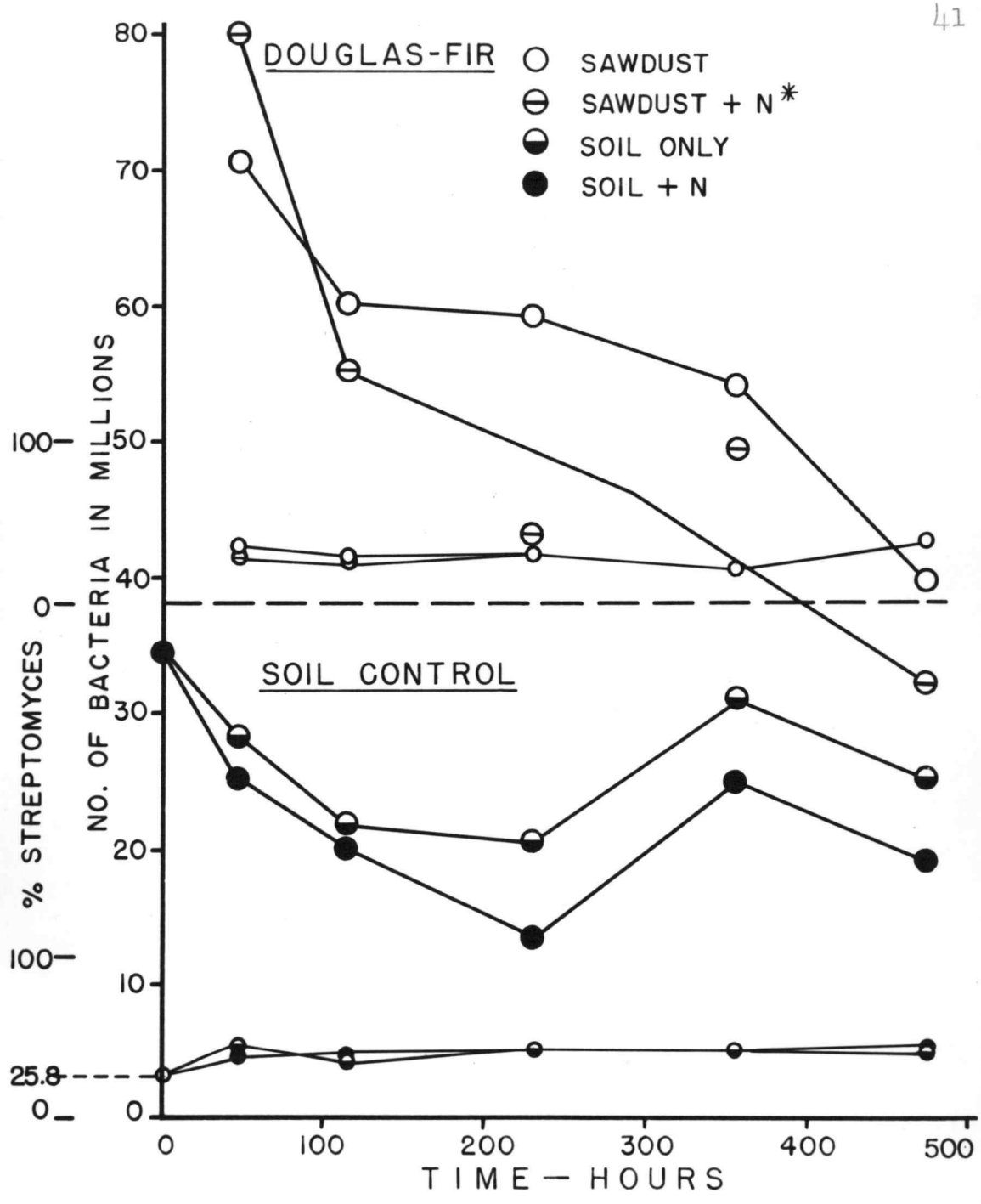

FIGURE 9: CHANGES IN NUMBERS OF BACTERIA & STREPTOMYCES DURING THE DECOMPOSITION OF DOUGLAS-FIR SAWDUST IN CHEHALIS SILTY CLAY LOAM SOIL

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON:NITROGEN RATIO OF 20:1

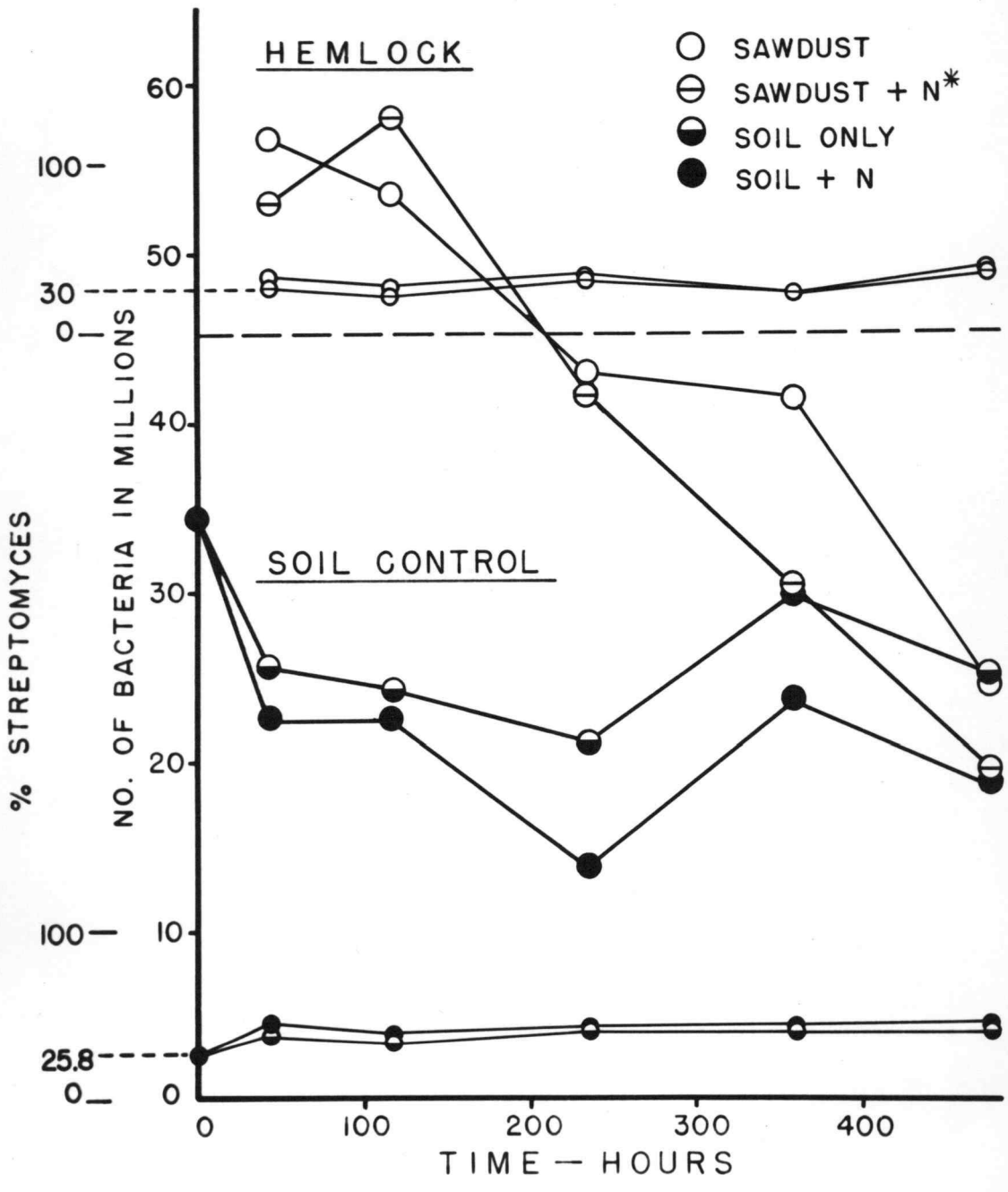

FIGURE 10: CHANGES IN NUMBERS OF BACTERIA & STREPTOMYCES DURING THE DECOMPOSITION OF HEMLOCK SAWDUST IN CHEHALIS SILTY CLAY LOAM SOIL

