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Date: June 17, 1976

Project Title: *Analysis and Synthesis of Data from Studies of a Subtropical Terrestrial Biome: Microbial Ecology*

Project No: *G-32-630 (Cont. of G-32-620)*

Project Director: *Dr. E. L. Fincher*

Sponsor: *Florida Power & Light Co., Miami, Fla. 33101*

Agreement Period: From *3/1/76* Until *8/31/76*

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Sponsor Contact Person (s):

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Contractual Matters
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Project No: G-32-630

Project Director: Dr. E. L. Fincher

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CA–4 (3/76)

G-32-630

SUMMARY REPORT

March 1, 1976 - August 31, 1976

Project No. G32-630

"ECOLOGICAL STUDIES OF A SUBTROPICAL TERRESTRIAL BIOME: MICROBIAL ECOLOGY"

Submitted to: Florida Power & Light Company

Attention: Mr. C.D. Henderson
P.O. Box 013100
Miami, Florida 33101

Report Prepared By: Edward L. Fincher, Ph.D.

School of Biology
Georgia Institute of Technology
Atlanta, Georgia 30332

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Members of the research group are:

Bopp, Cheryl A., B.S., Research Technician I (Bacteriology)
Bramlett, Royce N., Ph.D., Research Scientist (Biochemistry)
Colley, Ann M., Ph.D., Research Scientist (Bacteriology)
DeFoor, Dorothy E., B.S., Assistant Research Scientist (Bacteriology)
Schaeffer, Charles F., B.S., Research Assistant (Computer Programmer)
Lee, Michael, M.S., Research Assistant (Computer Programmer)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Foreword

This report presents a summary of the results of the project, "Ecological Studies of a Subtropical Terrestrial Biome: Microbial Ecology," in non-technical terminology and format for purposes of general review by persons not professionally oriented to the subject. Technical data and analyses of results of this study have been reported in the Annual Reports to the Florida Power and Light Company, Miami. The Annual Report under Project G32-620 (March 1, 1975 - February 29, 1976) was written to summarize the technical findings of this study and should be consulted for details. This report was submitted to the Florida Power and Light Company, Miami, in August, 1976.

As a general summary, efforts have been made to minimize detailed tabulated and graphical data that can be found in the Annual Reports, and to present such data in summary form only when essential to the discussion here. References are made to specific sections of the Annual Reports when it is deemed that a subject and related data under discussion might not be fully presentable in limited non-technical terminology. Additional references are made to published studies in the biological literature which are pertinent to the discussion of results obtained in this study, as well as general references of assistance to the reader which provide a technical background in general ecological and bacteriological studies related to the summary of results discussed in the following pages.

I. INTRODUCTION

1.1 Outline of General Microbial Ecology. The microbial population of soil includes fungi, algae, protozoa, and bacteria. Bacteria are the most abundant numerically, but probably account for less than 50 per cent of the microbial biomass. In non-aerated soil, e.g., wetlands, bacteria account for almost all biological and chemical changes, whereas in aerated soil both bacteria and fungi are dominant. The absence of a single inclusive procedure for estimating mass or numbers of bacteria in the soil indicates the importance and diversity of taxonomic types.

The physiological basis of differentiating the soil bacteria includes the nutritional and metabolic characteristics of types of energy sources utilized to drive metabolism, the types of carbohydrates used for growth, and sources of nitrogen required in the synthesis of cell substance, including the ability to utilize atmospheric nitrogen. The requirements for oxygen results in several categories of bacteria which include the aerobes that require atmospheric oxygen for metabolism, the anaerobes that grow in the absence of oxygen, and those bacteria capable of metabolism and growth either in the presence or absence of atmospheric oxygen - the facultative anaerobes. In addition to physiological characteristics, taxonomic types of bacteria are differentiated by the size and shape of the cell, motility, and the ability to form spores which enhance the resistance of the organism to environmental stresses of heat, drying, and antimicrobial chemicals.

The distribution and abundance of bacteria in soil indicates a highly heterogeneous population, the total number of which only can be estimated by conventional bacteriological techniques. Although no single culture medium

is adequate for all nutritional types of bacteria, the numerical determination of viable bacteria and their biochemical potential as colonies on broad-spectrum nutrient agar remains one of the more useful ecological measurements available. Various numerical ranges of the viable bacterial content have been reported and are of the order of 10^5 to 10^8 (hundred thousand to hundred million) bacteria per gram of dry soil. Such numbers represent an unknown fraction of the total bacterial population present.

The in situ relationship of bacteria and soil indicates that bacteria are rarely free in the liquid phase of soil, most of the bacteria adhering to soil particles and are probably in association in definite colonies in favorable ecological sites. The bacterial flora is viewed in microecological rather than in gross terms. Frequent occurrence of bacteria as colonies in soil aggregates which may not disintegrate is a source of sampling errors in obtaining and processing soil samples.

