NOTE TO USERS

SOIL MOISTURE TENSION AND MICROBIOLOGICAL ACTIVITY

by

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INTRODUCTION

Moisture is recognized as essential for the biological transformation of complex organic material in soil. Definition of the soil moisture content that may be considered optimal for microbiological activity in any given soil is complicated by a number of factors. At the outset, some particular aspect of microbial activity suitable for measurement must be selected. Most commonly employed for study have been extent of population changes, rate of carbon dioxide evolution, formation of ammonia and nitrate, and fixation of elementary nitrogen. To the experimental difficulty of separating the effects of moisture from other inter-acting factors, such as temperature, level of nutrient and energy materials, and particularly, aeration, there has been added at least in some instances failure to insure a constant moisture content throughout an experimental period or to effect an even distribution of moisture throughout the soil employed. Finally, there has been considerable dissimilarity in the manner in which soil moisture content is expressed; some workers have expressed water content as the percentage of oven-dry soil, others, as percentage of the maximum water holding capacity, and others, in terms of thickness of the moisture film around the soil particles.

During recent years, the energy concept of soil moisture has been more widely developed. Under this concept, soil moisture is expressed in terms of the physical forces by which it is held in soil and not in terms of actual percentage content. The present study has been undertaken to find out whether the energy concept could be applied to a

better definition of the range of moisture suitable for microbiological activity in differently textured soils.

REVIEW OF LITERATURE

Publications on the influence of moisture on microbiological activity in soil may be grouped for the most part according to the observed effects of differing soil moisture contents upon (a) microbial numbers, (b) carbon dioxide evolution, (c) ammonification and nitrification, and (d) nitrogen fixation.

It has been noted frequently that the number of bacteria in a soil changes from season to season. Few bacteria may be found in a soil after a long dry period, but after the first rainfall thereafter the numbers rise rapidly. Engberding (11) found moisture to have a greater influence than temperature on bacterial numbers in soil. Whenever a difference in bacterial number was observed under different cropping, it could be accounted for by the differing moisture contents of the cropped soils. King and Doryland (24) reported a reduction in both number and activity of soil microorganisms as a result of excessive moisture. Hoffman (21) found a gradual diminution in microbiological activity as the soil dried out, the larger forms such as the fungi were the first to die, followed by the smaller and more resistant forms such as actinomycetes and spores of bacteria. Remy (33), Fisher, Lemmermann, Kappen and Blank (13), Prescott (30), Feher (12), Rahn (31, 32), and many others have reported an effect of soil moisture on bacterial numbers in soil. Lohnis (29) and Waksman (46) have summarized much of the earlier literature on this subject.

A few recent references may be cited in more detail. Eggleton (10) concluded that moisture exerts a controlling influence on the numbers of

bacteria, actinomycetes, and fungi in soil. A statistical analysis was made of the data for regressions of numbers of bacteria, actinomycetes, and fungi on moisture, temperature, and pH of soil during 24 weeks of study. In the case of bacteria, no effect of temperature was observed which could not be accounted for in terms of moisture. For actinomycetes and fungi there was a less definite relation between numbers and moisture content. Smith and Gall (38), on the contrary, found numbers of bacteria to remain uniform throughout a number of seasonal soil samples, whereas numbers of actinomycetes varied considerably. Taylor (41) and Cutler (9) have expressed the opinion that there are short term fluctuations of bacteria in soil, and that these variations may occur independently of moisture, inasmuch as they are to be encountered even in the presence of constant temperatures and moisture. James and Sutherland (22, 23) and Harmsen (18) doubt the actual existence of such fluctuations, providing adequate sampling procedures are employed.

There appears less agreement as to the amount of moisture required for optimal microbial development in soil. Stokes (39) found that bacteria and actinomycetes as well as algae met optimum moisture for growth at from 40 to 60 per cent of the moisture holding capacity of the soil. Engberding (11) reported maximum development of aerobic bacteria when the moisture content was between 50 and 70 per cent of capacity. Bollen (2), working with two soils differing in texture, Chehalis loam and Sifton gravelly fine sandy loam, found that in the former, the maximum number of bacteria and actinomycetes were found at 25 per cent of saturation, and of molds at half saturated soil, while in the latter, maximum number of organisms were encountered at 75 per cent of saturation. Chehalis loam had a maximum moisture holding capacity of 40 per cent and a wilting percentage of 8 per cent, whereas

the corresponding values for the Sifton soil were 98 per cent and 14 per cent, respectively.

Investigations of the influence of soil moisture on microbiological activity in soil, as revealed by the quantity of carbon dioxide evolved, likewise fail to show agreement as to the optimum moisture required. Lohnis (28, 29) found that moisture content of from 60 to 80 per cent of the water holding capacity was most desirable; Stoklasa and Ernst (40) observed that carbon dioxide evolution from soil increased with increasing moisture up to 50 per cent of the saturation capacity. Bollen (2), in studying Chehalis and Sifton soils, concluded that maximum evolution of carbon dioxide occurred at 75 per cent of saturation capacity.

The rate of transformation of organic nitrogen compounds in soil has also been studied as an indication of microbial response to differing soil moisture contents. Fraps (14) reported maximum nitrification with soil moisture content at 55.6 per cent of saturation. Above or below this optimum, nitrification diminished--the former condition being more injurious than the latter due to limiting oxygen supply and to denitrification. Lipman, Brown, and Owen (27) noted that the optimum moisture content for nitrification was considerably lower than that for ammonification. Extensive ammonification occurred even in saturated soils. Greaves and Carter (15, 16) encountered maximum ammonification at 60 per cent saturation, and maximum nitrification, at from 50 to 60 per cent of saturation. Bollen (2) found little difference in amounts of nitrate produced in soils at 25, 50 and 75 per cent of saturation during incubation periods of 30 days; after 68 days, nitrate content was found higher with increasing moisture up to 75 per cent of saturation.

Moisture content optimal for fixation of elementary nitrogen in soil has been reported both as 70 per cent (16) and as complete saturation (27). Greaves and Carter (16) believe that there are two maxima for nitrogen fixation, the first of which represents the conditions favorable for activity of aerobic microorganisms, and the second, for anaerobic activity.

Rahn (31, 32) found a definite correlation between bacterial activities in soil and the thickness of the moisture film around the individual soil particles. He expressed the opinion that moisture influences the course of microbiological development in two different ways, by controlling (a) aeration or diffusion of gases and (b) thickness of moisture film. Increased thickness of moisture film is favorable for bacteria in general but it diminishes aeration, making conditions deleterious to the growth and activity of aerobic organisms, although promoting the development of anaerobic bacteria. Decrease in moisture content of the soil is accompanied by a reduction in the thickness of moisture film. As a consequence, the rates of diffusion of food materials towards the microbial cell and of metabolic products away from it are materially affected. The result is an insufficient food supply, accompanied by an accumulation of harmful products, and there follows an inhibition of bacterial activity in spite of abundant oxygen supply. The optimum thickness of film in the case of Bacillus mycoides, Bacterium aceti, and Azotobacter was found to be between 10 and 20μ ; such thickness of moisture film was obtained in sand of average grain size of 1 mm. at a moisture content of from 5 to 10 per cent.

From the literature cited thus far, the lack of agreement concerning responses of the soil microflora to differing soil moisture contents is

readily apparent. Inasmuch as moisture has been expressed as (a) percentage of oven-dry soil, (b) as per cent of the maximum water-holding capacity of the soil, and (c) as thickness of the moisture film developed around the soil particles, it is difficult to compare, or to summarize briefly, much of the published data.

In recent years, the concept of using energy relations to characterize soil moisture phenomena has become quite generally accepted. Under the energy concept, problems of soil-moisture retention and movement have been placed on a fundamental basis, and suitable techniques have been developed for measuring the forces by which water is held in soil (1). The tenacity with which moisture is retained in a particular soil by surface force action depends on the texture and can be measured directly by equivalent negative pressure or tension in the soil moisture. The tenacity with which moisture is held in the soil cannot be fully explained by the curvature of the moisture film around the inorganic soil particles, or by the so-called "capillary tube" hypothesis of Briggs (3). Surface tension forces are only a part of the total forces involved in the attraction of water by soil. The organic matter fraction of the soil, osmotic pressure due to salt concentrations and other factors play a significant role in influencing soil-moisture relationships (1).

There are several equilibrium points that are commonly used in describing soil-water relationships. There may be mentioned (a) the permanent wilting percentage (5), (b) the field capacity (44), (c) the moisture equivalent (4), and (d) the "aeration porosity limit" (25). These points, in the order named, correspond to pF values of 4.2, 3.0, 2.7, and 1.7 (1). The term pF, introduced by Schofield (37) in pursuance of the ideas of Buckingham (6) on the energy concept or

capillary potential of soil moisture, designates the logarithm of the height in centimeters of a liquid (water) column that is necessary to produce the desired suction (1). At a particular pF value, differently textured soils do not necessarily have the same per cent saturation of their maximum moisture-holding capacity. Physical forces with which moisture is retained in the soil may be conveniently divided into two classes (34): (1) those arising from dissolved materials expressed in terms of osmodic concentration of an extracted sample of soil solution; (2) all other forces measurable by use of membranes permeable to soil solution, including tensiometer, suction plate, pressure membrane, centrifugation apparatus and equivalent to the negative pressure or tension in the soil moisture and independent of concentration of soluble materials. Unfortunately, usefulness of the terms "capillary potential" (6) as well as of pF (37) is considerably lessened by their indefiniteness inasmuch as they have sometimes been used to include and at other times to exclude osmodic effects. If pF is to be used as a free energy scale, it should be correctly used and should be clearly distinguished from pressure deficiency or soil moisture tension. In leached soils, the osmodic component of pF may be negligible but in soils from arid or semi-arid regions effect of dissolved materials on the free energy of soil water may be appreciable. There is appearing information concerning the responses of plants to differing moisture tensions in soils. Investigation, however, has not been made of the response of the soil microflora to differing soil moisture contents expressed in terms of the equivalent moisture tension values.

MATERIALS AND METHODS

Sources of Soil Samples

Soil samples used in the present investigation were selected with respect to textural characteristics with a range in clay content. Top soil was desirable in order that a large and flexible microbial population would be present. The samples as described below were all collected on 31 July, 1946, from Story County, Iowa, in sufficient amounts, with but one exception, for all experiments. The one exception was in the case of Wabash silty clay. An additional lot of this soil was collected in December, 1946, from the same site as the initial lot.

Thurman fine sand: vegetative cover weeds, following a 1946 crop of oats; previous field history unknown. From N.W. ¼ of Sec. 7, T83, R23W, Story County, Iowa.

Clarion loam: vegetative cover soybeans, following oats the previous year. From Plot 816, Agronomy Farm, Iowa State College.

Clarion fine sandy loam: vegetative cover soybeans, following corn the previous year. From west side of Agronomy farm, at crest of contoured area just on the Agricultural Engineering side of boundary roadway.

Webster silt loam: Vegetative cover corn, following meadow the previous year. From Plot 1400, Agronomy Farm.

Wabash silty clay: vegetative cover corn, following corn the previous year. From S.W. ¼ of Sec. 18, T83, R23W, Story County, Iowa.

Moisture Retention Characteristics of Soils

In the present work, the term "soil moisture tension" has been used to designate the net tension in terms of centimeters of water with which soil moisture is in equilibrium, as determined both directly and indirectly, and is the resultant of all the factors in operation under the conditions of the determinations. Inasmuch as the soils employed were from a humid region, an appreciable salinity is not to be expected.

Establishment of "0" and "1" cm. of water tension

One of the most familiar laboratory determinations of soil moisture is that of so-called "water holding capacity" (also called water retaining capacity, moisture holding capacity, moisture capacity). This term was introduced by Hilgard (20) and its determination usually is made as follows: A shallow container with perforated bottom is filled with air-dry soil, then saturated by immersing in water, then allowed to drain free of all the water which will drip out under the influence of gravity. The moisture content of the soil is then determined by usual oven drying.

It has often been assumed that such a determination would be an indication of the amount of water which would be held in that soil in opposition to the pull of gravity alone, or in other words against a tension of "0" cm. of water, but such an assumption may be unjustified in view of the fact that the lowest layer of soil particles is in contact not with soil but with the atmosphere. At the soil atmosphere interface, films of water develop which interfere with the free drainage of moisture. Removal of these films of water may involve

application of some suction.

In the present work, 30 grams of air-dry soil, screened to pass a 2 mm. sieve, were placed on filter paper within a standard "moisture equivalent box", levelled by gentle tapping and then placed overnight in a tray containing distilled water to slightly above the surface of the soil. Next day the boxes were allowed to drain by gravity for 30 minutes, with the lids in place, after which free water drops at the bottom of the box were wiped off but once with a paper towel. The moisture content of the soil on an oven dry basis was taken to represent "0" cm. of tension.

It was noted that during the weighing of the boxes, more drops of water tended to drain out of the samples. Removal of the free drops of water by blotting several times with blotting paper, or until no more free water drops appeared, would result in a different moisture content. Such blotting involved a certain amount of suction force, and the moisture content determined was expressed as that corresponding to "1" cm. of water tension. At the high moisture range, soil moisture is very loosely held, and a very small change in tension causes a large variation in the moisture content of a soil.

Establishment of 10 and 50 cms. of water tension

A manometric device was employed in order to bring the soil samples previously saturated with water to equilibrium with tensions of 10 and 50 cms. of water.

The apparatus, as shown in Figure 1, consisted of two arms of glass tubes A and B, clamped in vertical position and connected together at the lower ends by rubber tubings through a glass stopcock C. A short piece of rubber tubing R was inserted at the top of the arm A for

holding a Buchner funnel F containing the soil sample, while the arm B was provided with a side outlet D towards the bottom. The Buchner funnel, 60 mm. in inside diameter and 35 mm. in depth, contained a closely fitting disc of blotting paper to cover the perforations of the funnel. A similarly-sized disc of filter paper was fixed in position on top the blotting paper with a few drops of distilled water. The filter paper disc was given a preliminary treatment with a dilute suspension of clay in order to minimize the pore sizes sufficiently to hold a length of water column in subsequent use without admitting air.

The funnel was filled to about 4/5th of its capacity with air-dry soil, screened to pass a 2 mm. sieve, and in order to insure uniform packing of individual samples, the funnel containing the dry soil was tapped 50 times by dropping it from a height of approximately 2.5 cm. The funnel was then placed over night in vertical position in a beaker containing cold freshly boiled distilled water to slightly above the surface of the soil. The next day the funnel with its contents was carefully taken out of the beaker and its stem introduced on the rubber tube R of the manometer tube, already filled with distilled water up to the brim of the rubber tube. Care was taken to avoid entry of air bubbles inside the funnel during the manipulation. The funnel was clamped in vertical position and covered by an inverted dish in order to prevent loss of moisture by evaporation.

During this manipulation, the stop cock C connecting the two arms was kept open, and water was slowly released through the side outlet D in a number of installments until the difference of level, measured from the center of the soil in the Buchner funnel to the level of the water in arm B, corresponded to the tension desired. This differential was maintained constant by releasing water through the side outlet from

time to time, until equilibrium was attained. The time required depended on the nature of the soil, the tension applied, and the size of the pores in the filter disc, but in general, could be attained within 2 to 3 hours.

After equilibrium was attained, the glass stopper C was closed, and the contents of the funnel removed by spatula into a tared container for determination of moisture content.

Determination of the moisture equivalent

The standard centrifuge method of Richards and Weaver (34) and Veihmeyer, Oserkowsky and Tester (45) was employed, with 30 grams of air-dry soil being used. The moisture tension at the moisture equivalent is generally agreed to correspond to pF 2.7.

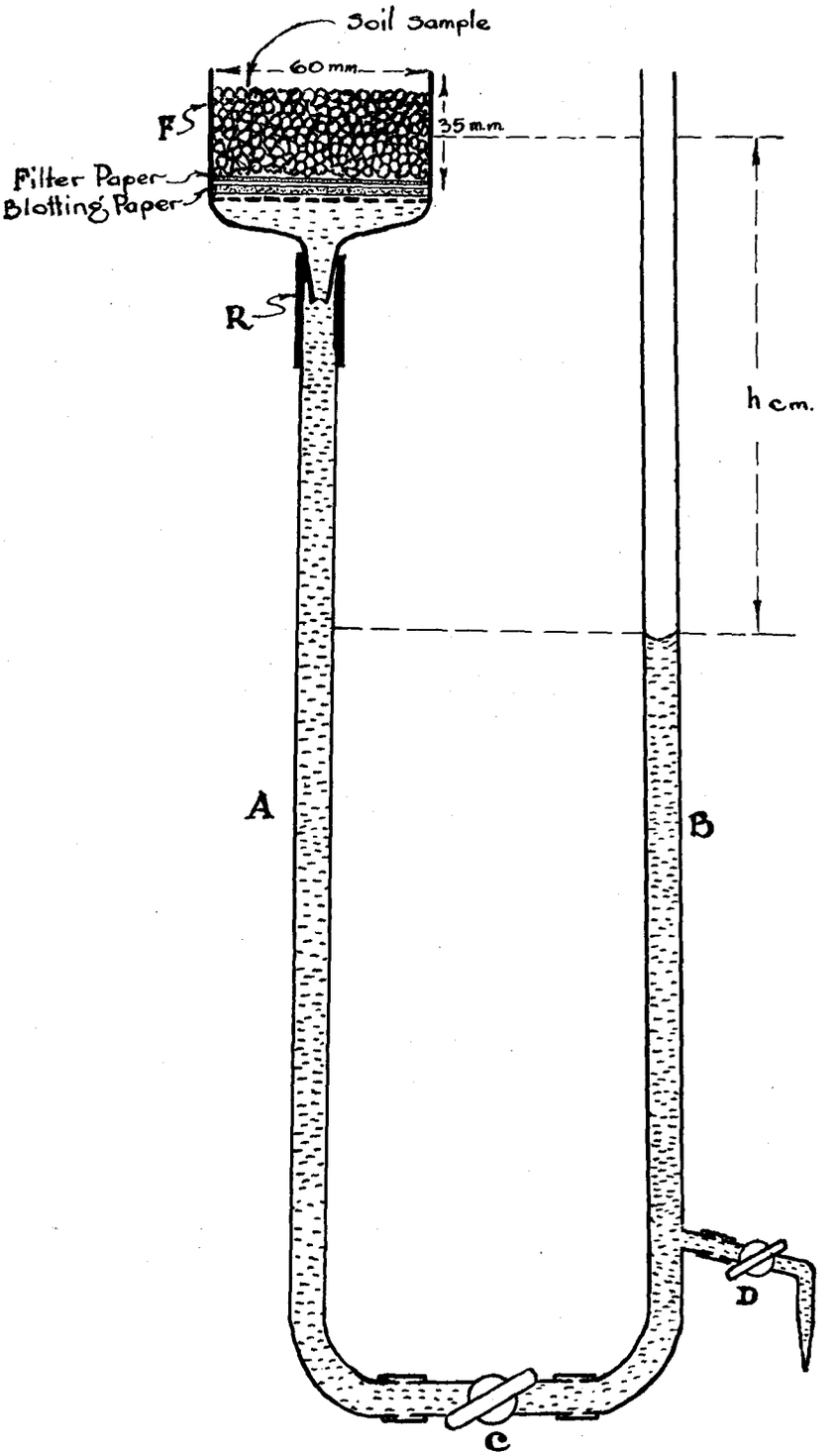
Determination of the permanent wilting percentage

Moisture content at the permanent wilting percentage was determined indirectly by growing sunflowers as the indicator plants in suitable containers (19). The moisture tension at the wilting percentage is controversial, but is generally believed to lie within the range of pF 4.1 to pF 4.2. According to Richards and Weaver (35) 15 atmosphere percentage is a better approximation.