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON:NITROGEN RATIO OF 20:1

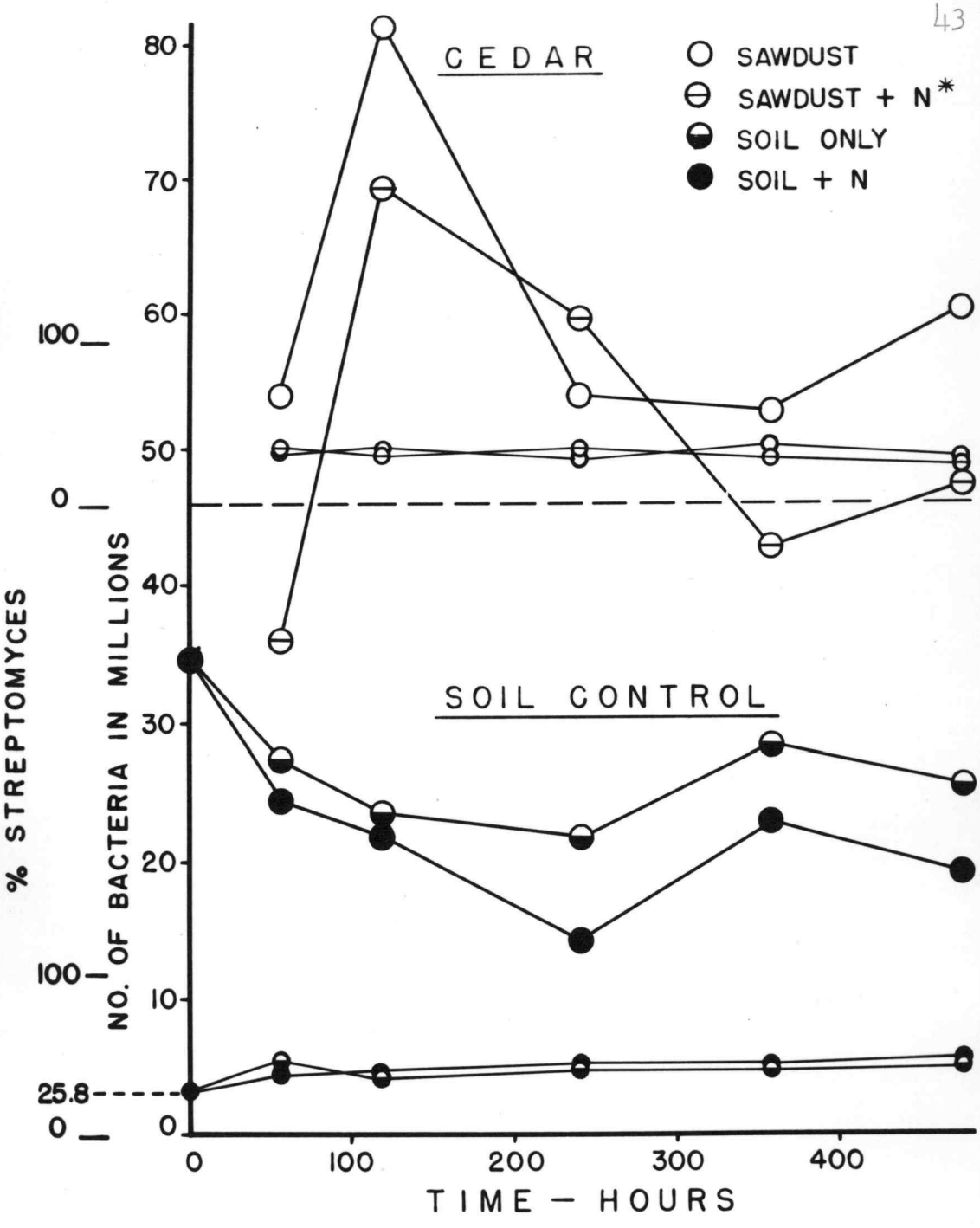

FIGURE 11: CHANGES IN NUMBERS OF BACTERIA & STREPTOMYCES DURING THE DECOMPOSITION OF CEDAR SAWDUST IN CHEHALIS SILTY CLAY LOAM SOIL

*NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF 20 : 1

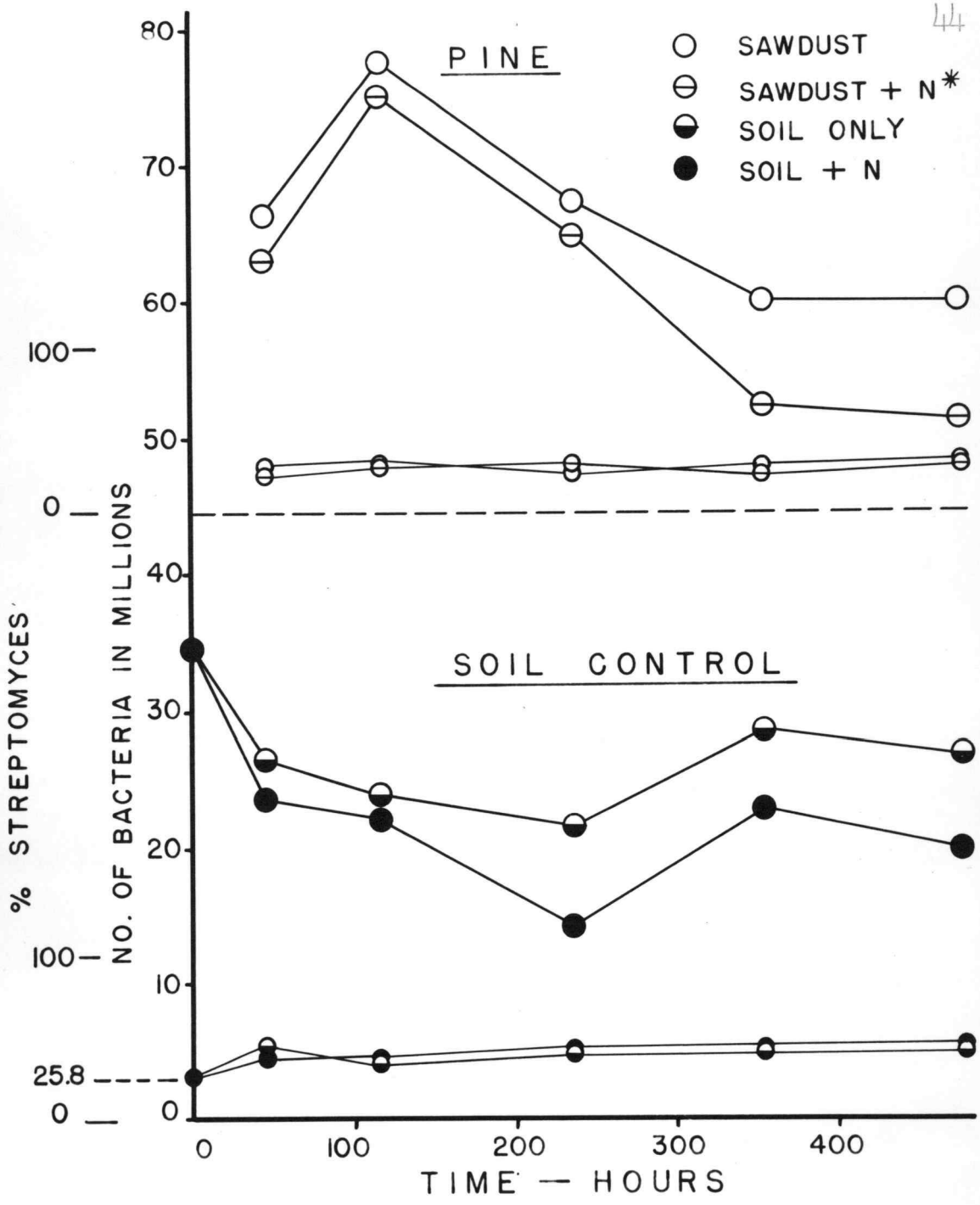

FIGURE 12: CHANGES IN NUMBERS OF BACTERIA & STREPTOMYCES DURING THE DECOMPOSITION OF PINE SAWDUST IN CHEHALIS SILTY CLAY LOAM SOIL