The numbers and types of bacteria are governed to a large extent by type of soil and cultivation practices, being higher in cultivated than in virgin land. The root-density effect resulting in higher numbers of bacteria associated with plant roots cause counts in grasslands to be higher than in comparable arable land. Two types or categories have been proposed for the microflora in soil. One category contains the population of microorganisms which is relatively stable in composition and not markedly influenced by treatment with organic amendments to the soil. This category of microflora is referred to as the autochthonous flora and is especially common in environments receiving no plant or animal remains. The second category of microflora, the zymogenous flora, is active in transformation of added organic matter, and also includes species responding to addition of

inorganic nutrients. Several groups of microorganisms have been proposed within the zymogenous flora to include organisms characterized by their physiological activities, including cellulose decomposers, bacteria utilizing atmospheric nitrogen, and those converting ammonia to nitrate; fungi that become active upon addition of organic matter; actinomycetes; the genus of bacteria Pseudomonas; and species of the bacterial genus Bacillus which, in the vegetative (non-spore) state, develop in the presence of suitable carbonaceous nutrients, noted most commonly when proteinaceous or other amino acid-rich nutrients are present.

Bacteria can be broadly divided into two groups according to the source of energy needed to drive metabolic processes. One group (phototroph) utilizes radiant energy, the second group (chemotroph) derives energy from oxidation and reduction reactions of chemical compounds. The chemotrophs can be subdivided into chemoautotrophs, which are characterized by using energy derived from utilization of inorganic chemical substances and the use of carbon dioxide to meet the need for carbon in cell synthesis, and chemoorganotrophs (heterotrophs). The chemoorganotrophs derive energy and required carbon for synthesis from the utilization of organic compounds.

Chemoautotrophy is limited to a relatively few bacterial species, yet it is of major agronomic and economic importance. Most chemoautotrophic bacteria are strict aerobes (living only in the presence of atmospheric oxygen), although some of the chemoautotrophs which can grow in the absence of atmospheric oxygen require oxygen-rich substrates, e.g., nitrate for Thiobacillus denitrificans, sulfate for Desulfovibrio sp., and carbon dioxide for Methanobacillus sp. In contrast, the chemoorganotrophs are more versatile in oxygen requirements, ranging from aerobe, facultative anaerobes, to

anaerobes, i.e., growing and metabolizing in the presence of atmospheric oxygen or in its absence, with some organisms growing under either condition (facultative anaerobes). The point in making this detailed distinction between the two groups is that aerobic bacteria were conspicuously absent from soil at the Turkey Point study-site, and the bacteriological procedures used were for the study of chemoorganotrophic flora.

A number of environmental variables influence the character of the bacterial population in soil. Moisture must be available for vegetative development, but excessive moisture (waterlogging) limits gaseous exchange and lowers the concentration of oxygen with the effects of limiting or preventing the development of aerobic bacteria. Size of the bacterial population is directly related to the organic content of soil, and stimulation of this population is most pronounced during the first months of organic decomposition followed by a decline in numbers.

1.2 Description of Ecological Study Site. The site area is approximately 10 miles southeast of Homestead, Florida and consists of a measured transect of land 100 meters wide and 3700 meters long which extends from a point south of the model Land Company Canal (Levee 31 canal) to a terminus in Card Sound. This transect extends from a northwest to southeast direction in an approximate parallel line to the Old Dixie Highway at an estimated distance of 1.5 miles east of the Highway.

The hydraulic and water quality studies reported by Mr. Charles P. Gupton, P. E., et al. (1) of Dames and Moore showed that the mean probability of water coverage along the transect, as a function of tide level at Station 37, ranged from coverage of Station 30 at low tide to Station 28 at

high tide during the months of January through July, or dry season. During the period between August and December water coverage of the transect reached at peak of inundation in October, which included all Stations in the transect at low and high tides.

Beginning in August, as tide levels increased, the inland intruded boundary of saltwater appears to reach as far as Station 21, and it is believed that the fluctuating boundary of saltwater occurs between Stations 30 and 21. Conductivity measurements of water during the wet season indicates an interfacial zone of mixing between saltwater water and fresher water in the transect boundary area of Station 21. Location of this zone is influenced by tide levels in Card Sound and quantity of rainfall. Inland from Station 21 the salinity profile reflects fresher water indicative of rainwater with some dissolved salts due to evaporative concentration of salts in the soil. Station 25 marks a generally inland boundary limit of a hypersaline zone under the influence of tidal fluxes of saltwater and evaporation with a build-up of concentrations of salts.

Cyclic movement under tidal influence of water covering the transect in the range of Stations 30 and 28 indicated that ca. 50-60 per cent of the water volume passed into Card Sound during most of the year. The volume exchange of water back to Card Sound during the wet season months of September through November was ca. 20-30 per cent of the water volume covering the transect. The exchange of water volume during the wet season at the 'upland' Stations 2 and 9 was small due to the low average slope of the ground producing low flow velocities and to the tidal lag phase (ca. 5-6 hours). The flushing effect of rainfall on the transect area is indicated to be a significant factor in transport from the land to Card Sound, which, depending

on tidal conditions, might result in a greater water volume transported to the Sound than occurs with normal oscillatory tidal motion.