Establishment of values for 3,160 cm. of water tension

Moisture content at this tension was determined by interpolation from curves constructed from data obtained in the several determinations just cited. This tension value corresponds to pF 3.5.

Fig. 1. Apparatus for determining moisture retaining capacity of soils at various tensions.



Apparatus for determining moisture-retaining-capacity of soils at various tensions.

Analytical Methods

Determination of moisture

The sample was dried to constant weight at 105° C. Moisture loss was determined and the initial moisture content was expressed as percentage of the oven-dry soil.

Estimation of carbon dioxide

Carbon dioxide was determined by volumetric method following absorption in standard sodium hydroxide solution in Pattenkoffer's tubes or in towers containing glass beads. Titration with standard hydrochloric acid was made in presence of excess of barium chloride solution (about 1 N) with phenolphthalein as the indicator.

Estimation of total carbon

Determinations were made according to the method of Salter (36) and Winter and Smith (49), with certain modifications. Instead of coarsely granular copper oxide, copper asbestos, made by soaking asbestos fibre in concentrated copper nitrate solution, drying, and igniting to remove all traces of oxides of nitrogen, was used as filling material in the combustion tube. It was found that due to fluctuations in temperature, the silica tube slags with fused copper oxide and cracks on cooling, whereas use of copper asbestos offers no such difficulty.

Estimation of carbonate carbon

Determination of carbonate carbon in soils was made by measuring the carbon dioxide given off during treatment with 12 per cent hydrochloric acid. About 10 grams of sample were used together with 50 ml. of twelve per cent hydrochloric acid in a small flask. Carbon dioxide liberated was swept up through a vertical condenser with carbon-dioxide free air, passed through a U tube containing granular zinc to remove any hydrochloric acid carried with the air stream, and then absorbed in standard sodium hydroxide solution in a tower packed with glass beads. After initial exposure to acid, the contents of the flask were heated just to boiling after which heating was discontinued. The contents of the tower were then washed down into a 500 ml. Erlenmeyer flask with carbon-dioxide free distilled water. Washing was continued about 14 times or until no trace of caustic soda was indicated in the washings when tested by a drop of phenolphthalein indicator. A blank determination was carried out concurrently under exactly similar conditions.

Estimation of ammonia and nitrate

The soil was neutralized with about 1 N hydrochloric acid and made acid to congo red. A measured volume (100 to 125 ml.) of 2 N potassium chloride was then added to the soil, followed by an amount of distilled water calculated to bring the concentration of KCl to 1 N. This could be done by determining the amount of water present in the sample and deducting it from the volume of water to be added.

The mixture was then shaken for a period of one hour in an end-over-end shaker, after which it was filtered through a dry fluted filter paper. An aliquot portion of the filtrate was distilled with an

excess of magnesium oxide, and the ammonia liberated was absorbed in standard hydrochloric acid solution and estimated by titration with standard sodium hydroxide solution using methyl red--methylene blue as the indicator.

The residue left after ammonia determination was diluted with water and distilled with Devarda's alloy. The ammonia liberated as a result of reduction of nitrates and nitrites was estimated in the manner just described.

Estimation of total nitrogen

Total nitrogen was determined by modified Kjeldahl method using sodium sulphate, sodium thiosulphate, salicylic acid, and copper sulphate in addition to concentrated sulphuric acid in the digestion of the sample, followed by distillation with excess of concentrated sodium hydroxide solution and collection of the ammonia liberated in standard hydrochloric acid. Excess of hydrochloric acid was titrated with methyl-red methylene blue as the indicator.

Estimation of microbial populations

Microbial populations were estimated by cultural procedures, according to the methods of Clark (7). Determinations were limited to numbers of fungi, actinomycetes, and bacteria in selected series of incubated soils.

Establishment and Maintenance of Incubation Conditions

Procedures for carbon dioxide studies

The additional amounts of water required to establish desired tension values were added to air-dry soils of known moisture contents. For establishing incubation experiments at the lower tensions, 0, 1, 10, and 50 cm., the required amounts of water could be added from a burette. In the case of the higher moisture tensions of 502 and 3,160 cm. of water, such a procedure would not insure an even distribution of moisture throughout the sample.

In an initial trial, a known weight of air-dry soil was evenly spread on a large enamelled tray and a known volume of water was added by spraying with an "atomizer" run by compressed air. The tray with its contents was then covered with a lid and allowed to stand at 6° C. for 45 minutes. Moisture determinations on samples collected from various places in the tray showed unacceptable variation. In further trials, similar procedure was employed but the required volume of water was added in 5 to 6 installments, and the soil was thoroughly mixed with a spatula after each addition of water. Moisture determinations on randomized samples showed close agreement amongst themselves, but they also showed that considerable amounts of moisture had been lost to the air during the spreying operation.

The procedure finally adopted as satisfactory is described as follows:

A known weight of air-dry soil (with or without admixture of plant material) was spread evenly on a large enamelled tray (an 8" x 12" photographic dish could be used for sample weights not exceeding 400

grams) and the required amount of water was applied by spraying in 5 or 6 installments, the sample being mixed thoroughly after each installment. The tray with its contents was weighed before and after the addition of water, and the loss of moisture during the operation was made up by the further addition of water by spraying. Moisture determinations on randomized samples showed close agreement to the moisture contents desired.

Incubation flasks received weighed amounts of moist soil, to correspond to 100 grams of oven-dry soil. In cases where one per cent of finely ground corn stover was admixtured with the soil prior to watering, the amounts of soil incubated corresponded to 100 grams of oven-dry soil plus 1 gram of corn stalk.

Incubations were made at 30° C. ± 1°. For incubation containers, 500 ml. pyrex Erlenmeyer flasks generally were used. In a few instances to be noted later, flint glass bottles approximately 5 x 5 x 15 cm. in size were used.

Incubation containers were fitted with 2-hole rubber stoppers, containing inlet and outlet tubes and connected with an air stream maintained at constant pressure by means of a water column. The air stream was passed through concentrated potassium hydroxide solution, concentrated sulphuric acid, soda lime, ascarite, and calcium chloride towers before it entered the incubator. After entering, the air stream was passed through a series of long, horizontal tubes, half filled with distilled water, in order to saturate the air stream to 100 per cent relative humidity at the temperature of the incubator. The air stream was finally broken into a number of different branches in order to provide aeration for a large number of flasks at one time.

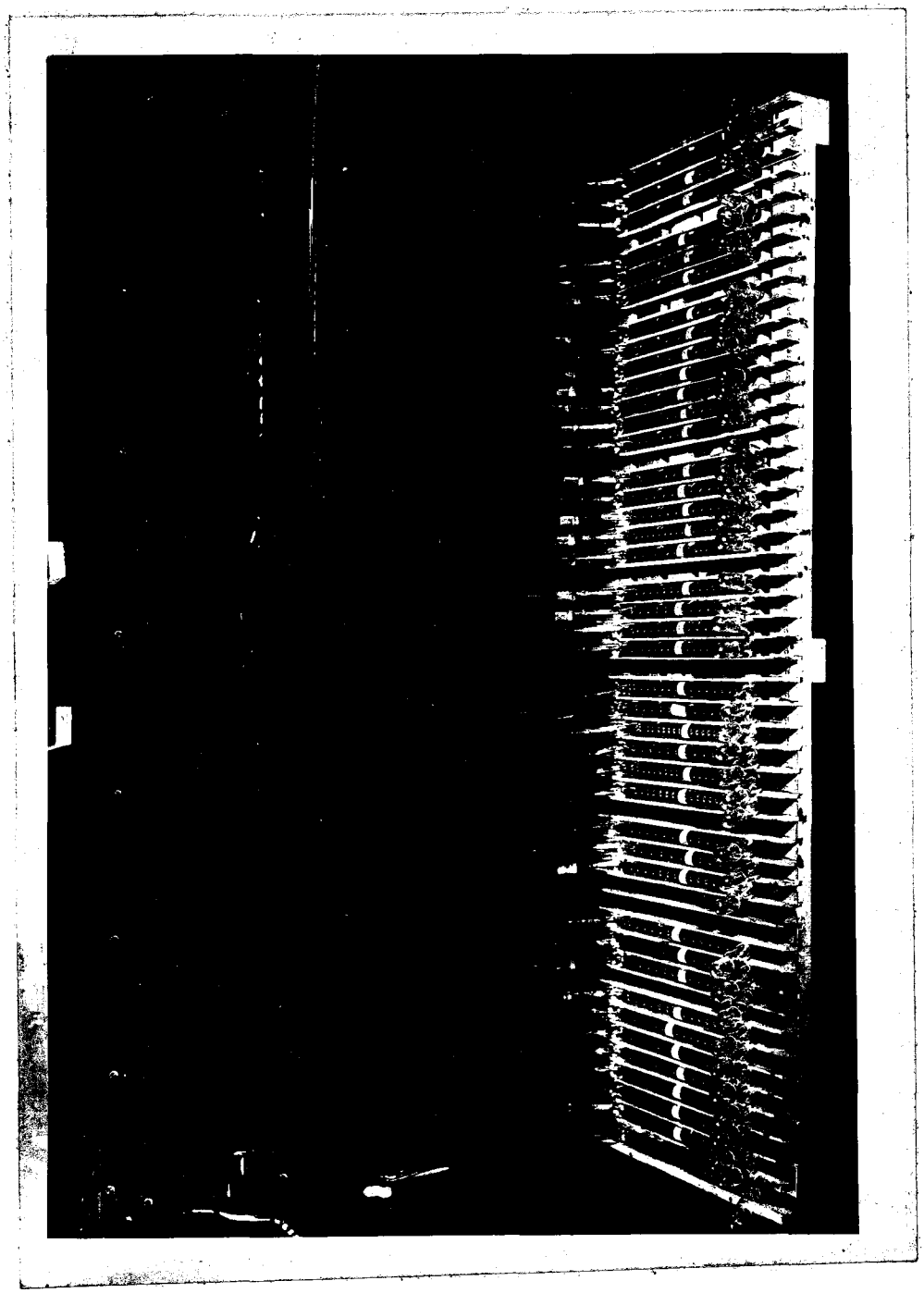
Aeration rate through the individual incubation flasks was regulated by restricting the flow of the air stream by means of a short length capillary glass tubing drawn at one end. In constructing capillaries for controlling the flow rate, one end of a tube of small bore was drawn out to a very small diameter. This capillary was then connected to an air stream maintained at constant pressure and the rate of flow determined with a bubble counter. Successive segments of the narrow end of the capillary tubing could be cut off until the proper rate of flow was obtained. About 3/4 liters of air passed through each incubation flask during a period of 1 hour.

The gases coming out of each incubation flask were led through rubber tubes passing to the outside of the incubator and connecting to Pettenkoffer tubes filled with standard sodium hydroxide solution.

Arrangement of the Pettenkoffer tubes and the passage of air bubbles through them are shown in Figure 2.

Estimation of carbon dioxide collected in the Pettenkoffer tubes was generally carried out after intervals of 1, 2, 3, 4, 6, 9, 12, and 15 days of incubation. From the values determined, the daily rates of evolution were calculated.

Fig. 2. Arrangement of Pettenkoffer's tubes used for incubation studies.



Procedures for studies on ammonification and nitrification

Studies on ammonification and nitrification at differing moisture tensions were limited to Thurman fine sand and Wabash silty clay, with 2 per cent of egg albumin added. Procedures employed in the establishment of inoculated soil lots and in the maintenance of incubation conditions were similar in principle, and in most details, to those employed for carbon dioxide evolution studies. Incubation intervals of 1, 2, and 4 weeks were employed.

In initial experiments on ammonification and nitrification, groups of flasks were connected in series to the air stream, and no attempt was made to collect the air emerging from the flasks. Erratic results were obtained between the duplicate flasks of Thurman fine sand, and also between those duplicates of Wabash silty clay in cases where the incoming air stream contained incubation gases from Thurman sand. In some instances, the ammonia recovered was more than could be accounted for assuming complete mineralization of the nitrogen in the original soil plus that added in the form of egg albumin. These findings indicated that there was appreciable volatilization of ammonia from at least some flasks.

The following procedure was then adopted. The individual incubation flasks were connected in parallel to the incoming air stream, and the outgoing gases from each flask were led separately through Pettenkoffer's tubes containing dilute hydrochloric acid in sufficient quantity to absorb all escaping ammonia. At the end of incubation, the ammonia absorbed was recovered by distillation with excess of alkali in Kjeldahl flasks and estimated by volumetric titration, as described under analytical methods.

Procedures for studying the activity of different types of micro-organisms in steam-sterilized soils

From Webster loam incubated 15 days at 50 cm. of water tension, the culturally predominant fungi, actinomycetes, and bacteria were isolated. Three members of each of these groups were cultured separately on agar slants, and the resulting surface growth of each was removed. All bacteria harvested were combined into one suspension. The actinomycetes were combined into a second suspension, and the fungi, into a third. One-fourth of each suspension was removed, and these three fractions were combined, providing a fourth suspension containing bacteria, actinomycetes, and fungi. One ml. portions from the four prepared suspensions were used to inoculate steam-sterilized soil.

Soil aliquots, corresponding to 100 grams oven-dry soil and 1 gram of air-dry cornstalk, were placed in 500 ml. Erlenmeyer flasks for adjustment of water content and for sterilization. Water content was adjusted by addition from a burette, or by the spraying procedure previously described. Inasmuch as some water was lost during sterilization, a number of preliminary trials were necessary to determine the exact amounts of water lost during sterilization. By proper standardization, moisture contents in the soil could be adjusted successfully. After sterilization, the maximum deviation from the desired values was not more than 0.2 per cent.

Prior to sterilization, incubation containers were fitted with rubber corks carrying inlet and outlet tubes plugged with non-absorbent cotton. Sterilization was at 15 lbs. pressure and 120° C. for 60 minutes. After sterilization, and inoculation, the containers with

their cotton plugs still in place were connected to a washed and filtered air stream.

Carbon dioxide evolved was collected continuously, and was determined after 2, 4, 6, 9, 12, and 15 days. At the end of 15 days, soils were removed for microbiological analyses.

EXPERIMENTAL RESULTS

Moisture Retaining Capacities of the Soils Employed

The moisture retaining capacities of the five soils employed are given in Table 1. At any particular tension, moisture retention increased progressively with fineness of the soil texture. Thurman sand invariably contained the lowest, and Wabash silty clay the highest, percentage of moisture at each tension for which determinations were made.

Curves showing the moisture relationships of the several soils are given in Fig. 3, in semi-logarithmic scale. As there is no provision in a logarithmic scale for a tension of "0" cm., tensions corresponding to "0" and "1" cm. of water have been plotted as 1 and 2 cm., respectively, for the sake of convenience. No alterations have been made for any other values.

The nature or shape of the tension/moisture content curves is also characteristic of a particular soil, and it is to be noted that the curves do not run parallel. In general, towards the wet end of the scale, moisture is very loosely held, and a small change in tension is accompanied by a comparatively large change in moisture content. Towards the dry range, soil moisture is more tightly held, and for extraction of small additional amounts of water, a great increase in suction force is found necessary.