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF 20 : 1

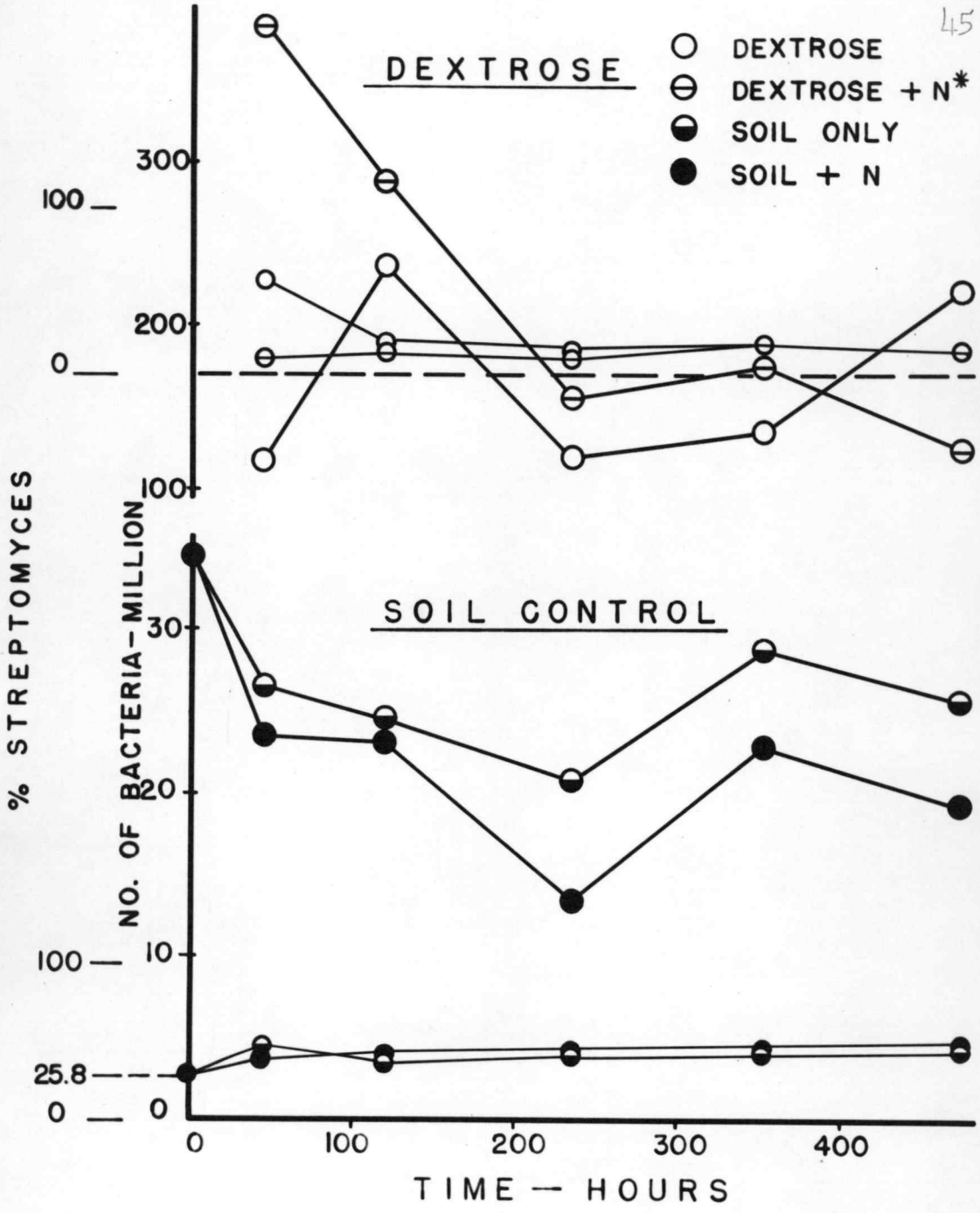

FIGURE 13: CHANGES IN NUMBERS OF BACTERIA & STREPTOMYCES DURING THE DECOMPOSITION OF DEXTROSE IN CHEHALIS SILTY CLAY LOAM SOIL

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF 20 : 1

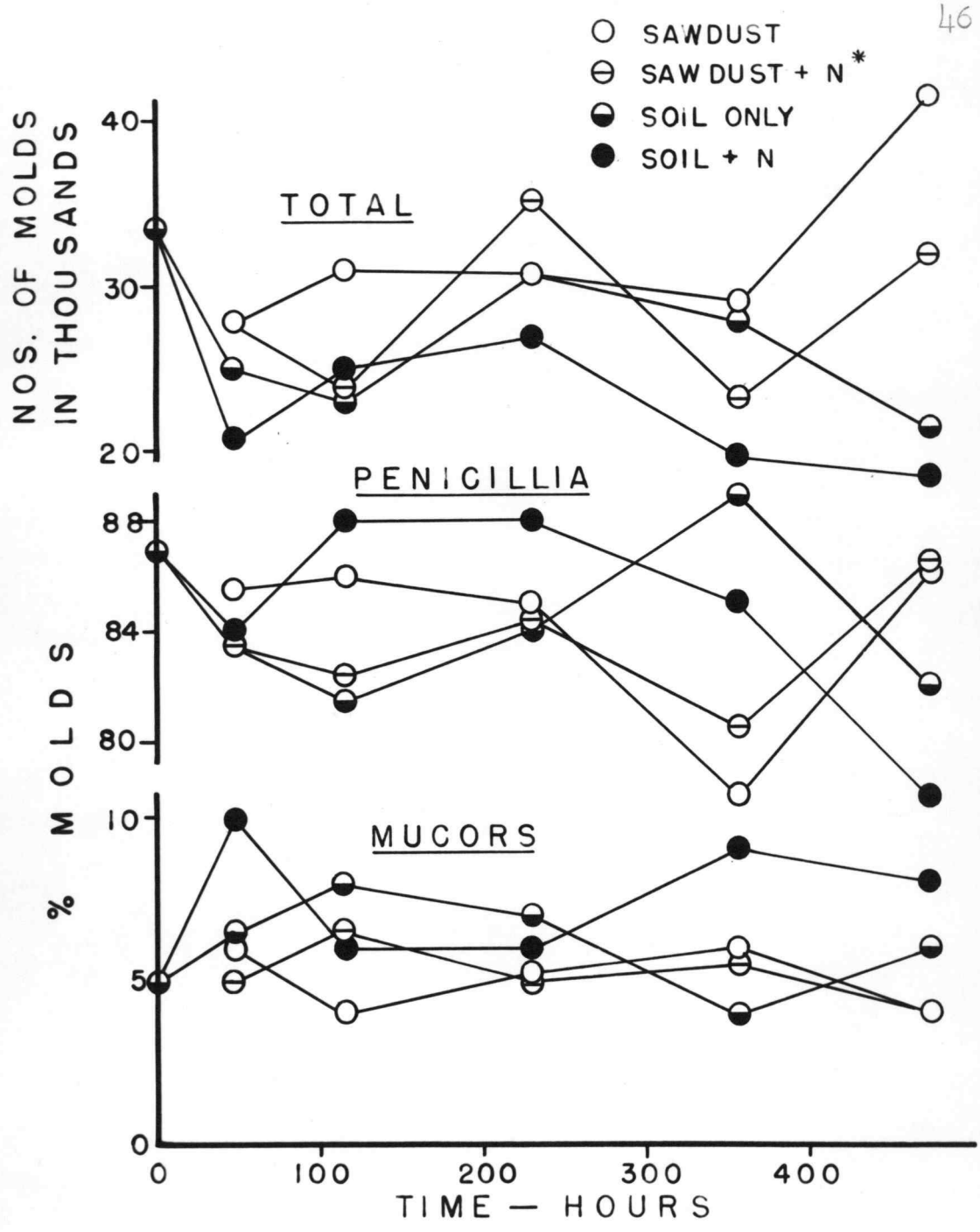

FIGURE 14 : CHANGES IN NUMBERS OF MOLDS DURING THE DECOMPOSITION OF DOUGLAS FIR SAWDUST IN CHEHALIS SILTY CLAY LOAM SOIL

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON: NITROGEN RATIO OF 20 : 1