Temperature ranges reported (Gupton, et al., 1976) for ground-water were 15.5-32 C and for surface water 9.5-45 C, broader ranges occurring at Stations 30 and 37. The pH of ground-water ranged from 3.9-9.6 and 2.7-12.0 in surface water. Dissolved oxygen concentrations in surface water ranged from 0-10 parts per million (ppm), but generally in the 0-5 ppm with highest values at Station 37.

1.3 Selection of Transect Sites for Soil Analysis. Since hydraulic and water quality studies (Gupton, et al., 1976) were initiated at the same time as the microbial ecology study reported here, selection of soil sampling sites was made on results of study of a composite aerial photograph and ground survey of the transect. These results indicated three broadly discernible zones which were arbitrarily designated as 'upland','midland', and 'lowland', and which are respectively characterized by sawgrass, Juncus distichlis, and low-growing mangroves in a hypersaline zone.

Stations 2 ('upland'), 18 ('midland'), and 30 ('lowland') were considered representative of the transect, exclusive of the isolated hammock areas, and were selected as sources of soil for bacteriological and other analyses. Representative photographs of these areas are shown in Plates 1A-3B. Pertinent to these sites was the application of appropriate culture methods for the isolation and growth of the bacteria present in the soil. Station 30 evidenced apparent characteristics of an area either marine or dominated by factors from a marine source. The estimated 'midland' zone (Station 18) was postulated to be under intermittent influences from the

PLATE 1A. STATION 2 - APRIL, 1974

PLATE 1B. STATION 2 - JANUARY, 1974

PLATE 2A. STATION 18 - APRIL, 1974

PLATE 2B. STATION 18 - APRIL, 1974

PLATE 3A. STATION 30 - OCTOBER, 1974

PLATE 3B. STATION 30 - MAY, 1974

marine area through tidal flooding for surface effects, and/or from sub-surface intrusion of seawater whose vertical diffusion, perhaps cyclical, in the soil column might be influenced by tidal effects. The deductions made from initial analyses of the area and estimates of the zonal characteristics of the transect are supported by the subsequent studies reported by Gupton, et al. (1).

Selection of these representative zonal sites (Stations 2, 18, 30) with their indicated differences in salinity was associated with the general and complex problem of the total recovery of all physiological and nutritional types of bacteria present in the soil. No single culture medium will support the growth of all soil bacteria, and the added parameter of differences in growth requirements for essential salts was present in the selection of 'non-marine' (Station 2) and 'marine' (Station 30) soil environments.

II. METHODS OF ANALYSIS

2.1 Introduction. The objective of this study was to provide information on the bacteriological content of the soil in the transect area using selective methods of analyses which would recover and characterize the broadest sample of bacteria utilizing organic compounds as sources of energy and growth. Such bacteria would have a significant role in the cycling of carbon compounds in the soil. Efforts were therefore directed to the determination of numbers and distribution of such bacteria in the soil, their degree of relatedness, and range of physiological activities which might indicate their qualitative contribution to the ecosystem. Additional analyses were made of the soil environment of these bacteria to measure certain fac-

tors which might delineate the nature of the ecosystem in which these organisms function. It should be pointed out that the results of these studies were produced under laboratory conditions with interpretations being extrapolated to their presumed functions in an undisturbed natural ecosystem existing in the soil.

2.2 Soil Samples and Non-Bacterial Analyses. Soil samples consisted of cylindrical cores ca. 2 inches (5 centimeters) in diameter and 10-20 inches (25-50 centimeters) in length. Soil texture and moisture content influenced the length of recoverable cores which represented an average sampled soil depth of ca. 15 inches (38 cm). Multiple sections of ca. 0.5 inch (ca. 1 cm) were taken down the length of the core for analysis. Sub-surface sampling was done in view of the presence of undegraded plant detritus at varying depths in test cores and the possible influence of a fluctuating water level, which might function as a cyclic exchange mechanism in the transport of nutrients and degraded products of metabolism.

Analyses of core sections by depth included the determinations of pH (acidity/alkalinity), moisture content, organic and inorganic carbon content, and biochemical analysis of interstitial water in soil samples.

Adenosine triphosphate (ATP) content of soil was also determined. ATP is part of a system found in all types of living cells and functions as a carrier of chemical energy. Measurement of the ATP concentration in a soil sample is an indirect measurement of the biomass, or mass of living cells, present in the sample, but does not discriminate between the cellular sources of the ATP as to whether they are bacterial, protozoal, or higher life forms. The relatively constant ratio of ATP to carbon in living cells

permits estimates to be made of living biomass which cannot be calculated from in situ measurements of any metabolic function because of unknown environmental factors. For bacteria, the ATP:carbon ratios have been reported in the range 1:250 (2) to 1:286 (3) for laboratory cultures.