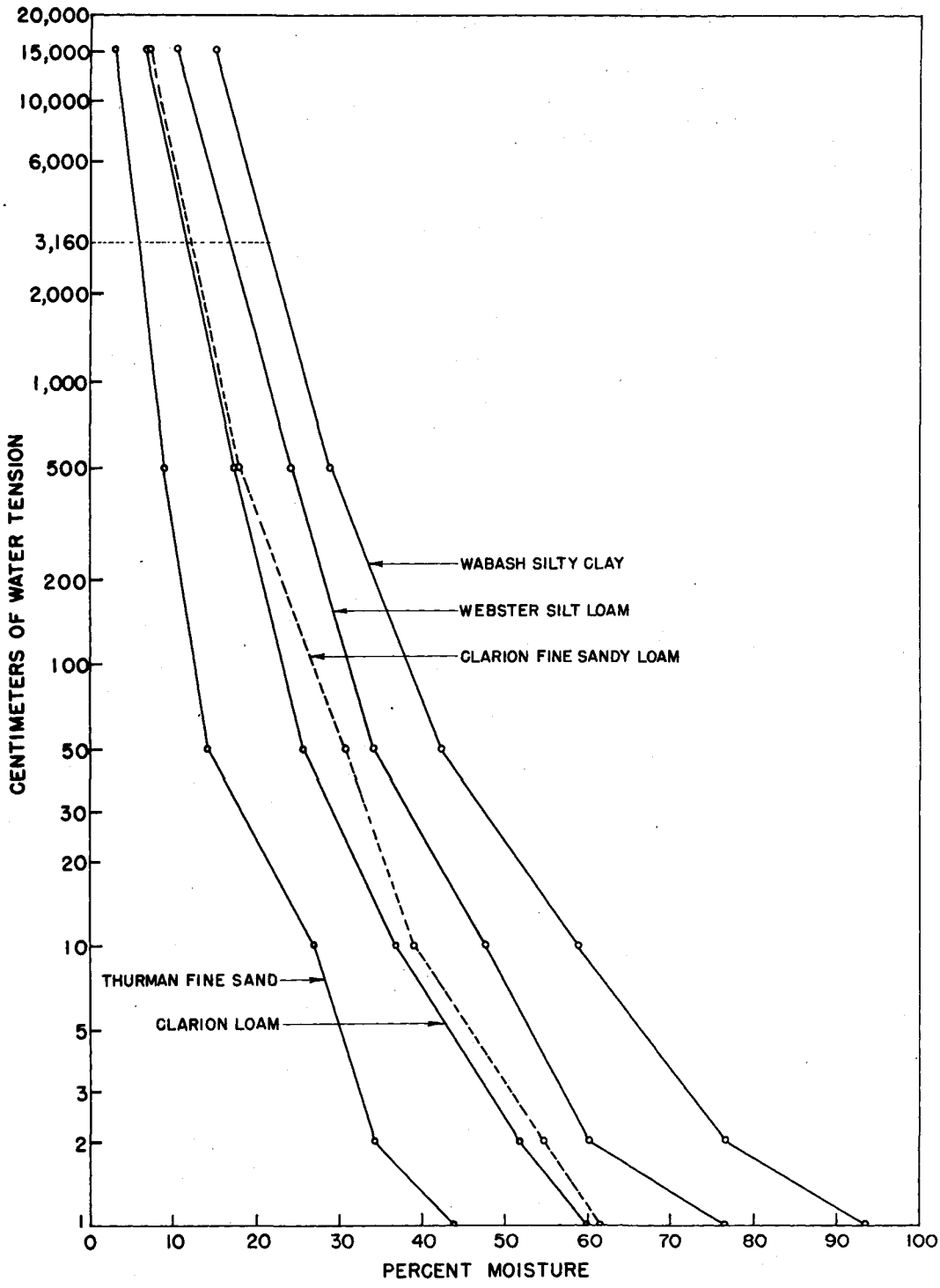
Table 1

Moisture retaining capacities of five Iowa soils at selected tension values

Soil type	Centimeters of water tension						
	0.0	1.0	10.0	50.0	502.0	3160	15,340
	Per cent moisture						
Thurman fine sand	43.69	34.31	26.86	14.12	8.98	5.60	3.04
Clarion loam	59.89	51.60	36.69	25.65	17.57	11.70	7.04
Clarion fine sandy loam	61.34	53.47	38.85	30.59	17.66	11.80	7.16
Webster silt loam	76.40	60.07	47.55	34.11	24.03	16.50	10.52
Wabash silty clay	93.11	76.40	58.55	42.25	28.79	21.10	15.09

Fig. 3. Moisture retaining capacities of five Iowa soils.

X - Moisture content on numerical scale.
Y - Centimeters of water tension on logarithmic scale.


20,000
15,000
10,000
6,000
3,160
2,000
1,000
500
200
100
50
30
20
10
5
3
2
1
CENTIMETERS OF WATER TENSION
0
10
20
30
40
50
60
70
80
90
100
PERCENT MOISTURE
WABASH SILTY CLAY
WEBSTER SILT LOAM
CLARION FINE SANDY LOAM
THURMAN FINE SAND
CLARION LOAM

Carbon and Nitrogen Contents of Experimental Materials

The carbon and nitrogen contents of the several soils, and of the corn stalk and egg albumin employed as amendments, are presented in Table 2. The carbon/nitrogen ratios of the several soils indicate that their native organic matter is in an advanced stage of decomposition. The total carbon values show that the coarse-textured soils contain less organic matter than do the fine-textured.

Carbon Dioxide Evolution from Incubated Soils

In order to determine whether the native organic matter directly determined the amount of carbon dioxide evolved during incubation, a preliminary experiment was performed with all soils, with and without added corn stalk, maintained at the moisture equivalent for 15 days. The amounts of carbon dioxide collected are shown in Table 3.

In the unamended soils, the rate of biological decomposition of native organic matter appeared very slow. There was no linear relationship between the organic matter content and the amount of carbon dioxide evolved. Thurman sand showed the lowest, and Clarion loam the highest quantity of carbon dioxide, when the five soils were incubated unamended. With added organic matter, Thurman sand showed the highest carbon dioxide evolution. These data indicated that the differences in organic matter content of the original soil were of little or no importance in determining the amounts of carbon dioxide evolved on incubation.

Table 2

Carbon and nitrogen contents of the experimental materials employed

Material	Total carbon	Carbonate carbon	Nitrogen	C/N
	Per cent	Per cent	Per cent	
Thurman fine sand	0.75[1,2]	0.0000	0.0669	11.21
Clarion loam	1.47	0.0015	0.1256	11.70
Clarion fine sandy loam	1.75	0.0052	0.1544	11.26
Webster silt loam	2.93	0.0034	0.2224	13.04
Wabash silty clay	2.92	0.0026	0.2330	12.53
Corn stalk	41.15	--	0.5603	73.44
Egg albumin	--	--	12.68	--

[1]Total carbon values includes carbonates in the case of soil samples.

[2]Results expressed as percentage of oven-dry material for soils, and of air-dry material, for cornstalk and albumin.

Table 3

Recovery of carbon as carbon dioxide from soils incubated 15 days at the moisture equivalent, with and without added cornstalk

Soil type	Soil alone			Soil plus 1% cornstalk		
	CO_2 evolved on incubation	Total CO_2 expected on chemical oxidation	Recovery of carbon	CO_2 evolved on incubation	Total CO_2 expected on chemical oxidation	Recovery of carbon
	mgms.	mgms.	per cent[1]	mgs.	mgs.	per cent[1]
Thurman fine sand	21.45	2750.0	0.78	508.02	4258.8	11.93
Clarion loam	34.98	5390.0	0.65	360.03	6898.8	5.22
Clarion fine sandy loam	69.44	6416.7	1.08	446.53	7924.8	5.63
Webster silt loam	46.71	10743.4	0.43	381.49	12252.2	3.11
Wabash silty clay	55.48	10706.8	0.52	332.35	12215.6	2.72

[1] Calculated from data presented in Table 2.

Total amounts of carbon dioxide evolved from the several soils, with 1 per cent of corn stover added, during 15 days of incubation at six different moisture contents are shown in Table 4.

It is apparent that for the several soils there is no regularity between total carbon dioxide evolved during the 15 day incubation and moisture content. In fact, no two of the five soils showed maximum carbon dioxide production at the same tension value. In the case of Thurman sand, activity appeared greatest at 3,160 cm. of water tension; it was gradually depressed with decreasing moisture tension. Maximum carbon dioxide evolution was exhibited by Clarion loam at 502 cm. of tension, by Clarion fine sandy loam at 50 cm., by Webster silt loam at 1 cm., and by Wabash silty clay at 10 cm. of water tension.

The deleterious effect of increased moisture beyond a certain maximum is believed generally to be due to its limitation of aeration. A supplementary experiment was performed with Webster silt loam incubated in dilution bottles (5 x 5 x 15 cm.) rather than in Erlenmeyer flasks. Thus a much smaller surface/volume ratio was established, but the same moisture tension values were employed for the two series of containers. The cumulative total amounts of carbon dioxide evolved in 15 days from deeply layered and shallow-layered soil are compared in Table 5. It was found that up to 10 cm. of water tension, there was no reduction in activity due to reduction in surface/volume ratio, whereas at 1 and 0 cm. of tension, there was a sharp drop in quantity of carbon dioxide evolved under incubation conditions of decreased surface and increased depth of soil column.

Table 4

Total amounts of carbon dioxide evolved from soils (100 grams, oven-dry basis, plus 1 gram cornstalk) incubated 15 days at selected moisture tensions

Soil type	Centimeters of water tension					
	0.0	1.0	10.0	50.0	502.0	3160.0
	Mgm. CO_2 during 15 days incubation					
Thurman fine sand	343.25	327.46	446.99	507.04	508.02	563.15
Clarion loam	322.99	327.81	316.43	312.91	360.03	299.07
Clarion fine sandy loam	380.16	393.99	418.89	503.96	446.53	416.86
Webster silt loam	431.32	513.98	426.28	408.75	381.49	370.55
Wabash silty clay	421.68	412.32	449.56	397.20	332.35	322.34

Table 5

Total amounts of carbon dioxide evolved from aliquots of Webster silt loam incubated in differently shaped containers

Centimeters of water tension	In bottles, 5x5x15 cm. in size	In 500 ml. Erlenmeyer flasks
	mgm. CO_2 during 15 days incubation	
0.0	188.67	431.32
1.0	409.36	513.98
10.0	501.49	426.28
50.0	450.91	408.75
502.0	397.08	381.49
3160.0	344.32	370.55

From the results presented in Table 4, it was observed that there was no regularity among the several soils with respect to total carbon dioxide given off during 15 days of incubation. It was, therefore, desirable to study the daily rates of carbon dioxide evolution, and to compare the peak rates, or maximum daily rates. Table 6 shows the peak rates of carbon dioxide evolution that were encountered for the five soils at each of 6 selected moisture tension values. Graphical presentation of experimental data calculated to daily rates is made in Figures 4 to 8, inclusive. For comparison of the daily rates of CO_2 evolution in differently shaped containers, Figure 9, prepared from data on Webster silt loam incubated in bottles, is included in order that direct comparison may be made for Figure 8, showing daily rates for this soil incubated in Erlenmeyer flasks.

As the general rule, peak rates were attained at or near a moisture tension equivalent to 50 cm. of water. For Thurman sand, the maximum peak rate was found at 502 cm. of water tension, but there was no material difference between the rates at 50, 502, and 3,160 cm. of tension. In the case of Clarion loam, the maximum peak rate was noted at 502 cm. of tension, but the difference from the rate at 50 cm. of tension was very slight. For the remaining three soils, the rate at 50 cm. was maximal.

Table 6

Peak rates of carbon dioxide evolution in the transformation of organic matter in various soils

Soil type	Centimeters of water tension	First peak rate		Second peak rate	
		Period of occurrence (days)	Magnitude (mgm.CO_2/ 24 hours)	Period of occurrence (days)	Magnitude (mgm.CO_2/ 24 hours)
Thurman sand	0.0	1-2	24.22	6-9	26.80
	1.0	"	26.92	"	28.63
	10.0	"	75.18		
	50.0	"	85.83		
	502.0	2-3	86.47		
	3160.0	3-4	85.66		
Clarion loam	0.0	3-4	22.75	6-9	23.48
	1.0	1-2	26.38	"	23.81
	10.0	0-1	54.33		
	50.0	"	66.88		
	502.0	"	71.25		
	3160.0	1-2	46.56		
Clarion fine sandy loam	0.0	4-6	31.84		
	1.0	"	33.95		
	10.0	0-1	51.64		
	50.0	"	81.97		
	502.0	0-2	68.20		
	3160.0	"	60.79		
Webster silt loam	0.0	4-6	32.14		
	1.0	1-2	36.35	4-6	40.45
	10.0	0-1	73.82		
	50.0	"	78.45		
	502.0	2-3	59.60		
	3160.0	"	52.64		
Wabash silty clay	0.0	6-9	32.61		
	1.0	1-3	29.22	6-9	33.83
	10.0	0-1	69.11		
	50.0	"	74.84		
	502.0	0-2	49.57		
	3160.0	1-2	45.02		

Fig. 4. Daily rate of carbon dioxide evolution from Thurman fine sand on incubation at selected moisture tensions.

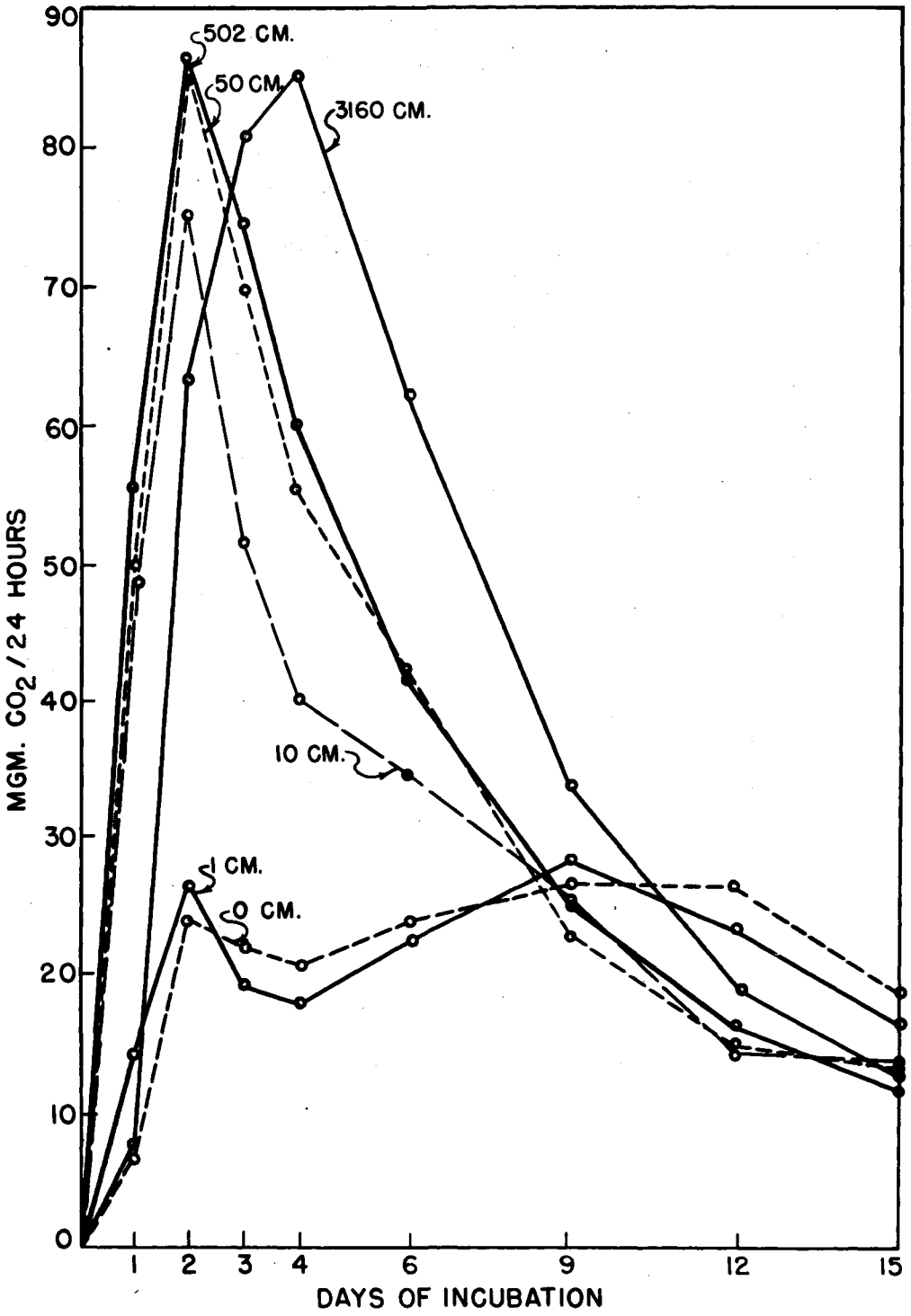

502 CM.
50 CM.
3160 CM.
10 CM.
1 CM.
0 CM.
MGM. CO_2 / 24 HOURS
DAYS OF INCUBATION
0
10
20
30
40
50
60
70
80
90
1
2
3
4
6
9
12
15

Fig. 5. Daily rate of carbon dioxide evolution from Clarion loam on incubation at selected moisture tensions.

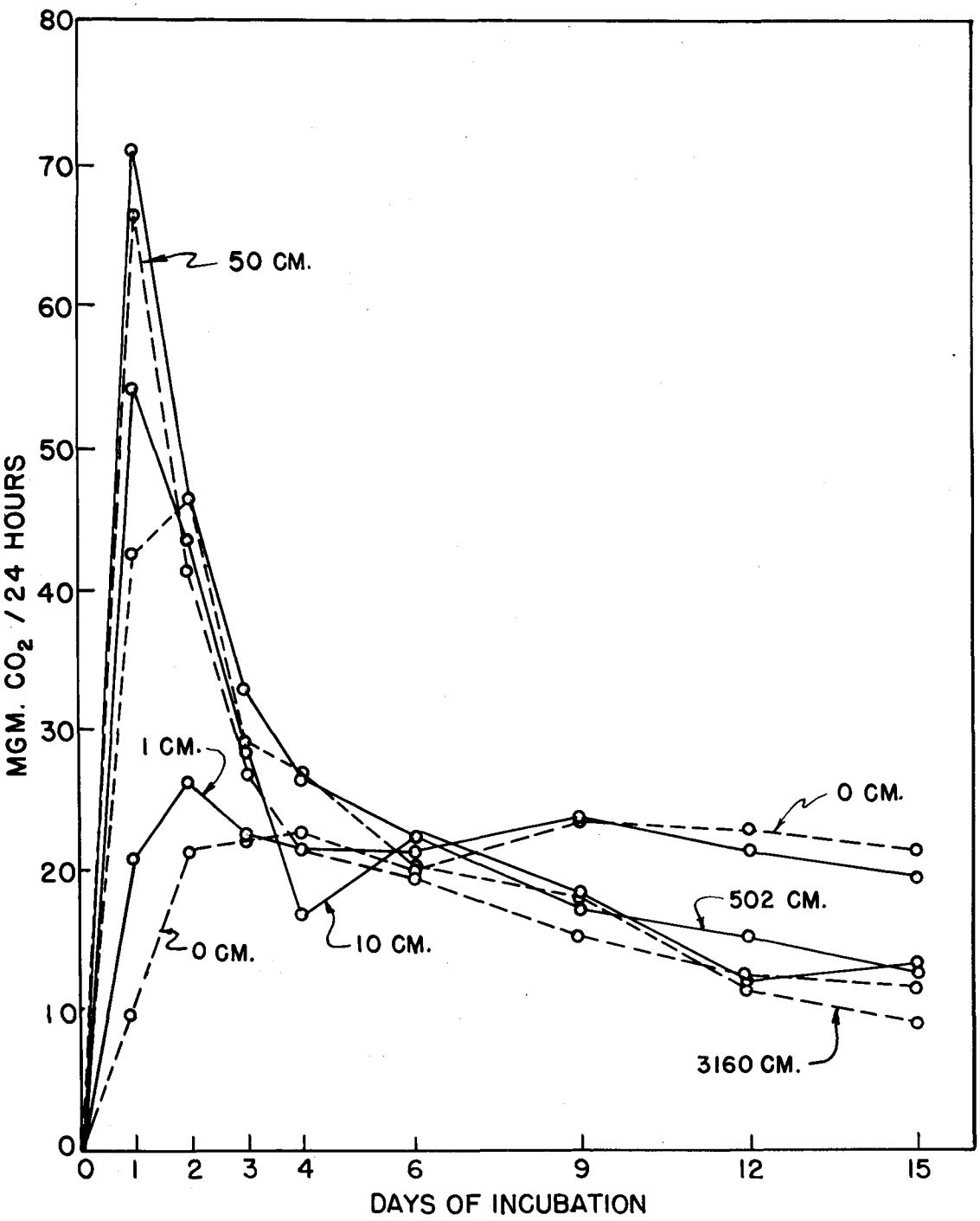

80
70
60
50
40
30
20
10
0
MGM. CO_2 / 24 HOURS
50 CM.
1 CM.
0 CM.
10 CM.
0 CM.
502 CM.
3160 CM.
0 1 2 3 4 6 9 12 15
DAYS OF INCUBATION

Fig. 6. Daily rate of carbon dioxide evolution from Clarion fine sandy loam on incubation at selected moisture tensions.