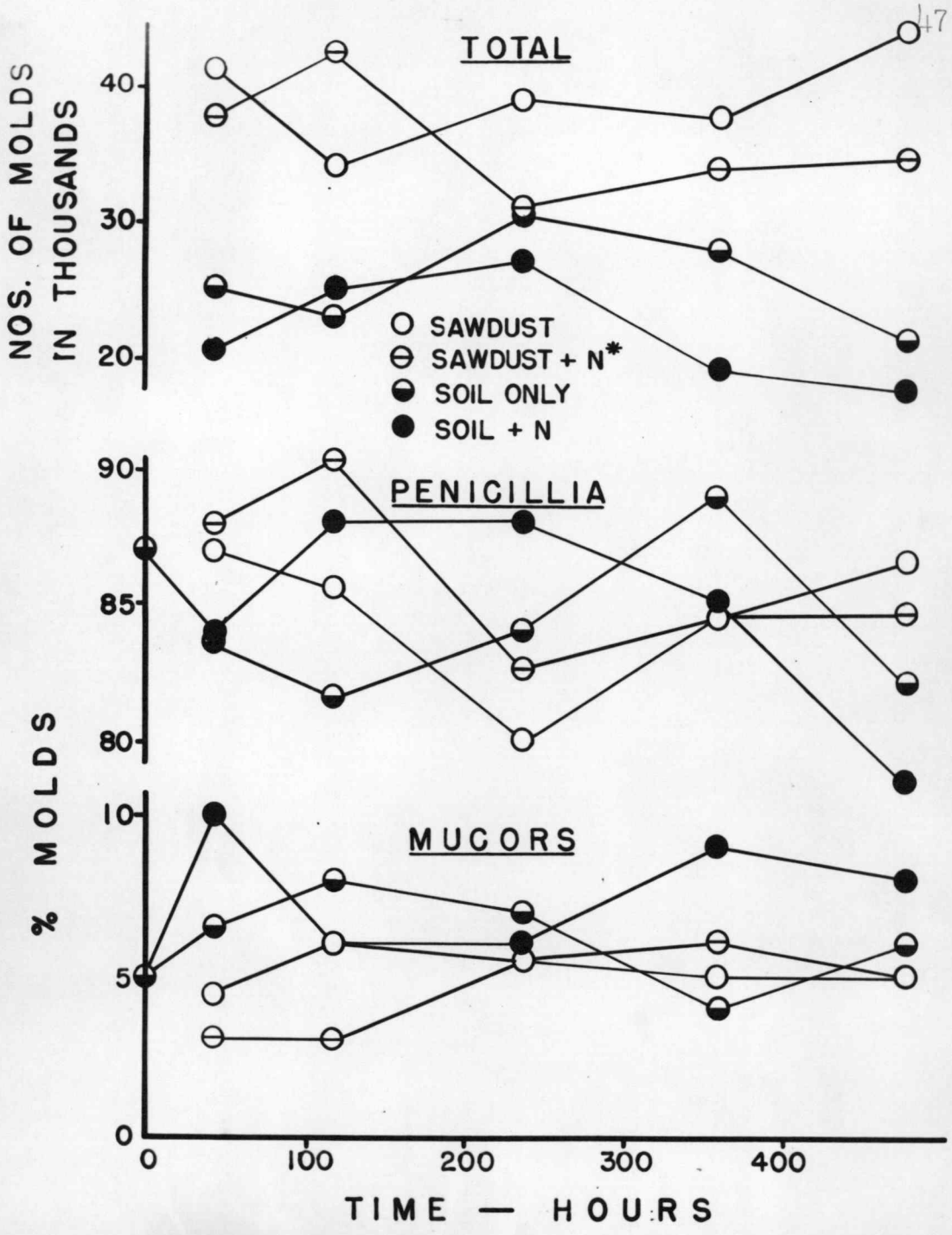

FIGURE 15: CHANGES IN NUMBERS OF MOLDS DURING THE DECOMPOSITION OF HEMLOCK SAWDUST IN CHEHALIS SILTY CLAY LOAM SOIL

*NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF 20 : 1

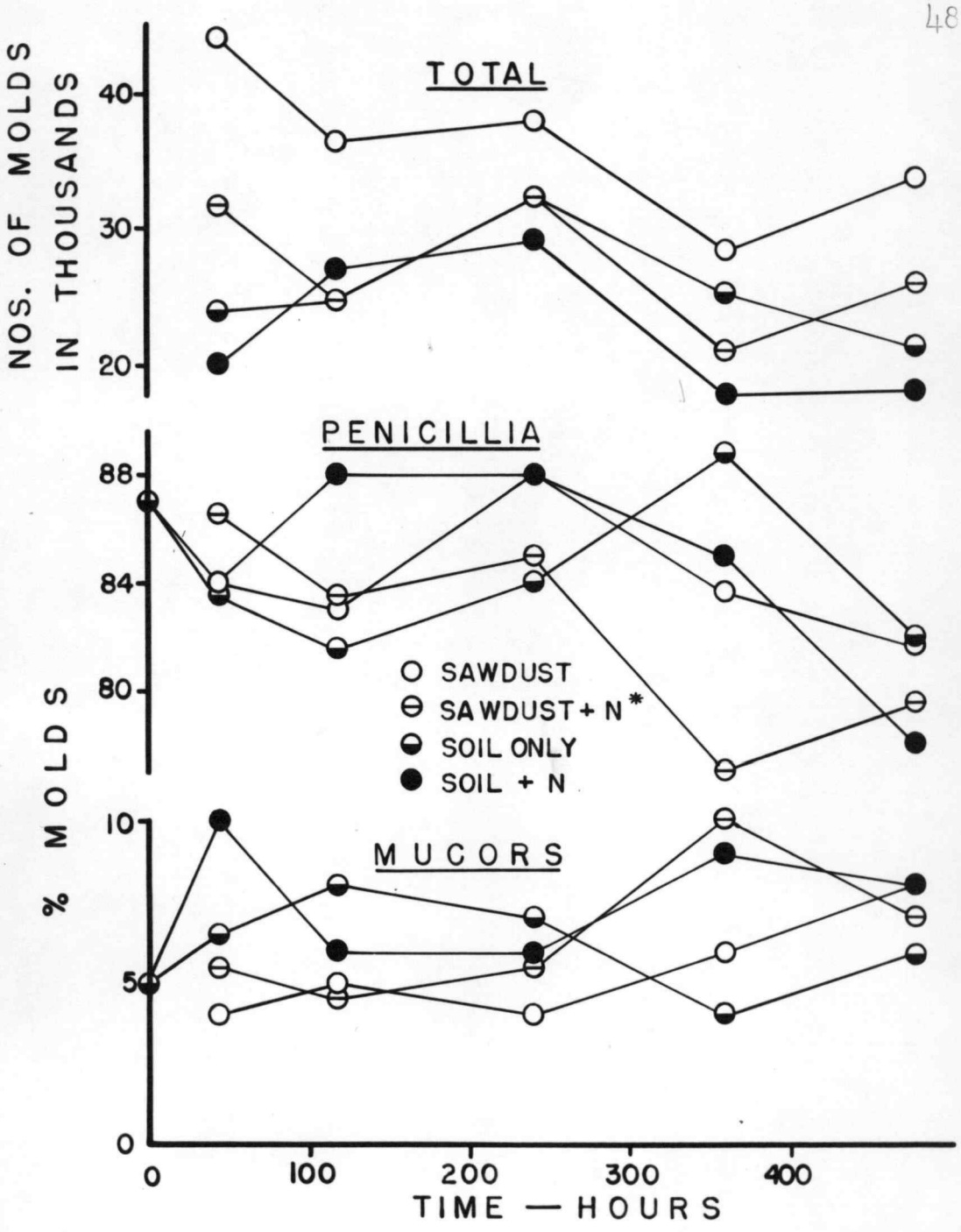

FIGURE 16 : CHANGES IN NUMBERS OF MOLDS DURING THE DECOMPOSITION OF CEDAR SAWDUST IN CHEHALIS SILTY CLAY LOAM SOIL

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF 20 : 1

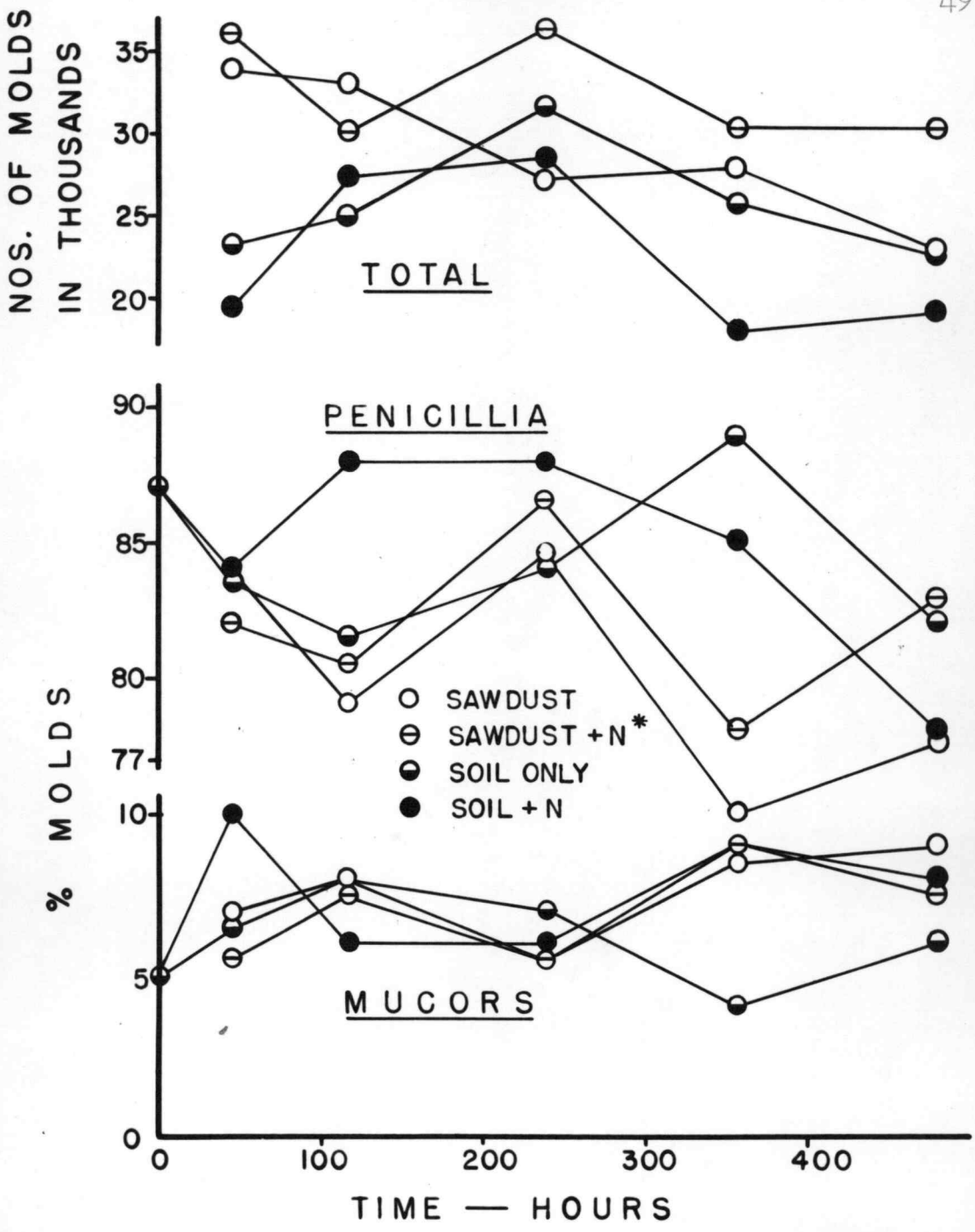

FIGURE 17: CHANGES IN NUMBERS OF MOLDS DURING THE DECOMPOSITION OF PINE SAWDUST IN CHEHALIS SILTY CLAY LOAM SOIL

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF 20:1

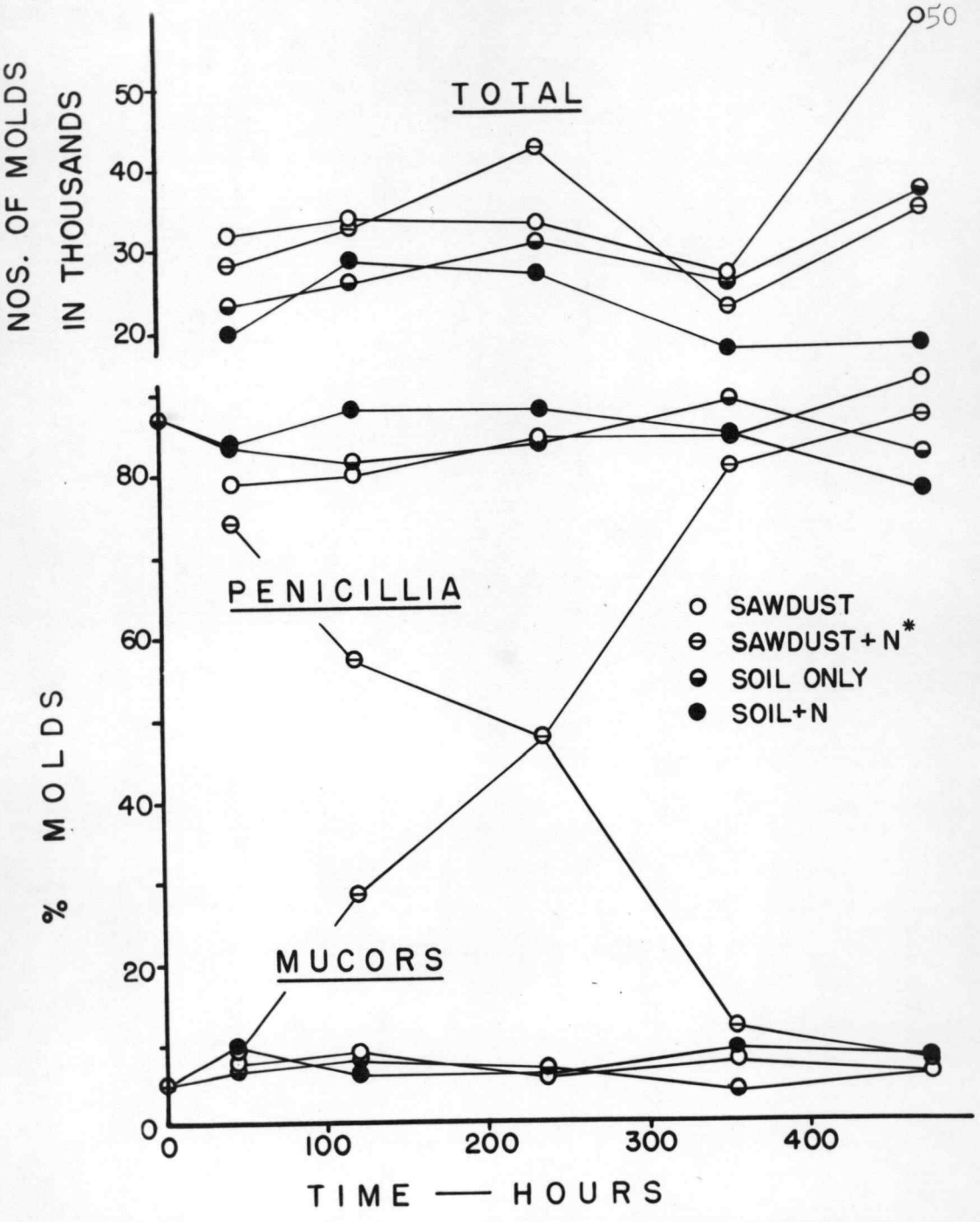

FIGURE 18 : CHANGES IN NUMBERS OF MOLDS DURING THE DECOMPOSITION OF DEXTROSE IN CHEHALIS SILTY CLAY LOAM SOIL

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF 20 : 1

Table 10: Influence of Available Nitrogen on Mold Respiration, Warburg Technique

Time (min.)	Endogenous			Dextrose			Dextrose plus N*		
	O_2 upt.	CO_2 evol.	R.Q.	O_2 upt.	CO_2 evol.	R.Q.	O_2 upt.	CO_2 evol.	R.Q.
	microliters			microliters			microliters		
10	2	0.5	0.25	4	2	0.50	4	6	1.50
20	1	0.3	0.30	6	6	1.00	10	16	1.60
30	3	2	0.67	12	13	1.08	18	21	1.17
40	2	- 1	-	16	18	1.12	21	26	1.24
50	6	6	1.00	20	24	1.20	28	35	1.25
70	9	10	1.11	34	30	0.88	50	54	1.08
90	11	12	1.09	44	42	0.95	62	66	1.06
110	14	18	1.28	52	50	0.96	77	82	1.06
130	19	18	0.95	64	59	0.92	98	100	1.02
160	24	25	1.05	81	76	0.94	128	142	1.11
190	27	26	0.96	94	92	0.98	166	170	1.02
220	29	28	0.97	107	106	0.99	199	196	0.99
250	33	33	1.00	124	124	1.00	242	251	1.04
280	37	36	0.97	140	142	1.01	283	294	1.04
310	39	36	0.92	152	158	1.04	329	342	1.07
340	41	38	0.93	170	174	1.02	374	392	1.05
370	48	44	0.92	184	194	1.05	416	439	1.05

*Ammonium nitrate added with glucose to give a C:N ratio of 20:1

Table 11: Influence of Available Nitrogen on *Streptomyces* Respiration, Warburg Technique

Time (min.)	Endogenous			Dextrose			Dextrose plus N*		
	O_2 upt.	CO_2 evol.	R.Q.	O_2 upt.	CO_2 evol.	R.Q.	O_2upt.	CO_2 evol.	R.Q.
	microliters			microliters			microliters		
10	4	5	1.25	12	10	0.83	14	13	0.93
20	7	9	1.28	20	18	0.90	24	22	0.92
30	12	16	1.33	30	28	0.93	32	30	0.94
40	14	16	1.14	40	40	1.00	46	40	0.87