2.3 Bacteriological Analysis. Enumeration of viable bacteria at various depth of soil samples from Stations 2, 18, and 30 was done under conditions to isolate aerobic, facultative anaerobic, and obligate anaerobic heterotrophic (organisms using organic carbon as energy source) bacteria. These conditions included studies on the distribution of obligate marine and facultative marine bacteria as determined by their requirement for various salts found in seawater. The sites sampled at the three Stations were away from the hammock areas where the plant root systems would present a more complex microbial ecosystem.

Approximately 870 individual bacteria, or cultures, were randomly selected as representative of the bacterial flora recovered from the soil. Each of these cultures were examined by a set of 46 tests which encompassed 252 possible characteristics of each bacterial culture. (See Annual Rpt., 2/29/76, pp. 10-12 for a list of these characteristics.) These characteristics included those of cell size and morphology, physiological, and biochemical functions which tested a variety of substrates to demonstrate the ability of the bacteria to break-down and/or utilize various carbon compounds. This type of information affords some basis of determining the role that these organisms might have in the cycling of carbon compounds in the ecosystem.

Identification of each of this large number of bacteria by name (nomen-

clature) was not done because of the practical difficulties of so treating this many organisms, and because of the inadequacies of bacterial nomenclature. A more useful and practical analytical method was the utilization of the large numerical array of characteristics in the method of numerical taxonomy. This computer-based procedure determines the level of relationship, in per cent, between individual cultures of bacteria. Clusters of related bacteria can be determined and a rapid comparison can be made between individual and groups of bacteria as a function of soil depth and of Station location on the transect. Numerical taxonomic analysis provided the most feasible technique of assaying a large number of organisms representative of the soil flora for their possible metabolic role in the ecosystem, which was the primary stated objective of this study.

III. RESULTS

3.1 Soil Analysis (Non-Bacteriological)

3.1.1 Moisture Determinations - Water content of soil cores was found to be 57.0 ± 5.9 per cent by weight, samples representing collection during the months of May 1974-5 and October, 1974. These determinations were made on standing core samples in the laboratory and the results are considered a measure of the water-retaining capacity of the soil. The small variation in measurement suggests a relatively constant high water content at Stations 2, 6, 10, 14, 18, 20, and 30. Higher percentages of water content (ca. 82 per cent) was formed in cores with a high peat content.

3.1.2 Soil pH - Different depth profiles of soil could be determined by the distribution of values of pH, organic carbon, and inorganic

carbon in soil at Stations 2, 18, and 30 where bacteriological analyses were done; and at Stations 6 and 14 for additional comparisons. Average values of the several parameters are shown in Fig. 1 where the interrupted vertical bars in the graph indicate statistically significant differences in values. The pH 7.66 at Station 2 is lower than the values at Stations 18 and 30 at all depths. At these latter two Stations a transitional zone was found at the depth of 9-14 cm (3.5-5.5 inches) the upper soil levels having a pH of 8.04-8.05 and the lower levels of pH 7.86-7.89.

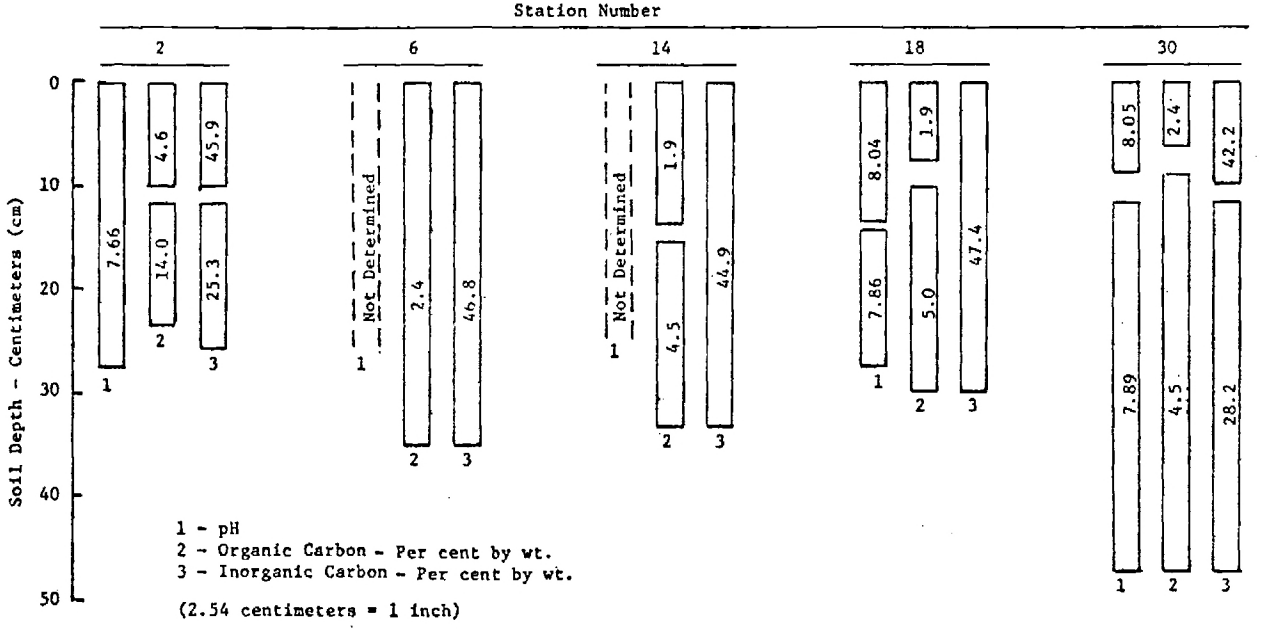
3.1.2 Organic and Inorganic Carbon - Concentrations of organic carbon showed a marked stratification in the soil. It is notable (Fig. 1) that the 4.6 per cent concentration in the top 10 cm (4 inches) of soil at Station 2 was equivalent to the values obtained below the depth interval of 10-15 cm (4-6 inches) at Stations 14, 18, and 30. Above this interval the organic carbon concentration (1.9-2.4 per cent) was equivalent to the uniformly constant value of 2.4 per cent at Station 6, all four Stations showing ca. 50 per cent lower concentrations of organic carbon than Station 2 in the upper 10 cm (4 inches) of soil. The highest concentration of organic carbon (14.0 per cent) was found below the depth of 10 cm (4 inches) at Station 2 in the 'upland' zone of the transect.