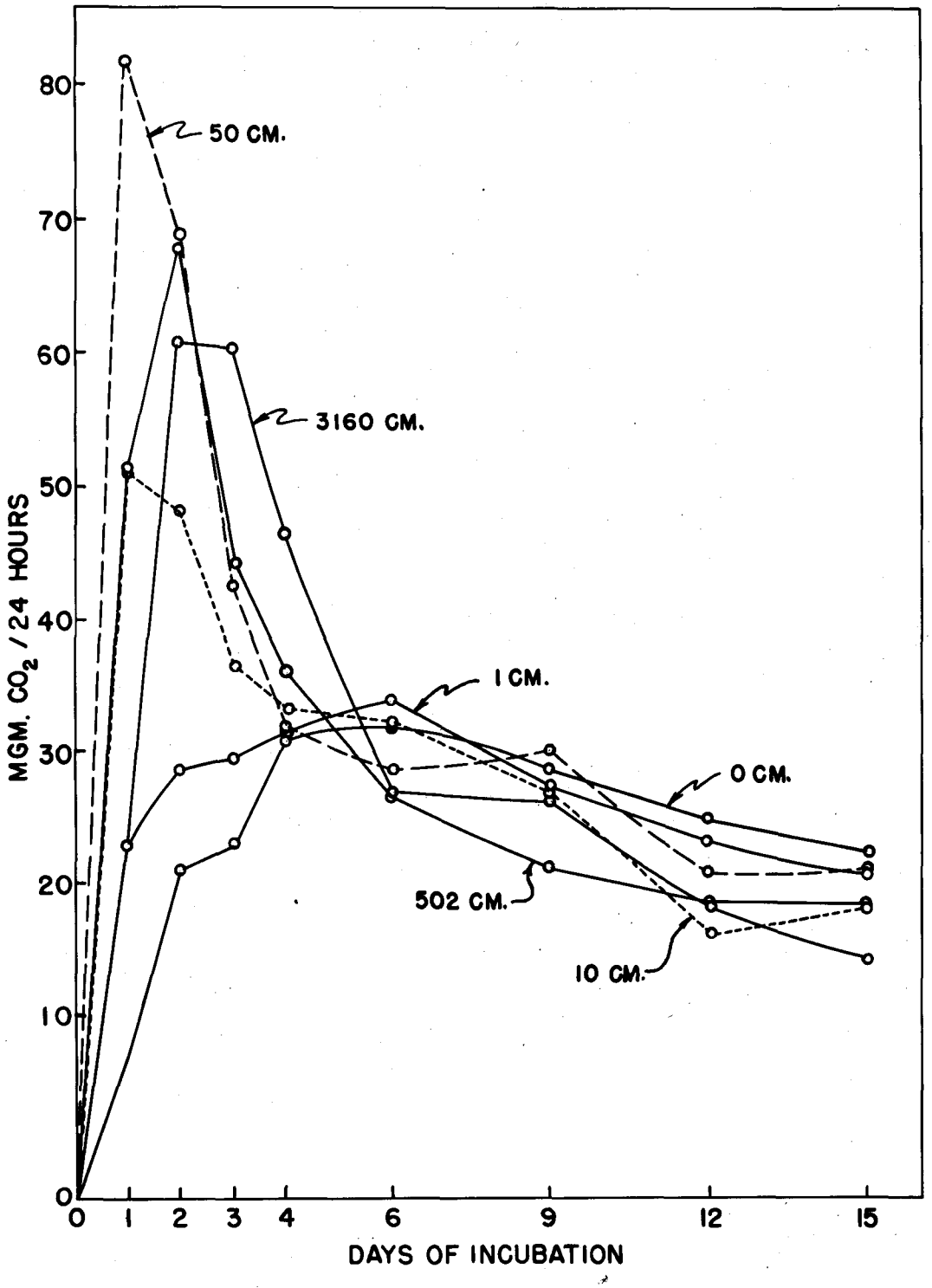

80
70
60
50
40
30
20
10
0
MGM. CO_2 / 24 HOURS
50 CM.
3160 CM.
1 CM.
0 CM.
502 CM.
10 CM.
0
1
2
3
4
6
9
12
15
DAYS OF INCUBATION

Fig. 7. Daily rate of carbon dioxide evolution from Wabash silty clay on incubation at selected moisture tensions.

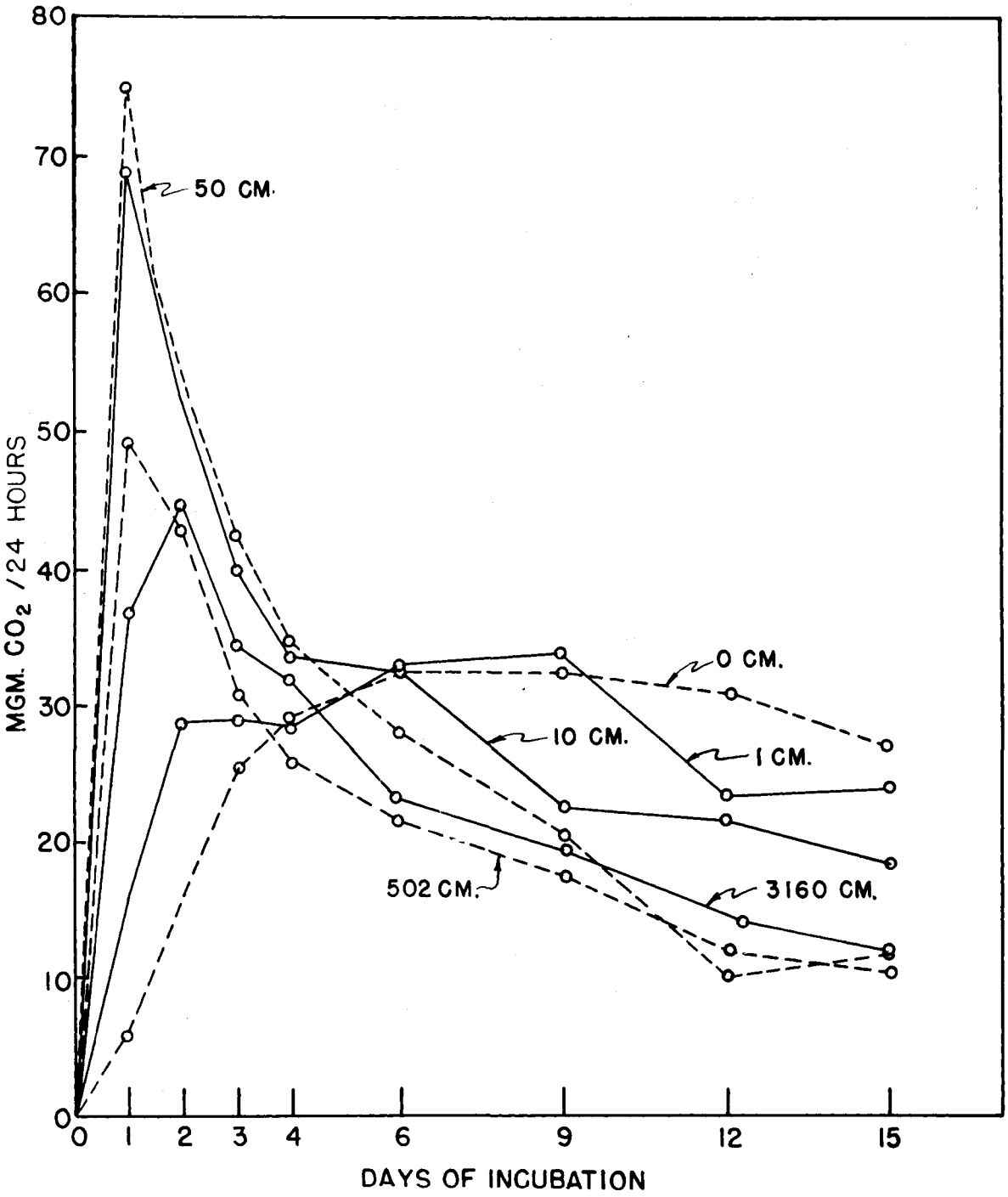

80
70
60
50
40
30
20
10
0
MGM. CO_2 /24 HOURS
50 CM.
0 CM.
10 CM.
1 CM.
502 CM.
3160 CM.
0
1
2
3
4
6
9
12
15
DAYS OF INCUBATION

Fig. 8. Daily rate of carbon dioxide evolution from Webster silt loam on incubation at selected moisture tensions.

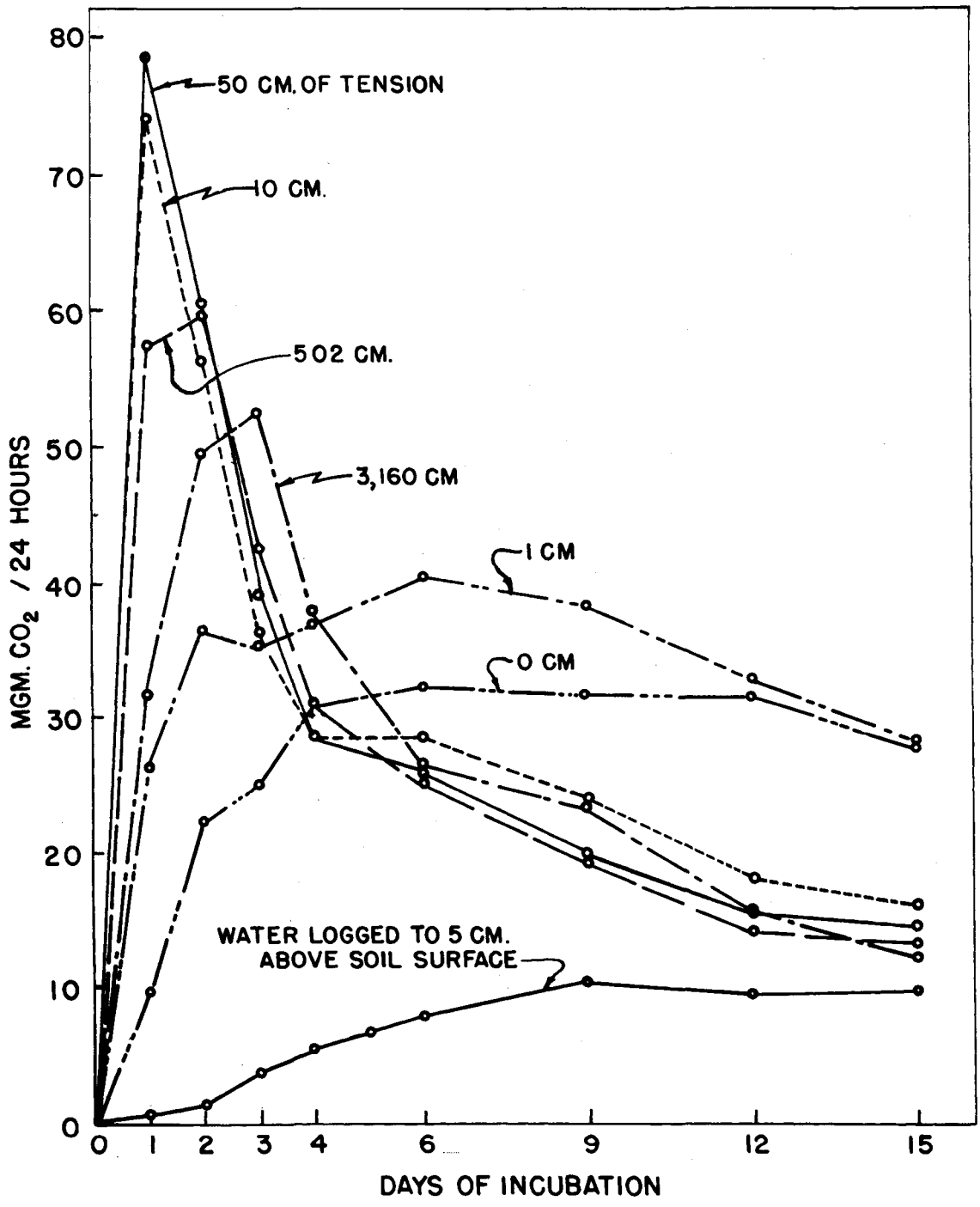
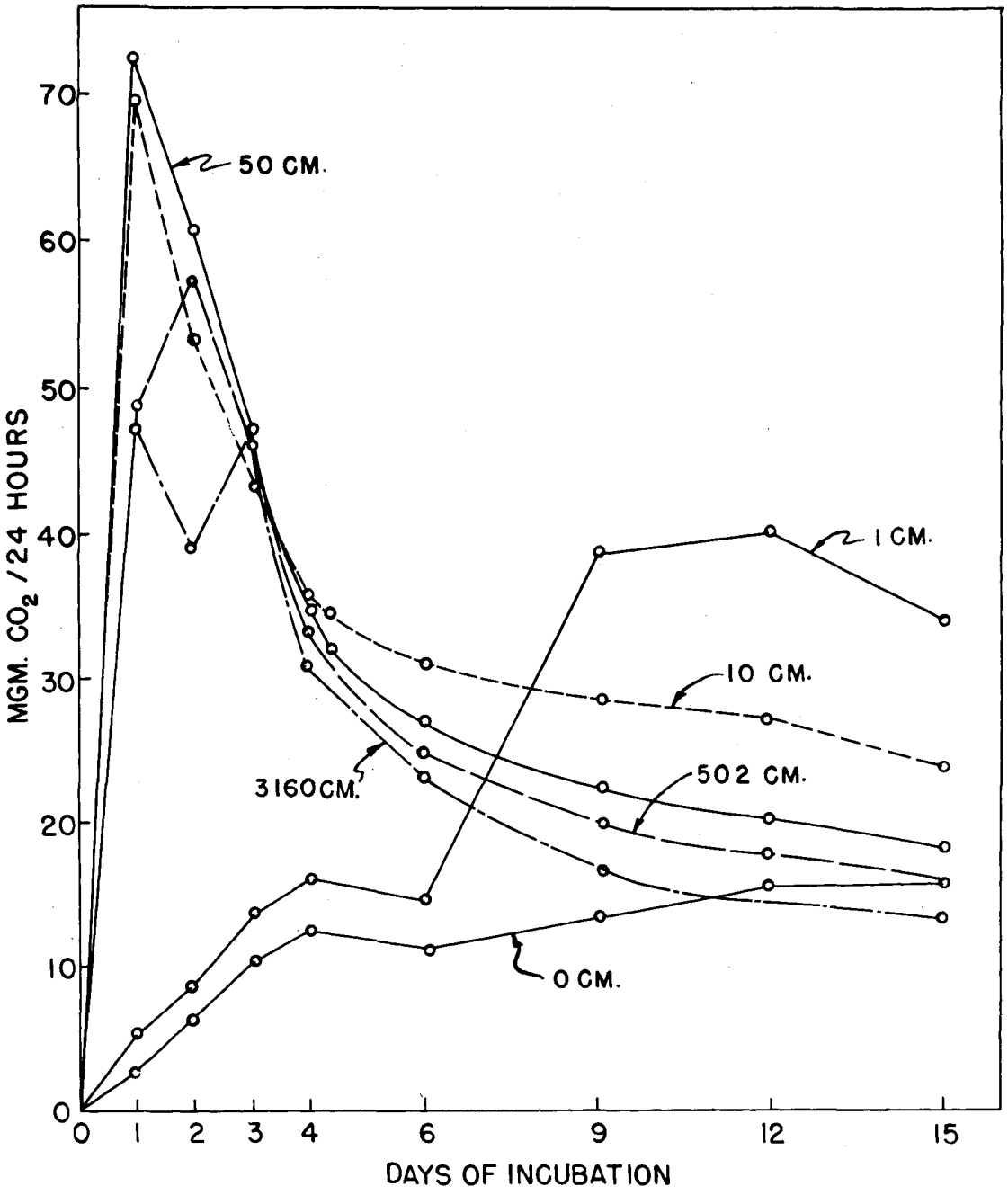

80
70
60
50
40
30
20
10
0
MGM. CO_2 / 24 HOURS
50 CM. OF TENSION
10 CM.
502 CM.
3,160 CM
1 CM
0 CM
WATER LOGGED TO 5 CM.
ABOVE SOIL SURFACE
0 1 2 3 4 6 9 12 15
DAYS OF INCUBATION

Fig. 9. Daily rate of carbon dioxide evolution from Webster silt loam incubated in differently sized containers (5 x 5 x 15 cm.) at selected moisture tensions.


70
60
50
40
30
20
10
0
MGM. CO_2 / 24 HOURS
50 CM.
1 CM.
10 CM.
3160CM.
502 CM.
0 CM.
0 1 2 3 4 6 9 12 15
DAYS OF INCUBATION

At a given moisture tension, the curves showing relation between daily rates of CO_2 evolution and period of incubation were similarly shaped for all soils. At higher moisture tension there is a rapid increase in the rate of CO_2 evolution during the initial stages of incubation, and the early maximum is followed by a rapid decrease (Fig. 10). The nature of the curve suggests a logarithmic ascent, followed by a similar descent, similar in characteristic to the growth curve of micro-organisms.