60	22	25	1.14	59	54	0.92	66	60	0.90
80	25	29	1.16	80	74	0.93	86	79	0.92
100	34	34	1.00	100	90	0.90	114	100	0.88
130	38	38	1.00	130	114	0.88	150	133	0.89
160	42	43	1.02	155	146	0.94	190	169	0.89
190	49	53	1.08	184	170	0.92	238	214	0.90
220	54	52	0.96	212	188	0.89	282	252	0.89
250	60	56	0.93	232	212	0.91	327	294	0.90
280	64	58	0.91	257	233	0.91	372	336	0.90
310	66	62	0.97	280	257	0.92	414	376	0.91
340	70	64	0.92	303	280	0.92	457	412	0.90
360	75	68	0.91	318	290	0.91	488	442	0.90

Ammonium nitrate added with glucose to give a C:N ratio of 20:1

Table 12: Influence of Available Nitrogen on Bacterial Respiration, Warburg Technique

Time (min.)	Endogenous			Dextrose			Dextrose plus N*		
	O_2 upt.	CO_2 evol.	R.Q.	O_2 upt.	CO_2 evol.	R.Q.	O_2 upt.	CO_2 evol.	R.Q.
	microliters			microliters			microliters		
10	4	4	1.0	9	8	0.89	8	6	0.75
20	4	6	1.5	19	16	0.84	16	12	0.75
30	8	8	1.0	28	24	0.86	26	18	0.69
40	10	12	1.2	38	34	0.89	40	30	0.75
50	12	14	1.17	44	43	0.98	48	39	0.81
70	15	18	1.2	64	60	0.94	82	64	0.78
90	18	20	1.11	79	74	0.94	111	94	0.85
110	20	22	1.10	98	94	0.96	152	129	0.85
140	22	25	1.14	115	115	1.00	200	177	0.89
170	28	30	1.07	138	134	0.97	259	232	0.90
200	30	34	1.13	154	151	0.98	313	285	0.91
230	34	36	1.06	170	167	0.98	367	342	0.93
260	35	38	1.08	186	188	1.01	422	400	0.95
290	40	40	1.00	203	196	0.97	475	452	0.95
320	42	44	1.05	221	215	0.97	528	494	0.94

*Ammonium nitrate added with glucose to give a C:N ratio of 20:1

nitrogen upon mold respiration. Values for oxygen uptake and carbon dioxide evolution were substantially higher in the presence of nitrogen and dextrose after the first twenty minutes. After this initial period, differences became increasingly greater so that after six hours total carbon dioxide evolved and oxygen taken up was approximately 2 1/2 times as much as values for dextrose without added nitrogen. The data are also representative of values obtained with bacteria and Streptomyces; these were not plotted but are shown in Tables 11 and 12. The effect of added nitrogen on respiration of Streptomyces was less pronounced than with the molds, while for bacteria the influence was greater. This is in accord with differences in their efficiency of utilization of carbon source.