Inorganic carbon (principally carbonates) concentrations were equivalent and in a uniformly high range (44.9-47.4 per cent) in the top 10 cm (4 inches) of soil at Station 2 and at 30-35 cm (12-14 inches) at Stations 6, 14, and 18. Station 30 showed significantly lower concentration (42.2 per cent) in the 0-10 cm (0-4 inches) depth and a markedly lower concentration (28.2 per cent) throughout the remaining depth interval to ca. 50 cm (20 inches) from the ground surface. This lower concentration compared

Figure 1

HYDROGEN-ION CONCENTRATION (pH); AVERAGE ORGANIC AND INORGANIC CARBON CONTENT OF SOIL

Stations 2, 6, 14, 18, 30



closely to the 25.3 per cent value in the depth interval below ca. 13 cm (5 inches) at Station 2.

Using the combined values as arithmetic means of per cent concentrations of organic and inorganic carbon values at all depth levels produced the results shown in Table 1, which includes the organic:inorganic carbon ratios as an indication of trends along the transect. Cummulative data in this form obscures the findings of stratification which appeared visually evident in dissection of a number of soil cores taken in the initial phase of this study.

This stratification is indicated by analysis of the carbon content of soil in relationship to depth intervals with results shown in Table 2. The amount of organic carbon in the top ca. 0-10 cm (0-4 inches) at Stations 2, 18, and 30 is apparently less than in the interval below ca. 11 cm (4 inches). An inverse relationship of inorganic carbon and soil depth appears to be the trend with decreasing concentrations with increasing depth, except Station 18 where stratification of concentrations appear absent. These relationships of concentrations of organic and inorganic carbon are also evident when expressed as ratios with the result of increasing ratio of carbon:inorganic carbon with soil depth. These results suggest significant differences in carbon concentration with depth, but there is considerable variation about the mean values of concentration based on a relatively few samples which are insufficient in number for rigorous statistical analysis to determine the differences between means. However, the results are considered meaningful in relationship to the results obtained in the bacteriological analysis reported below.

Such stratification of carbon with soil depth is suggestive of inflood-

Table 1

CONCENTRATION OF ORGANIC AND INORGANIC CARBON IN SOIL

Station No.	N	Per Cent By Weight in Dry Soil: Organic Carbon Mean	Organic Carbon S.D.	Inorganic Carbon Mean	Inorganic Carbon S.D.	Ratio of Means Org:Inorg C
2	13	9.67	7.21	34.13	14.56	1:3.5
6	19	2.42	0.59	46.88	4.73	1:19
14	18	3.27	1.85	44.90	6.42	1:13
18	16	4.10	1.78	47.40	4.58	1:11
30	25	4.06	1.23	33.25	9.17	1:8

N = Number of samples (core sections); S.D. = Standard deviation

Table 2

DEPTH ANALYSIS OF ORGANIC AND INORGANIC CARBON CONTENT OF SOIL

Station No.	Soil Depth Interval - cm	Carbon Content of Soil - mgm/gm Dry Soil: Organic Carbon N	Organic Carbon Mean	Organic Carbon Max - Min Range*	Inorganic Carbon N	Inorganic Carbon Mean	Inorganic Carbon Max - Min Range*	Ratio of Carbon Means Organic:Inorganic
2	0 - 10	6	46.0	57.4 - 34.6	6	459.2	501.5 - 416.9	1:10
	12 - 24	7	140.1	214.4 - 65.8	7	253.3	387.6 - 119.0	1:2
18	0 - 8	5	19.6	25.9 - 13.3	16	474.0	519.8 - 428.2	1:24
	11 - 30	11	50.8	62.0 - 39.6				1:9
30	0 - 10	6	24.0	27.6 - 20.4	6	442.0	488.5 - 395.5	1:18
	12 - 24	7	36.0	38.5 - 33.5	7	327.0	403.5 - 250.4	1:9
	26 - 48	11	51.5	56.7 - 46.2	11	260.4	360.3 - 160.4	1:5