The shapes of the curves towards saturation are entirely different (Fig. 11) from those for the drier range (cf. Fig. 10). The sharp peak is absent and the curves are more or less flat. In most cases there are two peak rates, the first one being attained during the early stages of incubation (generally within 2 days) and the second one at approximately 6 to 9 days. The second peak rate is entirely absent in the case of drier soils.

Fig. 10. Daily rates of carbon dioxide evolution from five Iowa soils incubated at 50 cm. of moisture tension.

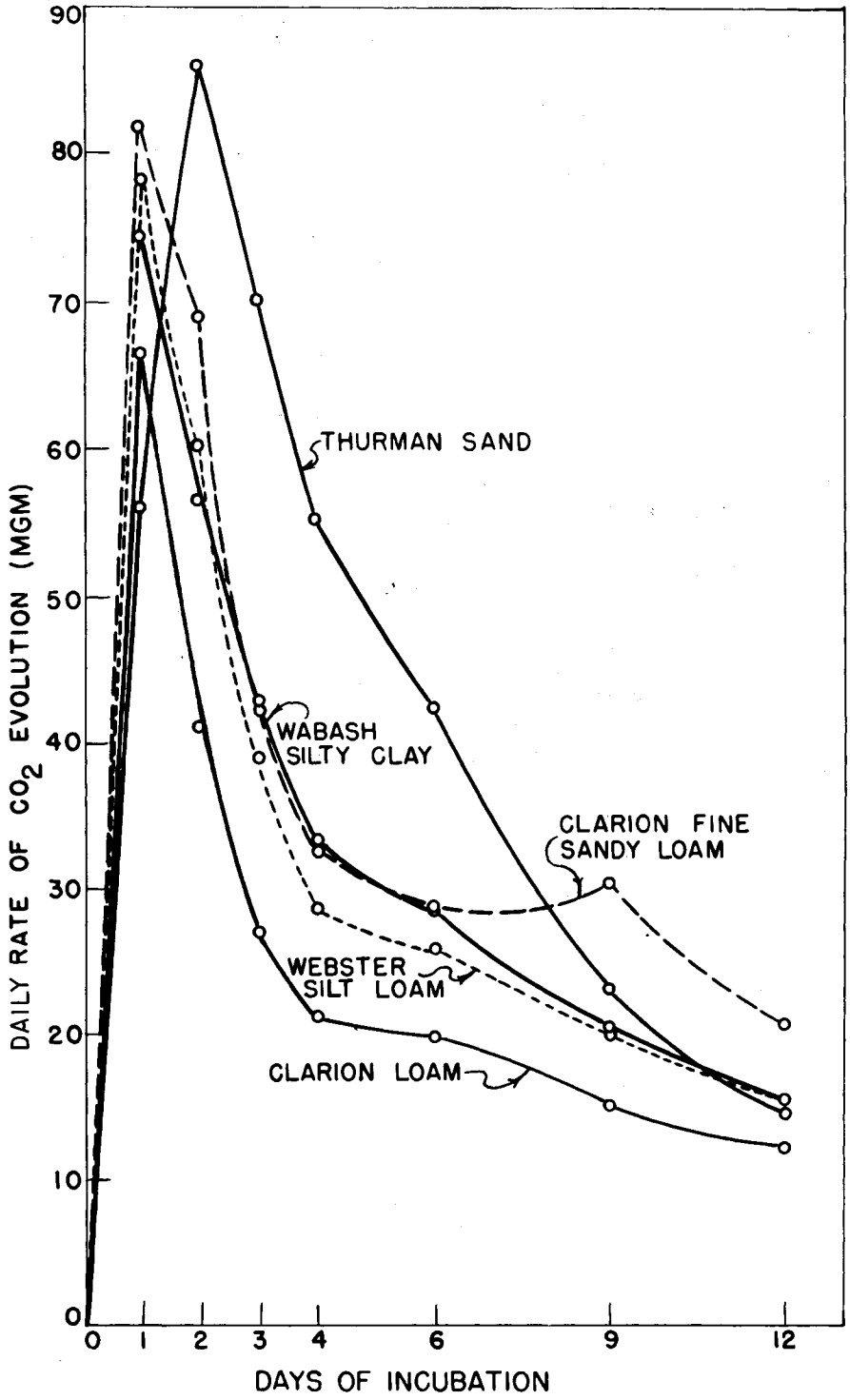
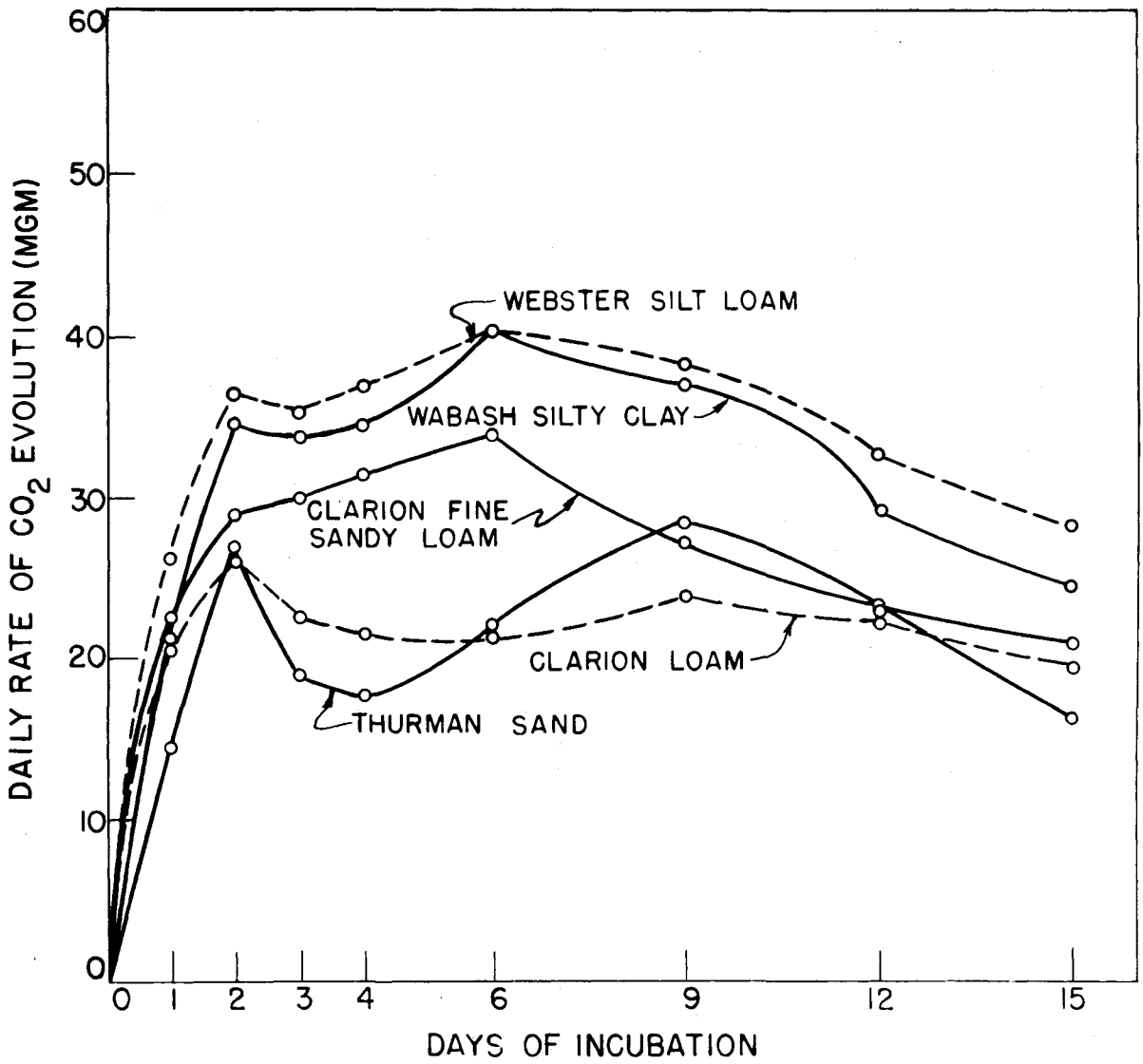

90
80
70
60
50
40
30
20
10
0
DAILY RATE OF CO_2 EVOLUTION (MGM)
THURMAN SAND
WABASH
SILTY CLAY
CLARION FINE
SANDY LOAM
WEBSTER
SILT LOAM
CLARION LOAM
0 1 2 3 4 6 9 12
DAYS OF INCUBATION

Fig. 11. Daily rates of carbon dioxide evolution from five Iowa soils incubated at 1 cm. of moisture tension.


DAILY RATE OF CO_2 EVOLUTION (MGM)
60
50
40
30
20
10
0
WEBSTER SILT LOAM
WABASH SILTY CLAY
CLARION FINE SANDY LOAM
CLARION LOAM
THURMAN SAND
0 1 2 3 4 6 9 12 15
DAYS OF INCUBATION

Relative changes in the magnitude of peak rate of CO_2 evolution with change in moisture tension have been presented graphically in Figure 12. Again (cf. Fig. 3), tensions corresponding to 0 and 1 cm. of water have been plotted as corresponding to 1 and 2 cm. of water, for the reasons stated previously.

To determine whether the peak rate for CO_2 evolution observed at 50 cm. of tension for soil with added organic matter would occur at the identical tension for soil containing only native organic matter, Wabash silty clay was incubated unamended for 15 days and the CO_2 evolved was collected at regular intervals. Observations are given in Table 7.

Although the cumulative CO_2 evolved in 15 days increased progressively with decreasing moisture tension, the peak rate of CO_2 evolution was again noted at 50 cm. of water tension.

Fig. 12. Peak rates of carbon dioxide evolution from five Iowa soils as influenced by moisture tension.
X - Peak rate in mgm. CO_2 per day
Y - Centimeters of water tension (logarithmic scale).

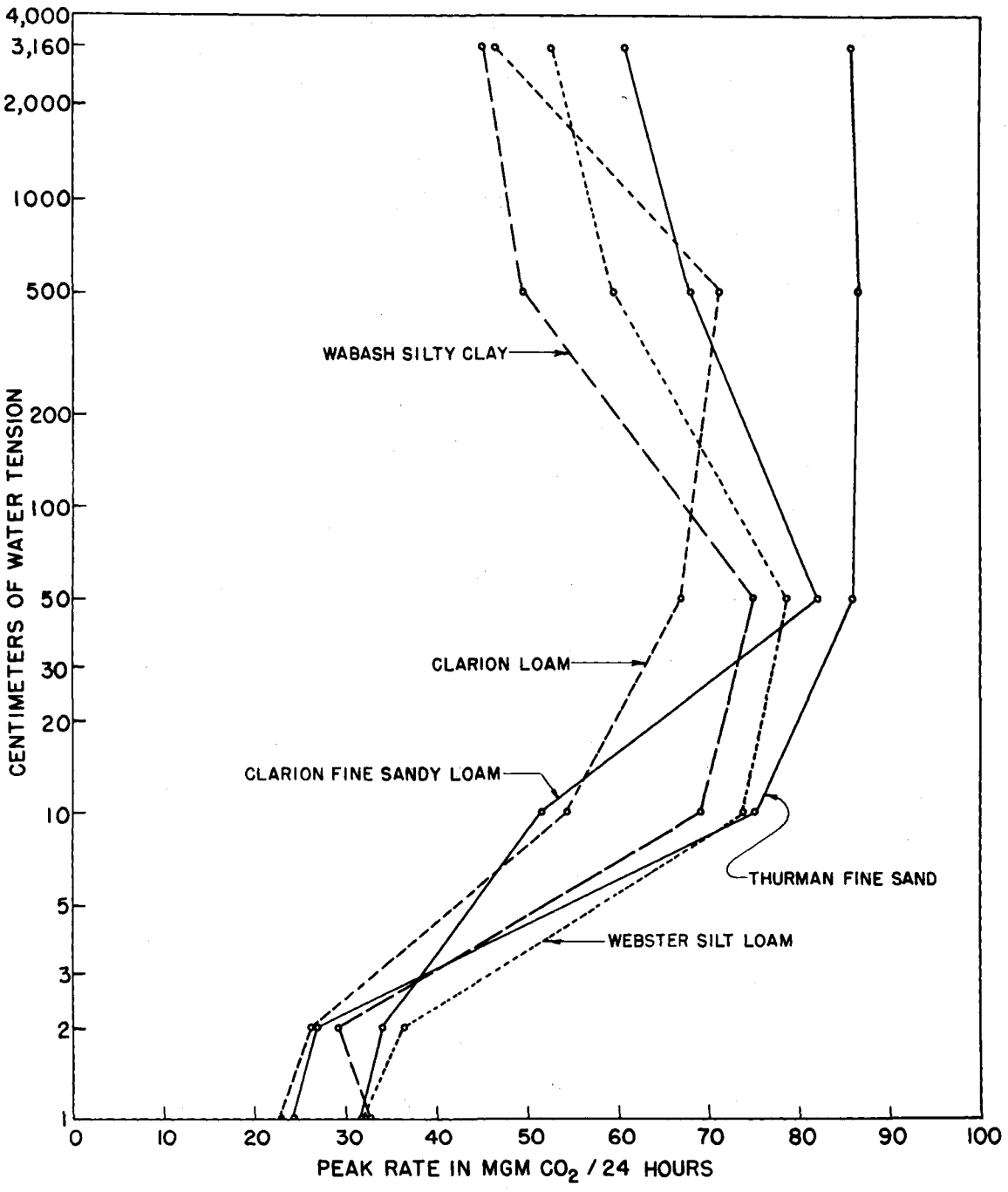

4,000
3,160
2,000
1000
500
200
100
50
30
20
10
5
3
2
1
CENTIMETERS OF WATER TENSION
WABASH SILTY CLAY
CLARION LOAM
CLARION FINE SANDY LOAM
THURMAN FINE SAND
WEBSTER SILT LOAM
0
10
20
30
40
50
60
70
80
90
100
PEAK RATE IN MGM CO_2 / 24 HOURS

Table 7

Evolution of carbon dioxide from Wabash silty clay incubated without addition of powdered corn stover

Centimeters of water tension	Cumulative CO_2 evolution in 15 days	Peak rate of CO_2 evolution
	mgm. CO_2	mgm. CO_2/24 hours
1.0	90.75	9.99
10.0	81.64	17.45
50.0	73.78	17.60
502.0	55.48	11.34
940.0	53.85	9.41

It was considered desirable to investigate the effect of water-logging of a soil on the rate and extent of liberation of carbon dioxide during incubation. Air-dry Webster silt loam, in quantities equivalent to 100 grams on an oven-dry basis, was mixed thoroughly with 1 gram of finely powdered corn stover and then placed in Erlenmeyer flasks. The surface was levelled, and 100 grams of coarse quartz sand, free from organic matter, was spread evenly on the top of the soil. Such a layer of quartz sand was used to keep the soil in position with the corn stalk powder, which would otherwise float to the surface. The contents of the flask were water-logged to about 5 cm. above the soil surface by addition of 180 ml. of carbon dioxide free distilled water. Flasks with their contents were then incubated in the usual fashion.

Data on carbon dioxide evolution from water-logged soil are given in Table 8. It may be seen that the rate and extent of carbon dioxide evolution was extremely slow in water-logged soil. In the presence of anaerobic conditions, other volatile products, as methane and hydrogen, and organic acids may accompany the transformation of organic matter (17), and the carbon dioxide evolved accounts for only a part of the total carbon transformations under water-logged conditions.

The peak rate of carbon dioxide evolution was found to occur after 6 to 9 days of incubation. This incidence corresponds to the second peak rate of carbon dioxide evolution observed in the case of most soils when incubated at low moisture tension (cf. Fig. 10).

Table 8

Evolution of carbon dioxide from Webster silt loam when incubated under water-logged conditions

Days from start	Incubation period	CO_2 liberated	Daily rate of CO_2 evolution
	hours	mgm. CO_2	mgm. CO_2/24 hours
1	0-15	0.35	0.57
2	15-39	1.54	1.54
3	39-63	3.78	3.78
4	63-87	5.32	5.32
6	87-135	15.66	7.83
9	135-207	31.55	10.52
12	207-279	28.71	9.57
15	279-360	32.97	9.77

The influence upon carbon dioxide evolution of a radical shift in the moisture status of a soil while active decomposition is in progress was investigated in parallel experiments upon Thurman sand and Webster silt loam.

For each of these soils, three series of incubation flasks were arranged. In one series, moisture tension was maintained constant at 50 cm. throughout the 15 days of incubation. In a second series, it was maintained at 0 cm. throughout incubation. In the third series for each soil, moisture tension was maintained at 50 cm. for 2 days, then by the addition of water the tension was changed to 0 cm., and for the remaining 13 days, the soil was incubated at this tension value. All flasks established received air-dry soil in quantities equivalent to 100 grams on an oven-dry basis, together with 1 per cent of ground corn stover.

The total amounts of carbon dioxide evolved under each of these three conditions are shown in Table 9. The daily rates of carbon dioxide evolution are presented in Tables 10 to 12, inclusive.

When the soil moisture was changed abruptly from 50 cm. to 0 cm. of water tension, carbon dioxide evolution was reduced to a rate below that occurring in soil maintained at a constant water tension of 0 cm. In the following days of incubation, however, there was a gradual increase in the rate of carbon dioxide evolution, and the shape of the curves (Figures 13 and 14) at 0 cm. for soil initially at that tension and for soil abruptly changed to that tension during incubation became roughly parallel.