Reciprocating Incubator Studies

Data from this intermediate approach to the main problem of the thesis, using actively proliferating cells and a carbon:nitrogen ratio of 5:1 to force the effect, are presented in Tables 13 and 14 and Figures 20 and 21. Results thus obtained at more frequent intervals early in the decomposition of dextrose show that carbon dioxide evolution is correlated with depletion of ammonia as well as with disappearance of substrate. The ammonia was preferentially utilized; nitrate concentration remained essentially constant throughout the experiment, the effective

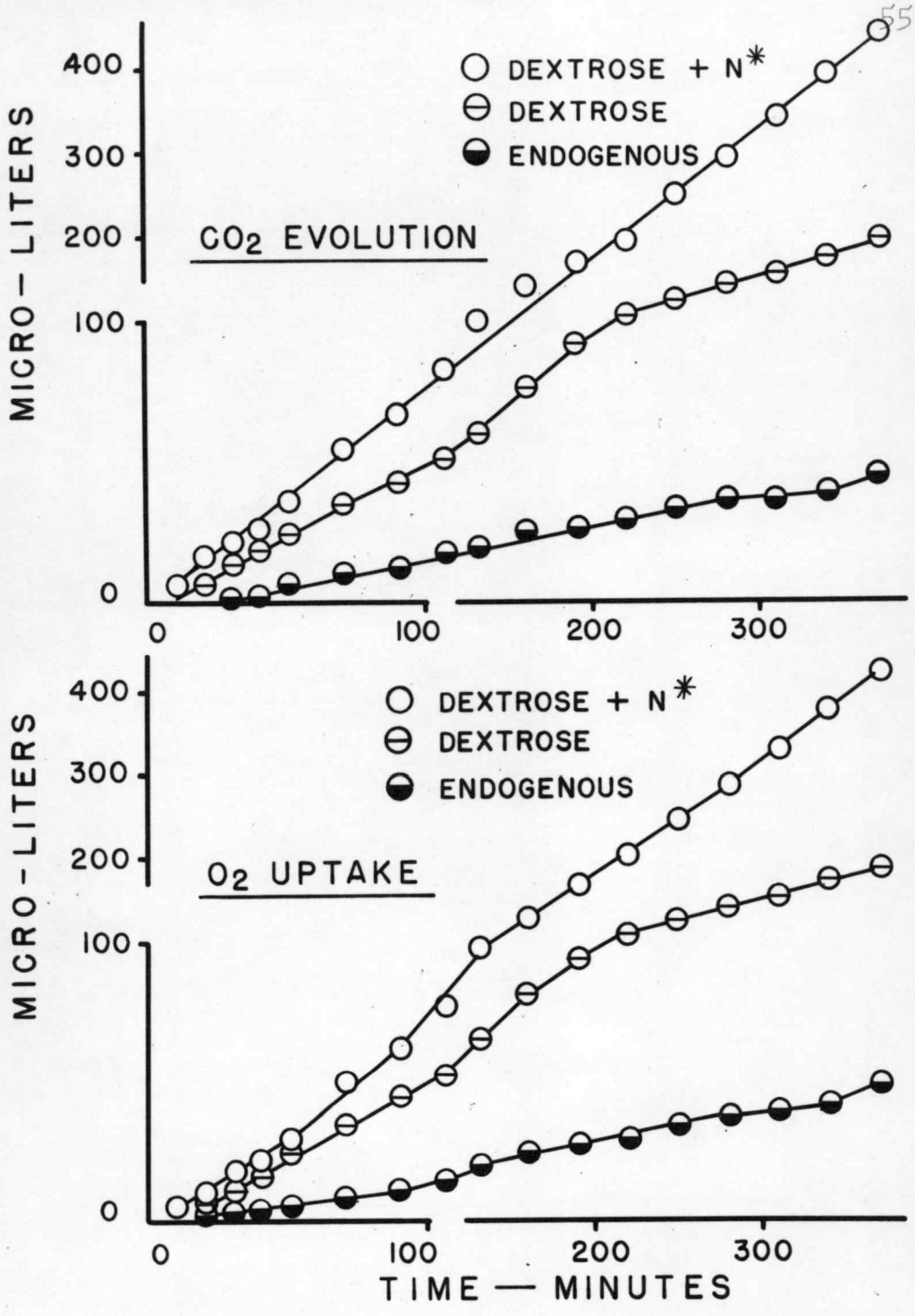

FIGURE 19: INFLUENCE OF AVAILABLE NITROGEN ON MOLD RESPIRATION

*NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF 20 : 1

carbon:nitrogen ratio therefore being 10:1.

Figure 20 represents a preliminary study with a single culture. The long lag was probably due to the use of an inoculum prepared directly from the bulk sample in which the microflora was resting or relatively inactive. Results in Figure 21 were obtained by using as an inoculum a suspension made from a sample of the soil previously incubated under optimum conditions for 5 days. A sufficient number of replicates also were included thus avoiding successive samplings and disturbances necessary with a single culture.

Without ammonium nitrate, little carbon dioxide was evolved and correspondingly, little or no dextrose was utilized, and the pH remained unchanged. With the active inoculum carbon dioxide was produced at a rapid rate from the beginning in the presence of available nitrogen; ammonia and dextrose decreased accordingly. Except for the lag in Figure 20, the two figures correspond in showing that available nitrogen causes an immediate rapid rise in substrate utilization and consequent carbon dioxide evolution, which continues until the substrate is virtually exhausted. Such depletion occurred with the concentration of dextrose employed.

In the soil respiration studies with the exception of dextrose, the nature of the organic additions was such that the concentrations of readily available carbon were

only a minor fraction of the total (2000 p.p.m.). Thus, analysis of Douglas-fir sawdust showed the presence of approximately 3.5 percent reducing sugars plus 4.8 percent substances soluble in cold water. In this case less than 80 p.p.m. readily available carbon was added to the soil. It is, therefore, apparent that with sawdust and other plant materials of mixed composition and low in available carbon, the initial increased carbon dioxide evolution caused by added nitrogen could well be overlooked if observations were not made at frequent intervals during the first 24 to 48 hours. Apparent also from the solution culture data is the fact that utilization of 120 mgm. carbon as dextrose results in the liberation of between 45 and 50 mgm. carbon as carbon dioxide. Since the substrate was exhausted at this time, the remaining 70 to 75 mgm. carbon must be accounted for in synthesized cell substance and incomplete oxidation products. Among the latter could be organic acids. The low pH, 2.98, at the close of the experiment, indicated that such acids might have accumulated in considerable amounts; although, residual nitrate ion could also contribute to the relative acidity. Calculations show that hydrogen ion equivalent to 0.225 mgm. nitrate-nitrogen per milliliter would lower the pH of an unbuffered solution to approximately 1.8. This same concentration of nitrate ion added as nitric acid to a sample of the culture medium was found to change the pH from 6.88

Table 13: Influence of Available Nitrogen on Utilization of Carbon Source* (Single Culture, Reciprocating Incubator)

Treat.	Time hrs.	Carbon as CO_2 interv.	Carbon as CO_2 cumul.	Carbon** resid.	Carbon** used	Ammonia resid.	Ammonia used
Dextrose	0			1.20	0		
	2	0.2	0.2				
	4	-	0.2				
	6	0.2	0.4	1.20	0		
	22	0.2	0.6				
	26	-	0.6				
	29	-	0.6	1.20	0		
	49	0.9	1.5				
	55	0.3	1.8	1.09	0.11		
	99	2.1	3.9	0.93	0.27		
Dextrose plus N°	0			1.20	0	0.22	0
	2	0.2	0.2				
	4	-	0.2				
	6	0.4	0.6	1.20	0	0.19	0.03
	22	0.2	0.8				
	26	-	0.8				
	29	-	0.8	1.20	0	0.19	0.03
	49	0.5	1.3				
	55	0.1	1.4	1.04	0.16	0.19	0.03
	99	44.1	45.5	0	1.20	0.08	0.14

*Values given as milligrams per milliliter
**Carbon as 40 percent of dextrose added
°Ammonia nitrogen added as ammonium nitrate; C:N ratio equals 10:1