*Range - ca. 68 per cent of samples can be expected to fall within this range.

ing of silt-bearing water, probably marine in origin, which, when it recedes, leaves a deposit covering existing vegetation. The resulting organic deposits beneath or mixed with the silt, and together with later development of plant growth on the surface, offer particular nutrient substrates for heterotrophic (carbon utilizing) bacteria. Subsequent bacteriological findings indicate that a significant portion of the bacteria active in utilizing an array of carbon compounds are found at the surface and at the lower ca. 10-48 cm (4-19 inches) strata of soil.

Detailed data and analysis of moisture, pH, and carbon content of soil samples can be found in the Annual Report (1976), pp. 18-31.

An indication of the range of organic constituents of soil is shown in Table 3 for Stations 2, 18, 30 and hammock areas adjacent to Station 18.

3.1.3 Adenosine Triphosphate (ATP) Measurements - Determinations of ATP content of soil collected from Stations 2, 18, and 30 in open land areas well away from the hammock area correspond to other samples taken for carbon analysis, pH, moisture, and bacteriological analysis. Such collection sites were considered representative of the predominant character of the area of the transect and were also considered to be a system of lesser complexity than hammock areas. Additional samples were taken of the 'drainage tail' of hammocks at Stations 2, 18, and 30 for comparative purposes to confirm an anticipated higher biomass in soil from areas of higher production of plant detritus.

Results of analyses of soil for ATP concentrations at 5 cm (2 inches) depth intervals of soil cores at Stations 2, 18, and 30 are shown in Table 4. These data were derived from non-linear regression analyses with determination of statistical variation of ATP concentration that could be expected in

Table 3

SUMMARY COMPARISON OF INTER-STATION BIOCHEMICAL PARAMETERS

Station No.	Soil Core Length (cm)	Aver. Micrograms/Gm Dry Wt. of Soil				
		Carbon		Carbo-hydrates	Lipids	Amino Acids
		Organic	Inorganic			
2	38	77.3	92.7	24.6	10.5	18.2
18	38	39.3	125.7	10.2	12.4	1.7
30	48	31.4	111.3	11.3	4.0	1.6
18HT*	28	127.0	262.7	58.5	2.5	2.1
18H*	26	467.7	564.0	132.7	0.0	22.0

*H - hammock; HT - hammock 'drainage tail'.

Table 4

ADENOSINE TRIPHOSPHATE (ATP) IN SOIL SAMPLES FROM NON-HAMMOCK AREAS

Stations 2, 18, 30

Soil Depth (cm)	Concentration of ATP - µgm/gm Dry Soil					
	Mean*			Maximum-Minimum Range**		
Station No.:	2	18	30	2	18	30
0	2.38	1.01	0.66	3.20-1.76	1.30-0.78	1.02-0.42
5	1.20	0.57	0.37	1.60-0.88	0.73-0.44	0.57-0.23
10	0.60	0.32	0.21	0.80-0.44	0.41-0.25	0.32-0.13
15	0.29	0.18	0.12	0.40-0.22	0.23-0.14	0.18-0.07
20	0.14	0.10	0.06	0.20-0.11	0.13-0.08	0.10-0.04
25	0.07	0.05	0.04	0.10-0.05	0.07-0.04	0.06-0.02
30	0.04	0.03	0.02	0.05-0.03	0.04-0.02	0.03-0.01
Rate of Decrease Per Depth Interval, %	50	43	44			

*Mean values: Station 2 - 4 soil cores; Station 18 - 3 soil cores; Station 30 - 2 soil cores.
**ca. 68 per cent of samples can be expected to fall within this range.

a randomly selected soil sample. Considering only the mean concentration of ATP (micrograms per milligram of dry soil) at all depths of soil, it appears that the concentration is indicated to progressively decrease from Station 2 to Station 30. However, the maximum-minimum ranges of ATP concentration at all soil depths at Stations 18 and 30 indicate that the mean values of ATP are not actually different, but fluctuation in ATP concentration in randomly selected soil samples suggests that equivalent concentrations would be found at these two Stations.

The concentration of ATP is significantly higher in the 0-10 cm (0-4 inches) soil depth interval at Station 2. In the 15-20 cm (6-8 inches) interval the minimum values of ATP concentration at Station 2 and the maximum values at Station 18 indicate that there is no real difference between the mean concentrations at this soil depth. All three Stations show an equivalent concentration of ATP in the 25-30 cm (10-12 inches, ca.) depth interval.

The relatively constant rate of decrease of ATP concentration as a function of soil depth is approximately the same at all three Stations, the concentration at any level being ca. 50 per cent of the preceding level.