Table 9

Cumulative total carbon dioxide evolved from soils at constant and at abruptly changed moisture tensions

Soil type	Moisture tension		
	50 cm. for 15 days	50 cm. for 2 days, then 0 cm. for 13 days	0 cm. for 15 days
	mgm. CO_2		
Thurman fine sand	507.04	381.80	343.25
Wabash silty clay	415.44	400.07	421.68

Table 10

Evolution of carbon dioxide from Thurman fine sand and Wabash silty clay incubated at a moisture tension of 50.0 cms. for 15 days

Days from start	Moisture tension	Incubation period	Total CO_2 liberated	Daily rate of CO_2 evolution
	cms.	hours	mgms.	mgm./24 hours
		Thurman Fine Sand		
1	50.0	0-24	56.18	56.18
2	"	24-48	85.83	85.83
3	"	48-72	70.54	70.54
4	"	72-96	55.50	55.50
6	"	96-144	84.81	42.41
9	"	144-216	69.86	23.29
12	"	216-288	43.87	14.62
15	"	288-360	40.45	13.49
		Wabash Silty Clay		
1	50.0	0-24	54.59	54.59
2	"	24-48	64.87	64.87
3	"	48-72	52.34	52.34
4	"	72-96	38.40	38.40
6	"	96-144	55.77	27.89
9	"	144-216	51.52	17.17
12	"	216-288	52.82	17.27
15	"	288-360	45.13	15.04

Table 11

Evolution of carbon dioxide from Thurman fine sand and Wabash silty clay incubated at 0.0 cm. moisture tension

Days from start	Moisture tension	Incubation period	CO_2 liberated	Daily rate of CO_2 evolution
	cm.	hours	mgm.	mgm./24 hours
		Thurman Fine Sand		
1	0.0	0-15	4.32	6.91
2	"	15-39	24.22	24.22
3	"	39-63	22.10	22.10
4	"	63-87	21.09	21.09
6	"	87-135	47.85	23.93
9	"	135-207	80.40	26.80
12	"	207-279	80.35	26.78
15	"	279-360	62.92	18.64
		Wabash Silty Clay		
1	0.0	0-15	3.90	6.24
2	"	15-39	16.07	16.07
3	"	39-63	25.40	25.40
4	"	63-87	29.30	29.30
6	"	87-135	64.63	32.32
9	"	135-207	97.83	32.61
12	"	207-279	93.34	31.12
15	"	279-360	91.24	27.03

Table 12

Evolution of carbon dioxide from Thurman fine sand and Wabash silty clay incubated at moisture tension of 50 cm. for 2 days, then at 0 cm. for following 13 days

Days from start	Moisture tension	Incubation period	CO_2 liberated	Daily rate of CO_2 evolution
	cms.	hours	mgm.	mgm./24 hours
		Thurman Fine Sand		
1	50.0	0-24	56.18	56.18
2	"	24-48	85.83	85.83
3	0.0	48-72	32.61	32.61
4	"	72-96	23.16	23.16
6	"	96-144	35.09	17.55
9	"	144-216	43.36	14.45
12	"	216-288	54.17	18.06
15	"	288-360	51.40	17.13
		Wabash Silty Clay		
1	50.0	0-24	54.59	54.59
2	"	24-48	64.87	64.87
3	0.0	48-72	11.94	11.94
4	"	72-96	13.59	13.59
6	"	96-144	42.18	21.09
9	"	144-216	76.68	25.56
12	"	216-288	67.81	22.60
15	"	288-360	68.41	22.80

Fig. 13. Daily rates of carbon dioxide evolution from Thurmen fine sand incubated under constant and abruptly changed moisture tensions.

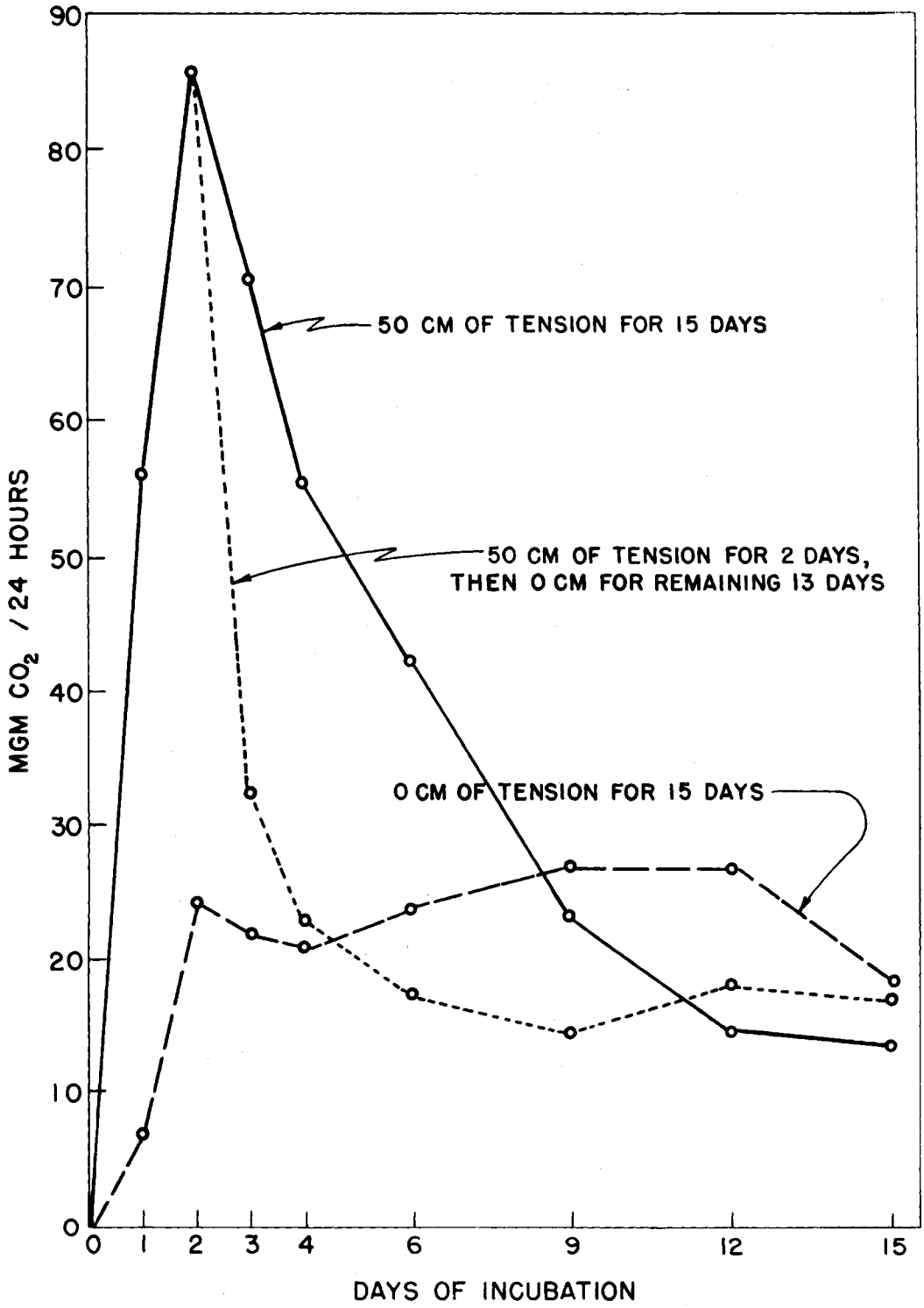
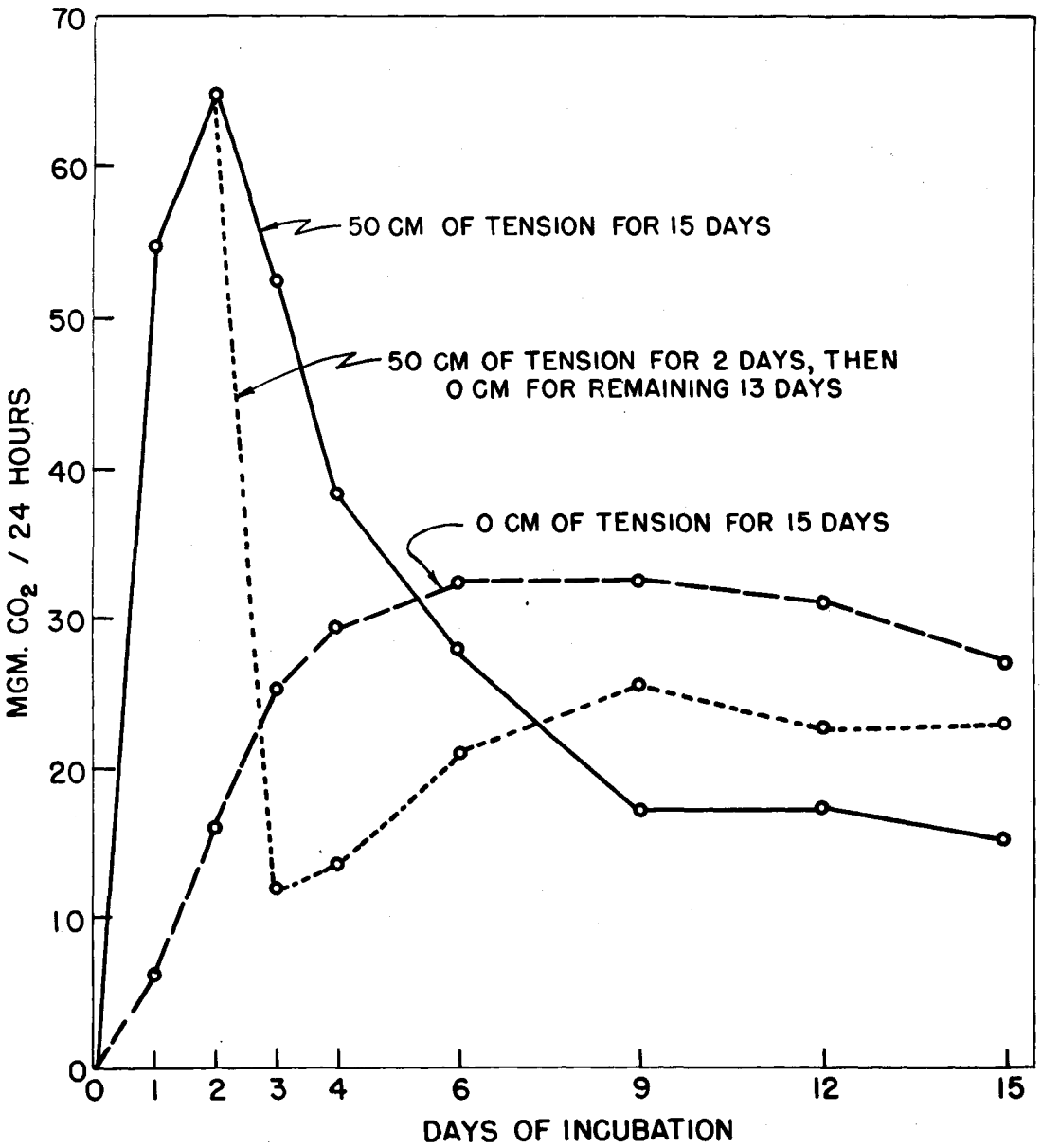

90
80
70
60
50
40
30
20
10
0
MGM CO_2 / 24 HOURS
50 CM OF TENSION FOR 15 DAYS
50 CM OF TENSION FOR 2 DAYS,
THEN 0 CM FOR REMAINING 13 DAYS
0 CM OF TENSION FOR 15 DAYS
0 1 2 3 4 6 9 12 15
DAYS OF INCUBATION

Fig. 14. Daily rates of carbon dioxide evolution from Wabash silty clay incubated under constant and abruptly changed moisture tensions.


70
60
50
40
30
20
10
0
MGM. CO_2 / 24 HOURS
50 CM OF TENSION FOR 15 DAYS
50 CM OF TENSION FOR 2 DAYS, THEN
0 CM FOR REMAINING 13 DAYS
0 CM OF TENSION FOR 15 DAYS
0 1 2 3 4 6 9 12 15
DAYS OF INCUBATION

Mineralization of Nitrogen in Soil at Differing Moisture Tensions

Observations on mineralization of nitrogen in soil were limited to Thurman fine sand and to Wabash silty clay, both with 2 per cent egg albumin added.

The extent of mineralization in these soils during 1, 2, and 4 weeks of incubation is shown in Tables 13 and 14. The values recorded are the averages of 2 to 4 replicate incubation flasks.

In Wabash silty clay, there was none or else only very little ammonia lost by volatilization. Failure to collect the amount volatilized would not have affected appreciably the total amount determined. In Thurman sand, however, the losses by volatilization were large. At four weeks, the ammonia volatilized accounted, in the majority of instances, for more than one-third the total amount formed. Without taking into account the amount lost by volatilization, the mean difference between replicates at four weeks was 15.53 mgm. of ammonia nitrogen. When the loss was taken into account, the mean difference was 2.55 mgm. The importance of determining the amount volatilized, both for accuracy in the total, and in obtaining agreement between replicates, is readily apparent.

Considering the total mineralization occurring in the two soils under differing moisture tensions, depression in rate of mineralization is seen both toward the dry range (3,160 cm. of tension) and toward the wet range (0 to 1 cm. of tension). The differences with moisture contents are significant only in the earlier periods; they tend to disappear with longer periods of incubation. At the end of 4 weeks, the amounts of nitrogen mineralized were practically the same under all

moisture treatments. This was especially true in the case of Thurman sand.

The maximum rate of mineralization of nitrogen in both soils occurred at 50 cm. of water tension. Such a maximum is in agreement with results obtained during the course of carbon dioxide evolution studies.

There was no nitrification of significance in Thurman fine sand even at the end of 4 weeks of incubation. In the case of Wabash silty clay, there was little or no nitrification up to a period of 2 weeks under any moisture treatment. At the end of 4 weeks, however, part of the nitrogen mineralized was in the form of nitrates (including nitrites) in the case of samples incubated under 10, 50, and 502 cm. of water tension. There was practically no nitrification at 0, 1, and 3,160 cm. of tension.

Comparison of values for total mineralization of nitrogen in the two soils shows that organic nitrogen added to Thurman sand in the form of egg albumin is more completely mineralized than is that nitrogen added to Wabash silty clay.

Comparing values for total mineralization obtained at 2 and 4 weeks for all moisture treatments, it is seen that at the end of 4 weeks, the values for mineralization in Wabash silty clay under moisture tensions of 1, 10 and 50 cm. of water are distinctly inferior to the values observed for these tensions at the end of two weeks. Apparently, nitrogen already mineralized has been lost. The loss cannot be explained by volatilization, inasmuch as all ammonia volatilized was subject to absorption in collecting tubes. Nor is there any reason to believe that any appreciable amount of nitrogen would be immobilized by the soil organisms, in the absence of any abundant supply of energy materials.

One interesting observation noted during the course of the chemical analyses may be mentioned. The acid potassium chloride extracts of the samples incubated at lower moisture tensions (0, 1 and 10 cm.) were colorless to greenish, whereas those for higher moisture tensions were strongly colored, either brown or deep brown. Neutralization of the extracts from soils at the lower moisture tensions with alkali yielded a greenish precipitate which turned to brown during filtration and washing. In the case of the higher moisture tensions, the precipitates initially obtained were deep brown. Solution of these precipitates in dilute hydrochloric acid and testing with $K_4Fe(CN)_6$ solution gave a positive "Prussian Blue" test for iron. The greenish color of the extract at low moisture tensions is undoubtedly due to ferrous (Fe^{++}) ions, whereas the brown color of the extracts from soils incubated at higher moisture tensions indicates the presence of iron in the ferric (Fe^{+++}) state.

Table 13

Formation of ammonia and nitrate (including nitrite) in Thurman fine sand amended with 2 grams of egg albumin and incubated for 1, 2, and 4 weeks at differing moisture tensions

Incubation period	Moisture tension	NH_3-N			NO_3-N in the soil	Total nitrogen mineralised
		In the soil	Volatilised	Total		
	cms.	mgms.	mgms.	mgms.	mgms.	mgms.
1 week	0.0	53.63	0.38	54.01	0.60	54.61
"	1.0	57.53	0.38	57.91	0.00	57.91
"	10.0	105.46	1.05	106.51	0.50	107.01
"	50.0	122.84	3.71	126.55	1.20	127.75
"	502.0	101.72	4.54	106.26	1.70	107.96
"	3160.0	89.13	3.45	92.58	1.20	93.78
2 weeks	0.0	165.83	3.00	168.83	1.95	170.78
"	1.0	161.79	2.06	163.85	0.60	164.45
"	10.0	174.07	10.64	184.71	1.50	186.21
"	50.0	147.41	42.06	189.47	1.40	190.87
"	502.0	137.52	30.71	168.23	2.40	170.63
"	3160.0	131.23	33.97	165.20	1.95	167.15
4 weeks	0.0	191.75	15.58	207.33	1.20	208.53
"	1.0	171.82	34.38	206.20	1.80	208.00
"	10.0	138.42	71.98	210.40	0.90	211.30
"	50.0	135.12	71.83	206.95	1.35	208.30
"	502.0	135.42	64.94	200.36	3.30	203.66
"	3160.0	118.05	87.56	205.61	2.70	208.31

Table 14

Formation of ammonia and nitrate (including nitrite) in Wabash silty clay amended with 2 grams of egg albumin and incubated for 1, 2, and 4 weeks at differing moisture tensions

Incubation period	Moisture tension	NH_3-N			NO_3-N in the soil	Total nitrogen mineralised
		In the soil	Volatilised	Total		
	cms.	mgms.	mgms.	mgms.	mgms.	mgms.
1 week	0.0	78.66	nil	78.66	1.21	79.87
"	1.0	141.51	"	141.51	1.61	143.12
"	10.0	145.12	"	145.12	2.41	147.53
"	50.0	147.57	"	147.57	3.00	150.57
"	260.0	142.88	"	142.88	2.11	144.99
"	502.0	142.31	"	142.31	1.20	143.51
"	3160.0	118.04	0.37	118.41	1.35	119.76
2 weeks	0.0	122.54	nil	122.54	1.50	124.04
"	1.0	143.51	"	143.51	2.40	145.91
"	10.0	145.36	"	145.36	5.56	150.92
"	50.0	159.23	"	159.23	4.43	163.66
"	260.0	157.88	"	157.88	3.76	161.64
"	502.0	166.80	"	155.80	2.10	157.90
"	3160.0	147.70	1.50	149.20	0.00	149.20
4 weeks	0.0	150.08	0.60	150.68	1.50	152.18
"	1.0	132.38	0.37	132.75	1.58	134.33
"	10.0	108.66	0.60	109.26	20.32	129.58
"	50.0	126.84	0.52	127.36	22.80	150.16
"	260.0	144.38	1.05	145.43	27.92	173.35
"	502.0	156.69	3.04	159.73	17.38	177.11
"	3160.0	161.34	2.33	163.67	1.80	165.47

Activity of Important Sub-groups of Soil Micro-organisms at Differing Soil Moisture Tensions

Population changes

Microbiological analyses of incubated soils showed that there were differences in the numbers of fungi, actinomycetes, and bacteria, not only between soils differing in texture and incubated at equivalent moisture, but also within aliquots of any one soil incubated at differing tensions.