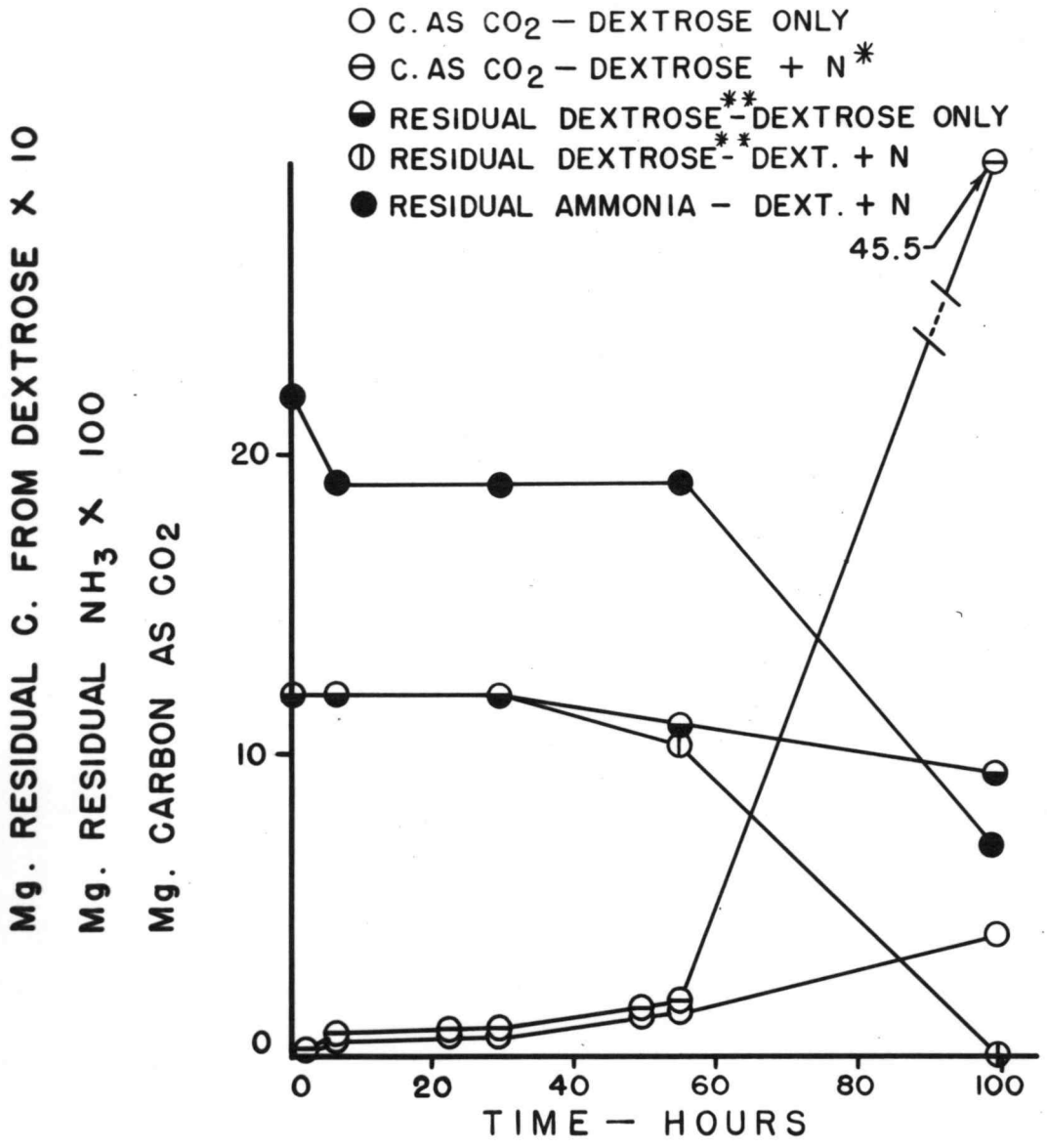

FIGURE 20: INFLUENCE OF AVAILABLE NITROGEN ON THE UTILIZATION OF CARBON SOURCE (SINGLE CULTURE RECIPROCATING INCUBATOR)

* NITROGEN AS AMMONIUM NITRATE ADDED TO GIVE A CARBON : NITROGEN RATIO OF – 5 : 1

** DEXTROSE AS CARBON (40 % OF DEXTROSE)

Table 14: Influence of Available Nitrogen on Utilization of Carbon Source (Replicate Cultures, Reciprocating Incubator)

Treat.	Time (hrs.)	Carbon as CO_2 interv. mg./100 ml.	Carbon as CO_2 cumul. mg./100 ml.	Carbon* resid. mg./ml.	Carbon* used mg./ml.	Nitrogen as Ammonia resid. mg./ml.	Nitrogen as Ammonia used mg./ml.	pH
Dextrose	0			1.20	0			6.88
	4	0.05	0.05					
	19.5	1.20	1.25	1.20	0			
	29.75	0.60	1.85	1.20	0			
	43.5	1.65	3.50	1.20	0			6.88
Dextrose plus N**	0			1.20	0	0.225	0	6.88
	4	0.95	0.95					
	19.5	15.25	16.20	0.64	0.56	0.134	0.091	
	29.75	2.80	19.00	0.42	0.78	0.116	0.109	
	43.5	28.45	47.45	0	1.20	0.073	0.152	2.98

*Carbon = 40 percent of dextrose added = 1200 p.p.m.
**Ammonia nitrogen added as ammonium nitrate to give a carbon:nitrogen ratio of 10:1

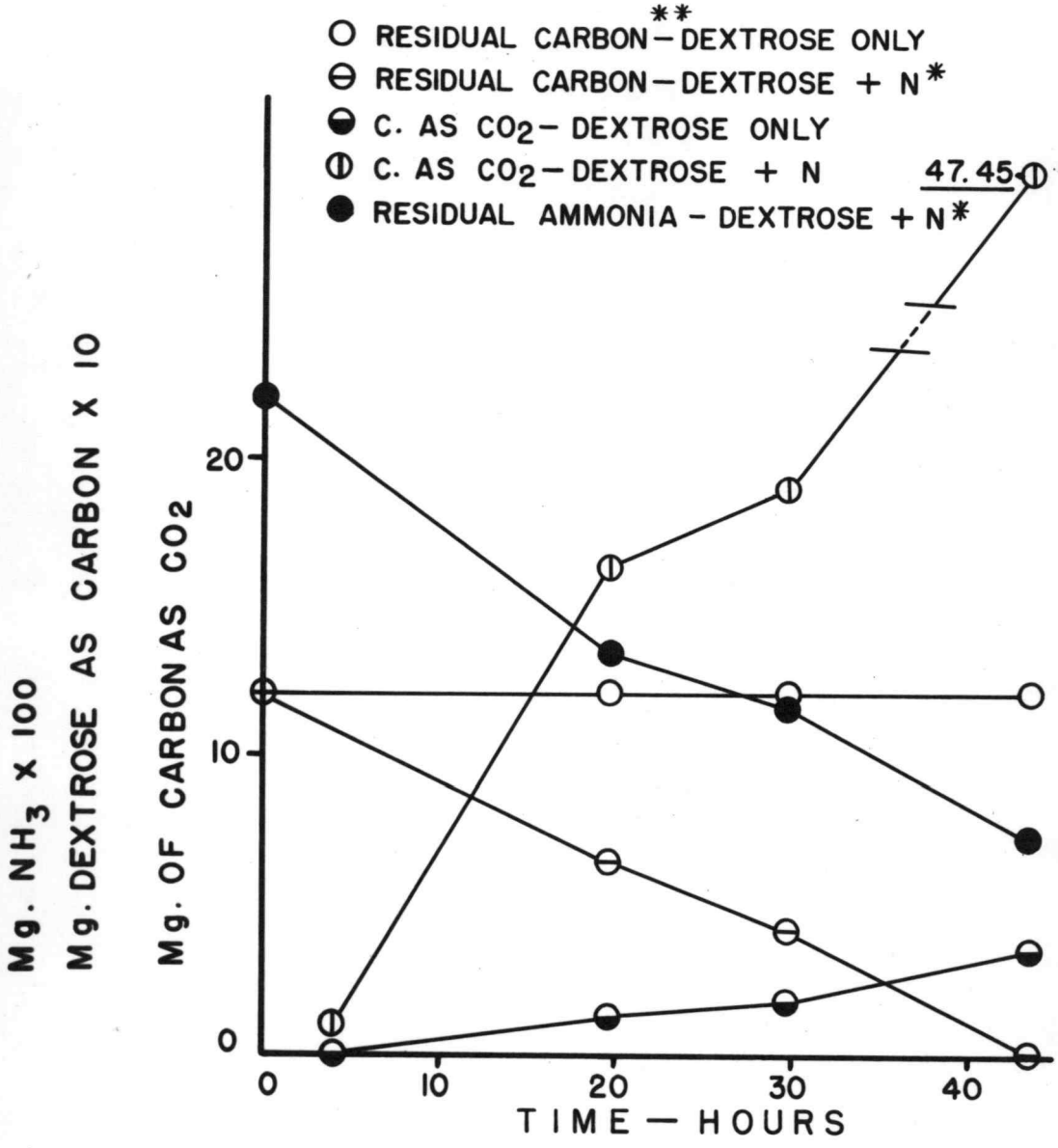

FIGURE 21: INFLUENCE OF AVAILABLE NITROGEN ON THE UTILIZATION OF CARBON SOURCE (REPLICATE CULTURES, RECIPROCATING INCUBATOR)

* NITROGEN ADDED AS AMMONIUM NITRATE TO GIVE A CARBON : NITROGEN RATIO OF – 5 : 1

** DEXTROSE AS CARBON (40 % OF DEXTROSE)

to 2.98, the same as found at the close of the experiment in the solution cultures with ammonium nitrate.