Similar determinations of ATP concentrations were made on soil cores from the 'drainage tail' of hammocks in the areas of Stations 2, 18, and 30. Expectations were for higher levels of ATP on the premise that these areas received higher quantities of plant detritus which would support a larger biomass, measured as ATP, than in the non-hammock or 'open' areas of the transect. Such expectations were found to be true at all Stations in the top 1 cm (0.4 inch)-0 cm of soil, as shown in Table 5. At Station 2, ATP concentrations in the 5-15 cm (2-6 inches) were equivalent in the 'drainage tail' and 'open' area sites. However, the ca. 2-fold higher concentration

Table 5

ADENOSINE TRIPHOSPHATE (ATP) IN SOIL SAMPLES FROM 'DRAINAGE TAIL' OF HAMMOCKS

Stations 2, 18, 30

Soil Depth (cm)	Concentration of ATP - µgm/gm Dry Soil					
	Single Core Values			Maximum-Minimum Range*		
Station No.:	2	18	30	2	18	30
0	4.78	4.47	1.66	5.00-4.56	6.46-3.09	1.99-1.38
5	1.70	1.90	0.66	1.77-1.62	2.57-1.23	0.79-0.55
10	0.60	0.76	0.26	0.63-0.57	1.02-0.49	0.31-0.21
15	0.21	0.30	0.10	0.22-0.20	0.41-0.19	0.12-0.08
20	-	0.12	0.04	-	0.16-0.07	0.05-0.03
25	-	0.04	0.01	-	0.06-0.03	0.02-0.01
30	-	0.02	0.006	-	0.02-0.01	0.007-0.005
Rate of Decrease Per Depth Interval, %	64	57	60			

*Range of values for single core samples; ca. 68 per cent of samples can be expected to fall within this range.

in the surface layer of the 'drainage tail' at Station 2 declines more rapidly with soil depth, the concentration being ca. 35 per cent of the preceding soil stratum as compared to ca. 50 per cent in the 'open', non-hammock location. The rate of decrease with soil depth is also higher in soil from the 'drainage tail' at Stations 18 and 30.

Concentrations of ATP at the same soil depths in the 'drainage tail' are equivalent at Stations 2 and 18, both locations showing higher concentrations than Station 30.

In summary, biomass in the soil, measured as ATP, tends to decrease in concentration in the sequence of Stations 2, 18, and 30, particularly in the surface layer of soil in the 'open' or non-hammock areas. It has been noted above that Stations 18 and 30 are probably not statistically different. Higher biomasses are evident at Station 2. Differences in biomass at the three Stations tend to become less as a function of soil depth, and at the 25-30 cm (10-12 inches) interval the biomasses are equivalent and ca. 2-3 per cent of the concentrations at the soil surface.

Although initially higher at the soil surface in the 'drainage tail' of the hammocks, the rate of decrease of biomass with soil depth is more rapid and is equivalent to the biomass found in lower soil strata in the 'open' or non-hammock areas.

Generally, these findings suggest a gradient of a biological system which tends to decrease from a comparatively high level in the 'upland' area to a lower level in the direction of Card Sound.

3.2 Soil Analysis - Bacteriological

3.2.1 Numbers of Bacteria in Soil - Determinations were made of

the aerobic and facultative anaerobic, chemoorganotrophic bacteria content of soil at Stations 2, 18, and 30. These bacteria include those requiring oxygen (aerobes) and those capable of living in the presence or absence of atmospheric oxygen (facultative anaerobes). Both groups utilized organic compounds for energy and growth. The method for isolating and culturing bacteria with these characteristics is restrictive insofar as certain nutritional types of bacteria and bacteria growing only in the absence of oxygen (obligate anaerobes) would not be included. There is as yet no single method of determining the total number of all types of bacteria in soil. The method used was considered to produce the maximum recovery of those bacteria utilizing various carbon compounds of interest in the cycling of such compounds in the ecosystem.

Derived counts were calculated from colony-forming units (CFU) representing viable bacteria capable of developing macro-colonies which are counted. Attention was given to the constant problem in soil bacteriology of dispersing clumps of bacteria and aggregates of soil particles containing bacteria, both systems being resistant to disaggregation and dispersion. Results of derived counts are shown in Table 6. In the surface layer there is an indication of greater numbers of bacteria at Station 2 than at Station 18. However, the expected range of numbers of bacteria at these sites indicate that there are no differences in counts, a result due to the random fluctuations in the numbers of bacteria recovered. A measure of this fluctuation is expressed as the standard error of estimate which shows a successive decrease from Station 2 to Station 30, i.e., 0.29, 0.20, 0.05. This result is interpreted as reflecting the relative homogeneity or distribution of the bacterial aggregates in soils which are more coarse with higher con-