Microbial numbers determined for the five experimental soils incubated for 5 and 15 days at 0 cm., and 3,160 cm., of water tension are shown in Table 15. At both 0 and 3,160 cm. of tension, Thurman sand showed more fungi on culture than any of the other soils. The greatest numbers of bacteria, however, were observed in Wabash silty clay. For all soils, the populations determined were greater in those portions incubated at 3,160 cm. of water tension than in those incubated at 0 cm. of tension.

Sufficient replicates were available on Webster silt loam incubated in bottles to permit microbiological analyses. The data accumulated in these studies are presented in Table 16. It was again found that the numbers of each of the three important sub-groups of soil micro-organisms were much greater in soil lots incubated toward the dry range than in those incubated at or near saturation.

Daily rates of carbon dioxide evolution, as previously recorded, had indicated that moisture at 50 cm. of water tension provided soil conditions most desirable for microbiological activity. In the data on microbial numbers, there is no indication that 50 cm. of water tension provide optimal conditions. This discrepancy will be considered in the general discussion below.

Table 15

Microbial numbers determined after 5 and 15 days of incubation at moisture tensions equivalent to 0 and 3,160 centimeters of water

	Fungi		Actinomycetes		Bacteria	
	5 days	15 days	5 days	15 days	5 days	15 days
	thousands per gram		millions per gram		millions per gram	
At 0 cm. tension						
Thurman fine sand	63	130	18	43	58	54
	63	115	14	30	61	33
Clarion loam	37	31	10	29	122	32
	17	27	15	39	77	25
Clarion fine sandy loam	10	21	11	38	128	45
	4	22	12	33	90	33
Webster silt loam	4	20	14	37	64	34
	10	25	17	41	88	43
Wabash silty clay	13	56	15	54	51	57
	3	43	14	40	84	50
At 3,160 cm. tension						
Thurman fine sand	6,000	11,700	18	30	81	38
	7,600	10,400	34	20	88	26
Clarion loam	243	420	63	72	145	113
	240	370	50	57	122	85
Clarion fine sandy loam	127	320	33	133	280	220
	147	380	43	70	296	230
Webster silt loam	233	620	47	62	143	148
	197	610	39	77	138	167
Wabash silty clay	150	620	38	93	316	360
	167	730	34	70	337	297

Table 16

Microbial numbers determined for Webster silt loam when incubated at selected moisture tensions

Moisture tension equivalent in cm. of water	Fungi		Actinomycetes		Bacteria	
	5 days	15 days	5 days	15 days	5 days	15 days
	thousands per gram		millions per gram		millions per gram	
0	25	25	7.3	6.5	16	28
	26	39	7.7	3.5	11	21
1	26	40	7.0	4.0	12	26
	21	34	5.0	3.7	9	18
10	35	74	13.3	8.3	66	60
	31	73	7.0	7.0	63	30
50	58	287	10.0	8.0	97	70
	62	250	8.0	4.0	82	66
500	113	463	41.5	28.0	85	105
	104	517	38.8	27.0	131	110
3160	107	593	76.1	67.0	105	97
	110	517	55.0	50.0	140	110

Carbon dioxide evolution by selected micro-organisms following their inoculation on sterilized soils

In the previous studies employing unsterilized soils, the amount of carbon dioxide evolved during incubation was the resultant of the activity of the many members of a heterogeneous soil population. To determine whether different sub-groups of micro-organisms were disproportionately affected by differing soil moisture tensions, pure cultures of soil organisms were introduced into sterilized soils. Incubation of soils and collection of carbon dioxide evolved were carried out in such a manner that any further contamination of the inoculated soils was avoided. Experiments with isolated cultures were carried out in Webster silt loam and at moisture tensions of 1, 50, and 3,160 cm. of water.

Almost without exception, uninoculated controls and experimentally inoculated soils remained free of incidental contamination. In one experiment with actinomycetes, for which results are recorded below, the flasks were found contaminated with bacteria at the end of 15 days. An abrupt change in the daily rate of CO_2 evolution after six days indicated the probable time at which contamination occurred.

The extent of carbon dioxide evolution from uninoculated, sterilized Webster silt loam is shown in Table 17. These results indicate complete sterilization. The amounts recovered during the first few days were doubtless present in the soil and in the containers at the time of establishment.

Table 17

Carbon dioxide evolved from uninoculated, steam-sterilized Webster silt loam

Hours of incubation	Mgm. CO_2 evolved
0 - 24	6.77
24 - 48	1.39
48 - 72	0.10
72 - 96	0.0
96 - 144	0.0

The total amounts of carbon dioxide evolved and the peak rates of evolution by representative soil micro-organisms at selected moisture tensions are presented in Tables 18-21, inclusive. The corresponding total values previously determined (cf. Table 5) for unsterilized soil are also entered for purposes of ready comparison.

It is observed that among the groups of micro-organisms studied, fungi exhibited the maximum activity, followed by the actinomycetes, and finally, by the bacteria. The latter two groups were especially favored by decreasing moisture tensions in the soil. It should be noted that the higher value for carbon dioxide evolution at 3,160 cm. of tension than at 50 cm. in the case of actinomycetes was due to contamination, as revealed culturally at the end of the incubation period. The contaminants were Gram-negative, rod-shaped bacteria.

The evolution of carbon dioxide in the case of bacteria was greatly depressed at a moisture tension of 3,160 cm., but was increasingly favored with decreasing moisture tension up to 1.0 cm., as shown both by total amounts and peak rates of carbon dioxide evolution. The activity of fungi on the other hand remained nearly constant throughout the entire moisture range studied. If anything, it was favored toward the dry range, or 3,160 cm. of water tension.

It is found, however, that in no instance do any of the inoculants, separately or combined, show the maximum peak rate of carbon dioxide evolution at 50 cm. of water tension, such as is shown almost without exception in unsterilized soil. In the case of bacteria and actinomycetes, the value for peak rate increases with decrease in moisture tension, in the case of fungi, it remains fairly constant, and in the case of mixed cultures (fungi, bacteria, and actinomycetes), it shows a tendency to rise with increase in moisture tension.

Table 18

Evolution of carbon dioxide from Webster silt loam following inoculation with important sub-groups of micro-organisms

Moisture tension	Treatment	Total CO_2 liberated in 15 days	Peak rate of CO_2 evolution
cms.		mgm.	mgm./24 hours
1.0	Sterilised and inoculated with bacteria	108.79	19.19
"	Sterilised and inoculated with actinomycetes	194.55	20.13
"	Sterilised and inoculated with fungi	275.95	49.32
"	Sterilised and inoculated with bacteria+fungi + actinomycetes	316.19	49.40
"	Unsterilised soil	513.98	36.35
50.0	Sterilised and inoculated with bacteria	88.83	11.31
"	Sterilised and inoculated with actinomycetes	127.86	10.62
"	Sterilised and inoculated with fungi	247.23	48.93
"	Sterilised and inoculated with bacteria+fungi + actinomycetes	298.17	50.29
"	Unsterilised soil	408.75	78.45
3160.0	Sterilised and inoculated with bacteria	21.69	2.24
"	Sterilised and inoculated with actinomycetes	228.15	28.64
"	Sterilised and inoculated with fungi	308.92	49.43
"	Sterilised and inoculated with bacteria+fungi+actinomycetes	305.00	51.23
"	Unsterilised soil	370.55	52.64

Table 19

Evolution of carbon dioxide from Webster silt loam incubated at a moisture tension of 1.0 cm., after steam sterilization and re-inoculation with important types of soil organisms

Incubation period	CO_2 liberated	Daily rate of CO_2 evolution
days	mgm.	mgm./24 hours
	Bacteria	
0 - 2	25.39	12.70
2 - 4	38.37	19.19
4 - 6	19.74	9.87
6 - 9	9.54	3.18
9 - 12	7.54	2.51
12 - 15	8.21	2.74
	Actinomycetes	
0 - 2	31.05	15.53
2 - 4	40.25	20.13
4 - 6	36.54	18.27
6 - 9	32.82	10.94
9 - 12	28.61	9.54
12 - 15	25.28	8.43
	Fungi	
0 - 2	56.33	28.17
2 - 4	98.63	49.32
4 - 6	37.59	18.80
6 - 9	32.94	10.98
9 - 12	25.29	8.43
12 - 15	25.17	8.39
	Bacteria + Fungi + Actinomycetes	
0 - 2	36.81	18.41
2 - 4	98.80	49.40
4 - 6	65.92	32.96
6 - 9	46.02	15.34
9 - 12	34.82	11.61
12 - 15	33.82	11.27

Table 20

Evolution of carbon dioxide from Webster silt loam incubated at a moisture tension of 50.0 cms., after steam sterilization and re-inoculation with important types of soil organisms

Incubation period	CO_2 liberated	Daily rate of CO_2 evolution
days	mgm.	mgm./24 hours
	Bacteria	
0 - 2	9.98	4.99
2 - 4	15.75	7.88
4 - 6	22.62	11.31
6 - 9	18.41	6.14
9 - 12	13.86	4.62
12 - 15	8.21	2.74
	Actinomycetes	
0 - 2	12.64	6.32
2 - 4	16.52	8.26
4 - 6	21.24	10.62
6 - 9	29.16	9.72
9 - 12	25.95	8.65
12 - 15	22.35	7.45
	Fungi	
0 - 2	33.49	16.75
2 - 4	97.85	48.93
4 - 6	44.69	22.35
6 - 9	27.06	9.02
9 - 12	24.84	8.28
12 - 15	19.30	6.43
	Bacteria + Fungi + Actinomycetes	
0 - 2	24.17	12.09
2 - 4	100.57	50.29
4 - 6	61.43	30.72
6 - 9	40.92	13.64
9 - 12	36.37	12.12
12 - 15	34.71	11.57

Table 21

Evolution of carbon dioxide from Webster silt loam incubated at a moisture tension of 3160.0 cms., after steam sterilization and re-inoculation with important types of soil organisms

Incubation period	CO_2 liberated	Daily rate of CO_2 evolution
days	mgm.	mgm./24 hours
	Bacteria	
0 - 2	5.32	2.66
2 - 4	1.17	0.59
4 - 6	1.00	0.50
6 - 9	2.05	0.68
9 - 12	5.44	1.81
12 - 15	6.71	2.24
	Actinomycetes	
0 - 2	3.61	1.81
2 - 4	1.66	0.83
4 - 6	1.67	0.84
6 - 9	81.61	27.20
9 - 12	85.93	28.64
12 - 15	53.67	17.89
	Fungi	
0 - 2	17.30	8.65
2 - 4	98.85	49.43
4 - 6	82.44	41.22
6 - 9	51.34	17.11
9 - 12	33.54	11.18
12 - 15	25.45	8.48
	Bacteria + Fungi + Actinomycetes	
0 - 2	16.64	8.32
2 - 4	102.46	51.23
4 - 6	73.07	36.54
6 - 9	50.56	16.85
9 - 12	35.60	11.87
12 - 15	26.67	8.89

From the values for cumulative total CO_2 evolution, it was observed that a mixed culture of bacteria, actinomycetes, and fungi is more efficient in the breakdown of organic matter than is any single group acting alone. The values for unsterilized soil go even higher, no doubt due to the existence of a greater variety of micro-organisms. A large proportion of the total carbon dioxide liberated was accounted for by the activity of the fungi alone. The difference between the values for fungi, mixed culture, and the general microflora found in unsterilized soil tend to diminish with increasing dryness of the soil. At a tension of 3,160 cm. of water, fungi alone were apparently responsible for most of the carbon dioxide evolved; the other groups of micro-organisms appeared nearly inactive.

Figures 15 to 18, inclusive, show the relative activity at differing tensions of soil moisture of representative soil fungi, actinomycetes, and bacteria, and of the general soil microflora (i.e., unsterilized soil), as revealed by carbon dioxide evolution.

Fig. 15. Relative activity, as revealed by carbon dioxide evolution, of representative soil fungi, actinomycetes and bacteria and the general microflora (i.e., unsterilized soil) when inoculated in Webster silt loam maintained at 50 cm. of water tension.

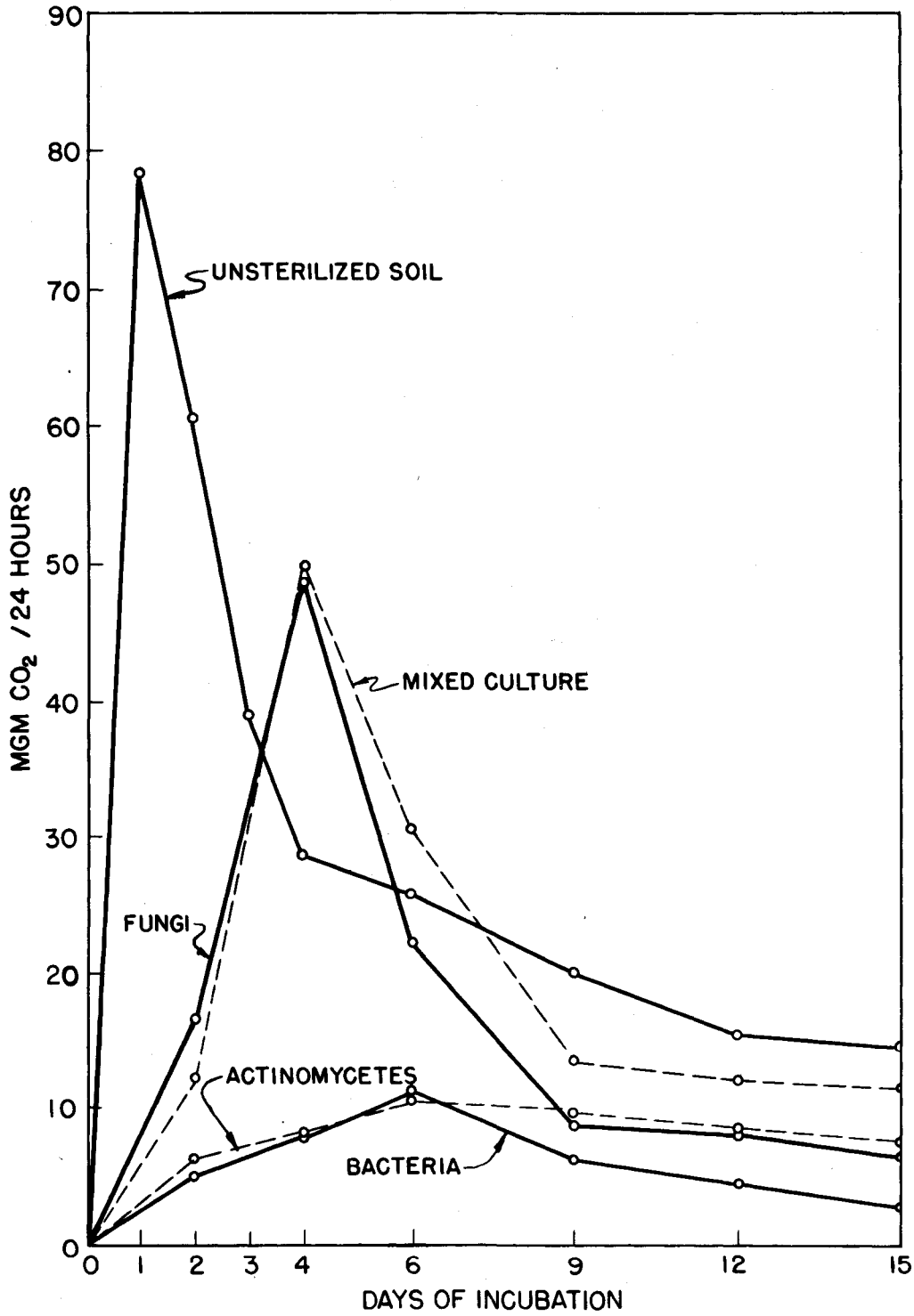

90
80
70
60
50
40
30
20
10
0
MGM CO_2 /24 HOURS
UNSTERILIZED SOIL
MIXED CULTURE
FUNGI
ACTINOMYCETES
BACTERIA
0 1 2 3 4 6 9 12 15
DAYS OF INCUBATION

Fig. 16. Relative activity, as revealed by carbon dioxide evolution, of representative soil fungi, actinomycetes and bacteria and the general microflora (i.e., unsterilized soil) when inoculated in Webster silt loam maintained at 0 cm. of water tension.