DISCUSSION

According to theory and practical observations adjusting the carbon:nitrogen ratio to an optimum value of approximately 20:1 increases the rate of decomposition. In the preliminary investigations, as well as in previous experiments in this laboratory, lower values for carbon dioxide evolution were observed in almost every case when available nitrogen was added to the different organic materials. These results would seem to correlate with the low proportion of available carbon in such materials and suggest that a brief but unobserved stimulation may have occurred in the usual time interval of 24 to 48 hours elapsing before the first carbon dioxide determination was made. Respiration data for Douglas-fir white rot, wheat straw, and corn cobs were more in accord with results to be expected from a wide carbon:nitrogen ratio. Since sawdust has even less nitrogen in proportion to carbon and also is lower in immediately available carbon compounds, it would seem that these characters may be involved in the anomalous results.

Many results showing long continued stimulation from nitrogen additions to different materials have been reported in previous literature by other investigators. In

these instances more substrate presumably was immediately available, thus enabling the nitrogen to effect a longer period of more rapid decomposition. The exact nature of the additions, such as chemical composition, stages of maturity, or other characteristics that could indicate amounts of readily available carbon often are not stated. In other instances, when such information is given, the duration of the experiment was often too short for development of apparent depressive effects. Moreover, in different soils the availability of the soil's own nitrogen was probably quite different.

Requirements for available nitrogen in the decomposition of organic material have long been recognized. Since some of the carbon source is utilized for cell tissue, nitrogen is required for the synthesis. Murray (15, p. 241) noted that when straw and nitrate were added to soil, the nitrate was assimilated although there was no decrease in total nitrogen. Heukelekian and Waksman (9, p. 341) observed correlation between carbon assimilation and the amount of nitrogen required for tissue formation. Because young cells contain more nitrogen than mature cells, they assimilate less carbon for each unit of nitrogen used. Nitrogen thus stored becomes unavailable until the cells die; ultimately, cell residues are decomposed by other soil organisms, but bacterial protein is especially resistant. Experiments by Sturgis (21, p. 696) showed that

decomposition of sugar cane trash, which is relatively high in available carbon compounds, was increased in the early stages by addition of ammonium sulfate. In comparing the decomposition of fresh leguminous and non-leguminous plant residues, Millar (13, pp. 87-88) found that more carbon dioxide was evolved from materials containing the greater amounts of nitrogen. He showed that under favorable conditions the rate of decomposition of organic matter was correlated with nitrogen content.

It is thus well established by laboratory and field observations that addition of nitrogenous fertilizers to plant residues of wide carbon:nitrogen ratio hastens decomposition. Carbon dioxide evolution should increase accordingly. More rapid and extensive carbon dioxide evolution in the absence of added available nitrogen, as indicated by the graphs in our experiments, therefore requires some explanation.

The possibility that a decrease in carbon dioxide could result from some detrimental effect of added nitrogen on the soil population must be considered. Our results show several examples of depression of bacteria and molds in the presence of added nitrogen. These seemingly correlate with the depression in carbon dioxide evolution. It is possible that the usual plating techniques employed are inadequate for indicating precisely certain significant effects of organic and other additions upon the soil

microflora. Norman (17, pp. 801-802) observed that plating techniques did not distinguish between inactive and active forms of organisms (3, p. 265), (19, pp. 465-467). This would apply particularly in the case of the molds; the first result of establishing conditions favorable for growth would be germination of spores and elaboration of mycelium. For the first few hours or days, therefore, increased mold activity would not be indicated by plate counts. Plate counts in the same soil under less favorable conditions could even be higher. Spores present at the beginning could germinate and produce mycelium which would be active for only a few hours because of a lack of sufficient substrate and then develop many more spores than the number originally present. Because of competition provided by the developing mycelia, molds could also contribute to a lower bacterial count in a soil more favorable for general microbial growth. These effects, however, cannot be considered as an over-all depressive influence on the activity of the soil population as a whole; hence, in the early stages of decomposition, plate counts would seem less reliable than carbon dioxide evolution as an index of microbial activity. Since no overwhelming differences were shown, the plate counts are considered not significant.

Another possibility to consider is the depressive effect of increased relative acidity that could result from the addition of ammonium nitrate or other nitrogen sources

that would leave a physiologically acid residue after assimilation of the nitrogen. In the solution culture study, pH values became quite low with preferential assimilation of ammonia from the ammonium nitrate, and this may have been due entirely to the residual nitrate anion; however, in the soil which is more effectively buffered, the effect on pH would be much less. As the results of Figure 8 show, the greatest pH change observed with ammonium nitrate additions to the soil was 0.4. It seems unlikely that this relatively small change in the region of neutrality would account for the differences observed in carbon dioxide evolution.

Time appears significant in the apparent depressive effects of added nitrogen upon carbon dioxide evolution. It is possible to have a rapid initial increase in activity that would be limited to a few hours duration by a limited amount of available carbon or nitrogen. In the case of sawdust and other plant residues of mixed composition the supply of soluble carbonaceous material is low, often less than two or three percent. Utilization of this small amount requires relatively little available nitrogen so that the soil itself might supply it; however, the soil's own nitrogen is largely in resistant organic combination and as such is much less available than added ammonia or nitrate. In different soils the supply of available nitrogen differs with the native store and the capacity of the

microflora to render it available. Whether or not nitrogen is adequate, a limited amount of available carbon in the organic additions would support increased activity and cell proliferation for only a brief time. It is entirely possible that increased growth and carbon dioxide evolution resulting from nitrogen added with sawdust and other similar materials would therefore occur within a period of a few hours, or some time before the first 48-hour observation as used in the preliminary experiments. Results from Warburg studies and reciprocating incubator respiration experiments show this to be true. Rapid carbon dioxide evolution and disappearance of dextrose occurred in the presence of available nitrogen. Under such conditions there is an increased amount of carbon metabolized; more tissue is formed and more carbon dioxide is evolved. This favorable state is maintained during a period lasting until virtual depletion of the readily available fraction of the organic material, regardless of its nature.

As a result of this comparatively rapid disappearance of available material, the microorganisms decrease in activity and numbers; accordingly, carbon dioxide evolution then falls sharply to a low, more or less constant rate determined by the higher resistance of the residual substrate, which now includes the carbon assimilated in cell substance. Without readily available nitrogen, on the other hand, even the little immediately available carbon of

sawdust and other similar additives are limited to slower decomposition. Since assimilation also must be slower, the time required to exhaust the soluble substrate is prolonged. At the same time, humus or other resistant nitrogen compounds in the soil are being slowly attacked by specific organisms of the autochthonous microflora; because of the narrow carbon:nitrogen ratio, approximately 12:1, ammonia as well as carbon dioxide appears as a waste product. The respiration curve without added nitrogen, therefore, rises with a lesser slope but extends farther before decreasing to the low, essentially constant rate characteristic of the residual substrate. The two curves therefore soon cross; the more soluble material originally present, the sooner the crossing. Unless observations are sufficiently close, this crossing will not appear with the data plotted.

CONCLUSIONS

It has been shown that failure to observe an increase in rate of carbon dioxide evolution from sawdust and similar materials following additions of available nitrogen can be attributed to an insufficient number of observations during the first few hours. The amount of readily available carbon compounds in these materials is so limited that their rapid decomposition is completed within a very brief time. This, with accompanying assimilation of substrate,

causes the respiration curve to decrease in slope more rapidly than the curve representing decomposition without added nitrogen. Concomitant decomposition of the soil's native organic matter contributed to the phenomena leading to early crossing of the curves.

By more frequent determinations of carbon dioxide evolution during the first few hours, the results of soil respiration experiments with sawdust should support field observations showing the stimulating effect of available nitrogen. In this they also should agree with similar experiments on materials containing more immediately available carbon.

SUMMARY

Results from preliminary investigations indicated that the addition of available nitrogen along with Douglas-fir bark, sawdust and other similar organic materials to the soil resulted in a reduction in carbon dioxide evolution. This is at variance with field observations which show that additional nitrogen must be added to avoid nitrogen deficiency in the soil solution as well as to encourage rapid decomposition.

Microbial population studies were employed in an effort to correlate numbers of molds, bacteria, and Streptomyces with the observed reduction in carbon dioxide

evolution. With several sawdust samples and dextrose as substrates with and without added nitrogen, carbon dioxide evolution, plate counts, and pH changes were followed over a 20-day period. While some correlations were obtained these were fortuitous and afforded no explanation.

Warburg studies with molds, bacteria, and Streptomyces isolated from the soil indicated that carbon dioxide evolution and oxygen uptake were markedly increased by available nitrogen.

These results support conclusions that an increase in carbon dioxide evolution from sawdust with nitrogen actually does occur. Because sawdust and other such materials are exceptionally low in immediately available carbon, the increase is so transient that more frequent determinations during the first few hours of decomposition are required for its detection.

It is concluded that the addition of available nitrogen to materials of wide carbon:nitrogen ratio is required for rapid decomposition. The immediate effect of the rapid depletion of available compounds is a reduction in numbers of active microorganisms accompanied by a reduction in carbon dioxide evolution.

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