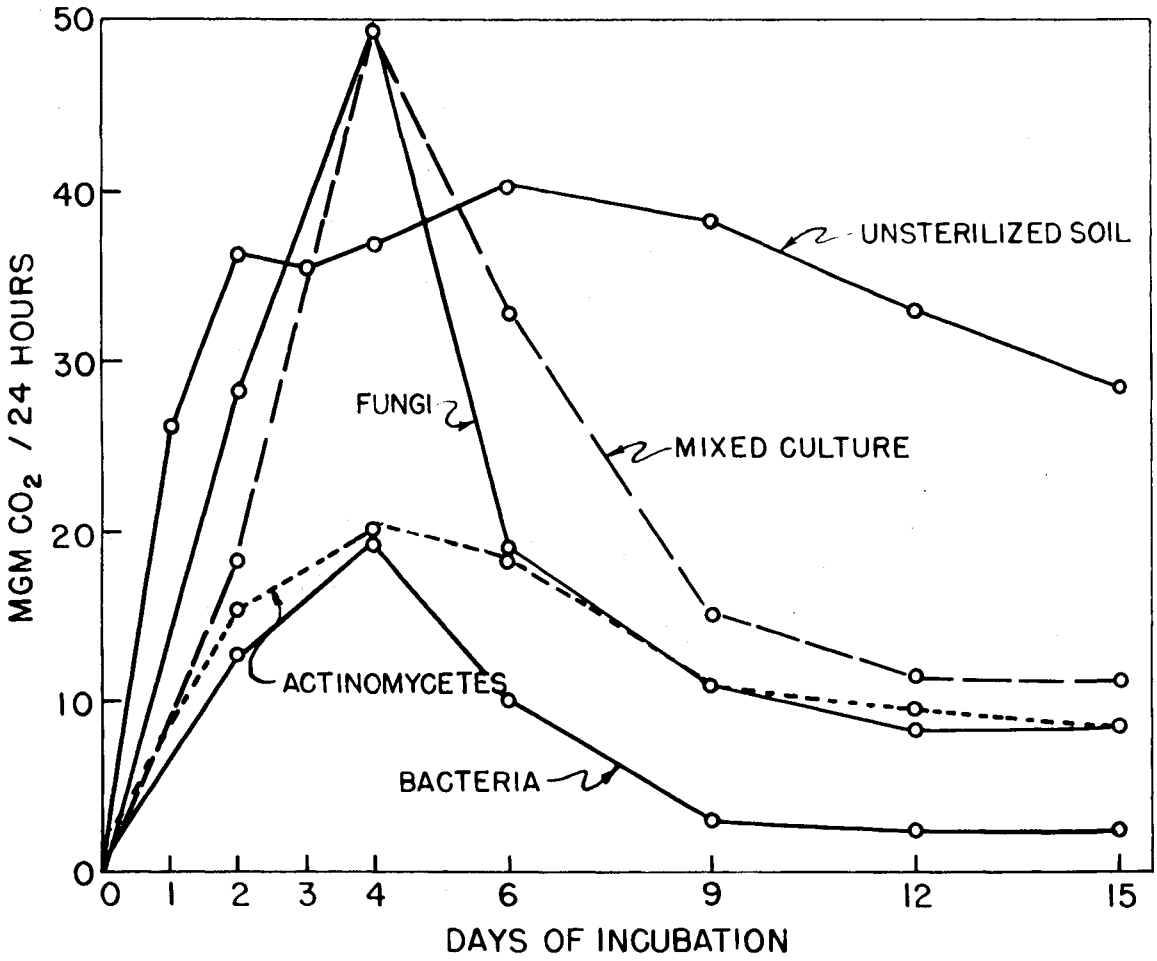

50
40
30
20
10
0
MGM CO_2 / 24 HOURS
UNSTERILIZED SOIL
FUNGI
MIXED CULTURE
ACTINOMYCETES
BACTERIA
0 1 2 3 4 6 9 12 15
DAYS OF INCUBATION

Fig. 17. Relative activity, as revealed by carbon dioxide evolution, of representative soil fungi, actinomycetes and bacteria and the general microflora (i.e. unsterilized soil) when inoculated in Webster silt loam maintained at 3130 cm. of water tension.

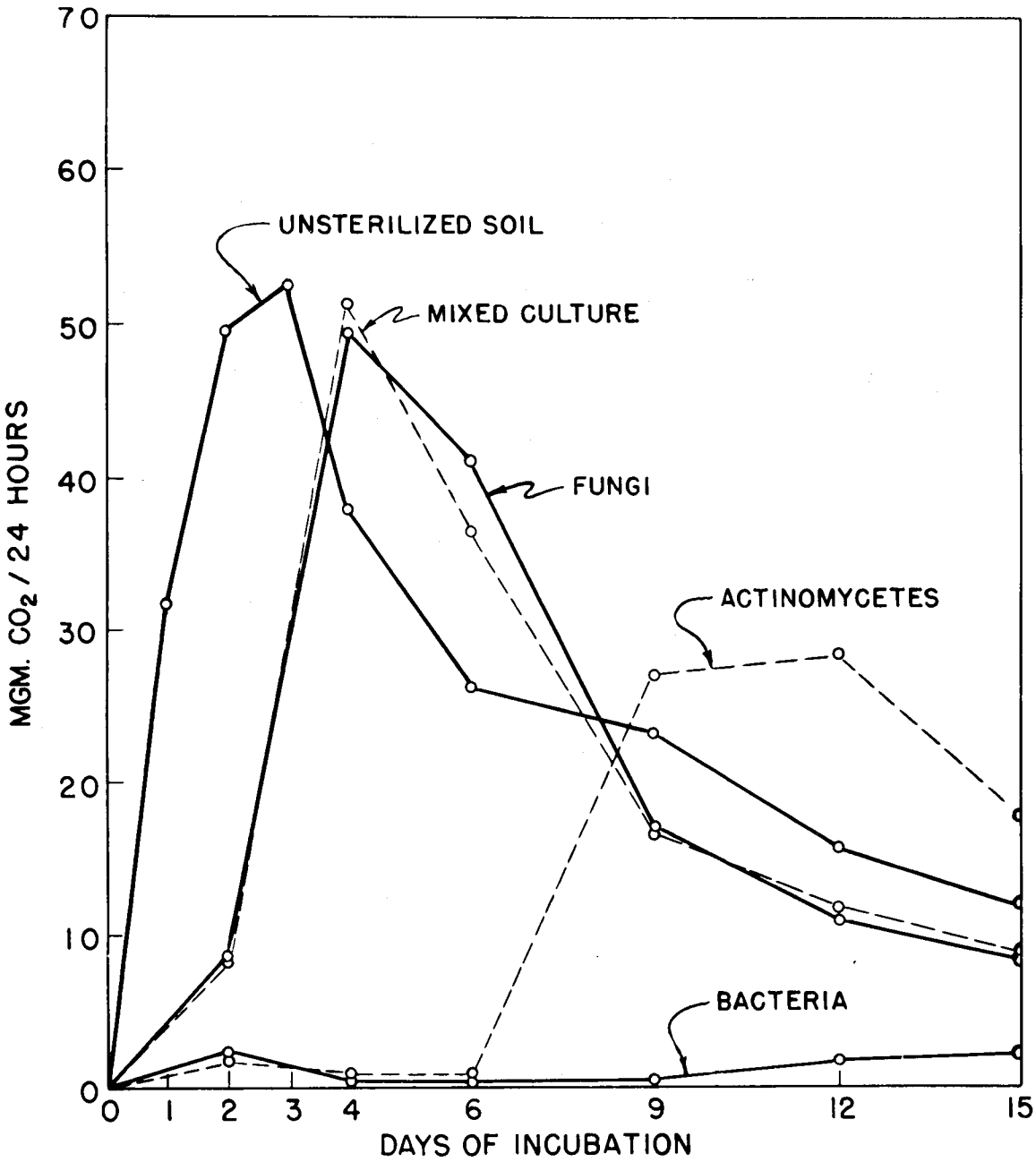
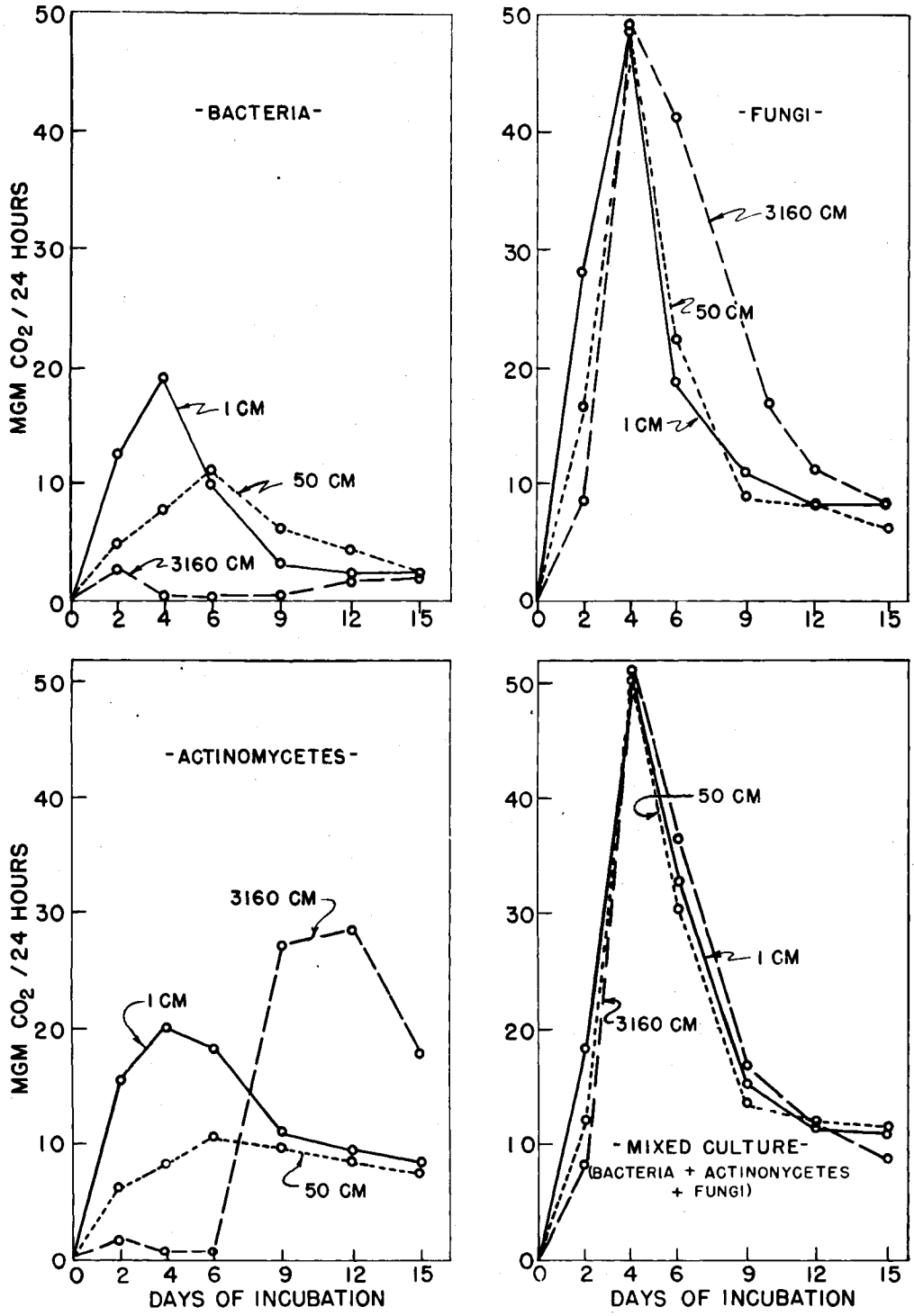

70
60
50
40
30
20
10
0
MGM. CO_2 / 24 HOURS
UNSTERILIZED SOIL
MIXED CULTURE
FUNGI
ACTINOMYCETES
BACTERIA
0 1 2 3 4 6 9 12 15
DAYS OF INCUBATION

Fig. 18. Influence of varying moisture tensions on the activity of representative soil fungi, actinomycetes and bacteria, as revealed by carbon dioxide evolution, when inoculated in sterilized Webster silt loam.


-BACTERIA-
-FUNGI-
-ACTINOMYCETES-
-MIXED CULTURE-
(BACTERIA + ACTINONYCETES + FUNGI)
1 CM
50 CM
3160 CM
MGM CO_2 / 24 HOURS
DAYS OF INCUBATION
0 2 4 6 9 12 15
0 10 20 30 40 50

DISCUSSION

Various attempts have been made to correlate microbiological activity in soil either to actual percentage of water on an oven-dry basis, to percentage of maximum water holding capacity of the soil, or to thickness of moisture film around the soil particles. It was soon realized that with a given per cent of water microbiological activity in a clay soil might be limited due to inadequacy of moisture, whereas in a sandy soil at the same per cent of moisture, activity might be depressed due to water-logging and inadequate oxygen supply. Thickness of moisture film around individual soil particles is beyond ready calculation for differently textured soils. The most satisfactory and most commonly accepted expression of suitable soil moisture conditions has been in terms of percentage of maximum water-holding capacity, but even on this basis there has been considerable lack of agreement concerning the value, or range of values, most satisfactory for microbiological activity. Moreover, there is no relationship between the maximum water-holding capacity and other soil moisture constants to justify such an expression of soil moisture.

In the present investigation, the data on the cumulative totals of carbon dioxide evolved show a difference between sandy soils on one hand and silt loam and silty clay on the other. The observation that at a given moisture tension (3,160 cm.), maximum number of fungi were found in Thurman sand, and the maximum number of bacteria in Wabash silty clay, suggests that soil texture not only determines the quantity of water that can be retained against a particular tension, but also

influences the qualitative nature of the soil microflora by modifying the physical and chemical nature of the environment. With this point of view, there need not be expected striking similarity in the amounts of carbon dioxide produced from differently textured soils. In many instances, the total amounts of gas produced, and not the rates of evolution, have been taken to indicate suitable soil moisture conditions, and it is not surprising that separate investigators have reached dissimilar conclusions.

When daily peak rates of carbon dioxide evolution are compared, there is seen a tendency for all the soils employed to show maximum rates of evolution at their respective moisture contents equivalent to 50 cm. of water tension. The mineralization of added organic nitrogen in Thurman fine sand and in Wabash silty clay was noted as more rapid at this particular tension than at any other moisture tension employed. The occurrence of peak daily rates of carbon dioxide evolution and of maximum transformation of added organic nitrogen at 50 cm. of water tension is interesting from the standpoint of the soil and water physical relationships. 50 cm. of tension represents the aeration porosity limit, and it is at this tension that the soil microflora is provided with maximum thickness of moisture film around the soil particles while at the same time some porosity is available for aeration.

Differences noted in carbon dioxide evolution from wet soil following alteration of the surface/volume ratio by use of differently shaped containers emphasize the importance of aeration on the activity of aerobic micro-organisms in wet soils. In the case of comparatively dry soil, the surface/volume ratio appeared much less important because there was much greater air space. Water-logging of Webster silt loam resulted in tremendous decrease in carbon dioxide evolution, no doubt

due to a limiting supply of oxygen.

Pure culture studies employing steam-sterilized Webster silt loam indicated that the complete native soil population was much more efficient in the disintegration of organic matter than were any single groups of microbes established by inoculation. The only exception was in the case of comparatively dry soil (3,160 cm. of moisture tension), wherein the fungi alone accounted for most of the carbon dioxide evolved by the native soil population, while the bacteria and actinomycetes showed very little activity.

Studies on the mineralization of egg albumin yielded certain observations on which comment can be made. The lack of any nitrification in sand at the end of four weeks is no doubt due to the toxicity of ammonia on the nitrifying organisms. Nitrification in clay, on the other hand, may be explained by the assumption that ammonia was held in the exchange positions of clay and was therefore less toxic than free ammonia in the sandy soil.

The loss of appreciable amounts of nitrogen in some unknown way was noted in the case of clay soil after 4 weeks of incubation at 1, 10, and 50 cm. of water tension. Moreover, this loss appeared to take place only in soil where nitrification was occurring. Whether this loss was due to biological activity or whether it occurred during chemical analyses of the samples is unknown. Wilson (48) has noted that micro-organisms reduce nitrates to nitrites and that this nitrite is free to react in an acidic environment with such substances as amines, amides and certain compounds containing sulphur with the result that gaseous nitrogen is liberated.

In the present study on mineralization of egg albumin in soil, nitrites may be assumed to be an intermediate product in the nitrification of ammonia. But in the presence of a large excess of ammonia from the decomposition of egg albumin, the environment will be alkaline rather than acidic. There remains a possibility of nitrogen loss during chemical analysis of the soil; during the analytical procedure dilute hydrochloric acid and potassium chloride are employed for extraction of soil, in which there may be an accumulation of breakdown products of protein, such as amines and amides.

Corbet (8) showed hyponitrous acid as an intermediate compound in the biological or photochemical oxidation of ammonia to nitrous acid. In routine analysis of soil, ammonia is estimated by distillation with magnesia and collection in standard acid, while nitrite and nitrate together are determined by measurement of the further ammonia liberated on distillation after reduction with Devarda's alloy. Hyponitrite is not estimated during such analysis, as the nitrogen is lost in gaseous form on warming.

SUMMARY

Five Iowa soils differing in texture were incubated under standard conditions at moisture tensions of 3,160, 502, 50, 10, 1, and 0 centimeters of water in order to study the effect of soil moisture tension upon microbiological activity.

Moisture retaining capacities of Thurman fine sand, Clarion loam, Clarion fine sandy loam, Webster silt loam, and Wabash silty clay were determined. Moisture content at any given tension increased progressively for these soils in the order named. The carbon/nitrogen ratios of all soils were of similar order and suggested that their native organic matter was in an advanced stage of decomposition.

Rate of carbon dioxide evolution following addition of one per cent ground corn stover, mineralization of organic nitrogen following addition of two per cent egg albumin, and population changes in the soil microflora as revealed culturally were taken as criteria for microbiological activity.

Carbon dioxide evolved from experimental soil lots was collected continuously and was determined after 1, 2, 3, 4, 6, 9, 12 and 15 days. The moisture tension at which the maximum cumulative total amount of carbon dioxide was evolved during 15 days differed for the several soils. No two soils showed total cumulative maxima at the same moisture tension. For all soils, however, the peak daily rate of carbon dioxide production was observed at or very near 50 cm. of moisture tension.

Alteration of surface/volume ratio by use of differently shaped incubation containers led to differences in carbon dioxide evolution

from wet soil. With comparatively dry soil, the surface/volume ratio appeared less important.

Mineralization of egg albumin was studied in Thurman fine sand and in Wabash silty clay, with determinations of ammonia, and nitrite and nitrate nitrogen being made after 1, 2, and 4 weeks. Differences in the rates of mineralization at differing moisture tensions were apparent only during the first week or two of incubation; they disappeared as the incubation period was continued to four weeks. Maximum rate of mineralization of nitrogen in both soils occurred at 50 cm. of moisture tension.

There was considerable loss of ammonia from the sandy soil due to volatilization but there was little or no loss from the silty clay soil. Mineralization of organic nitrogen was more complete in Thurman sand than in Wabash silty clay at the end of four weeks of incubation.

Microbiological analyses by cultural methods revealed differences in the abundance of microbial groups both at differing tensions of moisture within the same soil as well as among the several soils when maintained at the same moisture tension. It is believed that differences in microbial populations in soils are at least partly responsible for differences in the cumulative total amounts of carbon dioxide evolved from the several soils. Pure culture studies with bacteria, actinomycetes, and fungi in steam-sterilized Webster silt loam showed that the activity of fungi, as shown by carbon dioxide evolution, remained fairly constant within the range of moisture tensions employed. Carbon dioxide production by bacteria and actinomycetes was greatly depressed with increase in moisture tension. Microbial populations, however, were found to be greater in the drier soils.

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