Table 6

DERIVED COUNTS OF COLONY-FORMING UNITS (CFU) OF AEROBIC AND FACULTATIVE ANAEROBIC BACTERIA

Stations 2, 18, 30

Soil Depth (cm)	Station No.:	Colony-Forming Units (CFU) - N x 10^3/Gm Dry Soil					
		Single Core Values			Maximum-Minimum Range *		
		2	18	30	2	18	30
0		2317	1824	854	4488-1197	2890-1150	958-761
5		1567	1404	551	3033-809	2220-885	618-491
10		1059	1081	356	2050-546	1710-681	399-317
15		716	832	229	1386-369	1316-524	257-204
20		484	641	148	937-249	1013-404	166-132
25		-	493	95	-	780-311	107-85
30		-	380	61	-	601-239	69-55
35		-	292	39	-	462-184	44-35
40		-	-	25	-	-	29-23
Rate of Decrease Per Depth Interval, %		32	23	35			

*Ca. 68 per cent of samples can be expected to fall within this range.

tent of fibrous plant root systems at Station 2 than was found in the silt-like soil deposit at Station 30. Although the fluctuation in bacterial counts was much lower (0.05), the bacterial population is lower at this site than in the two upper Stations.

It should be noted that the bacteriological recovery and growth system used at Stations 2 and 30 contained synthetic seawater, whereas the system used at Station 18 contained distilled water. Comparative results of these two systems indicate numerically equivalent bacterial populations at Stations 2 and 18 that do not require seawater for growth.

The rate of decrease of bacterial populations with depth of soil is approximately the same at the three Stations, a rate of decrease that appears less than the rate of decrease of ATP at the same locations (Table 4). It should be noted that these measures, i.e., bacterial numbers and biomass (ATP), appear in an inverse relationship to the soil content of organic carbon which increases with the depth of soil (Table 2.)

A count of numbers of bacteria at different depths of soil include groups that form heat-resistant spores during some time of their existence and other vegetative organisms, known as thermodurics, which can endure exposure to elevated temperatures without lethal effects. Exposure of soil samples to 80 C for 10 minutes will not distinguish between these two heat-resistant groups, but results of exposure will provide some information on the presence of the frequency of the spore stage which is relatively low in metabolic activity. Soil samples processed in the laboratory contain bacterial spores which will germinate under appropriate conditions, thereby giving a possible erroneous interpretation of their contribution to bacterial activity in the soil under natural conditions.

The highest frequency of bacterial spores and/or thermoduric bacteria of the aerobic and facultative anaerobic group was found in the 0-15 cm (0-6 inches) depth interval, the frequency fluctuating between 10-50 per cent. Below this depth there appear a consistent trend of these bacterial forms to occur at a frequency of 10 per cent or less, indicating that the lower depth populations are in a vegetative state which is sensitive to thermal treatment. This population, although less numerically than at upper soil depths, was found to be more versatile in the range of different carbon compounds which they utilized in metabolism.

Certain bacteria that do not grow in the presence of atmospheric oxygen are termed obligate anaerobes, and some of these organisms will develop heat-resistant spores. The average frequency of occurrence of obligate anaerobic bacteria, as a percentage of the total bacteria recovered, increases progressively from Station 2 (ca. 8%), to Station 18 (ca. 23%), to Station 30 (ca. 50%), results interpreted as reflecting the increasing water content of soil with accompanying low oxygen tension (anaerobic conditions). The frequency of obligate anaerobes associated with soil depth showed that ca. 80-90 per cent of the total bacteria in the depth interval of 20-40 cm at Station 30 was composed of these organisms. At Station 2 a high incidence of obligate anaerobes was found in a cluster at two depth levels (16 cm - 57%; 18 cm - 15%), but quite low (< 1%) at a depth of 21 cm. At Station 18 soil depths of 8, 16, and 20 cm showed the highest incidence (ca. 20 - 50%) of obligate anaerobes and an absence of these organisms in the 24-32 cm depth interval.

Although the per cent frequency of obligate anaerobes in the total bacterial population tends to increase as soil samples are taken from the

uplant (Station 2) area toward Card Sound (Station 30), the proportion of heat-resistant (spores) and thermoduric bacteria in these populations remains relatively constant (ca. 26%). These results indicate that the majority of these organisms are in a vegetative state and, therefore, inferentially assumed to be in an active metabolic state in soil.

3.2.2 Taxonomy of Soil Bacteria - A study of the possible role of soil bacteria in such an extensive ecosystem as represented by the study site indicated the need for a broad range assay of the physiological and metabolic activities of the organisms present. Identification or nomenclature of the bacteria was considered to contribute less to study objectives than a more extensive characterization and grouping (taxonomy) of the bacteria in terms of metabolic activities and their distribution in the soil. To provide the broadest sample base for this information ca. 870 bacteria were isolated and studied in pure culture, each culture being examined for possible 252 characteristics included in morphological, physiological, and metabolic categories. This data was processed by computer analysis in the calculation of the coefficient of association, which is a numerical value (percentage) expressing the degree of overall relatedness of one bacterium to another, the formation of clusters of related groups, and the intercluster comparisons of bacterial groups. This analysis permitted comparison of bacteria within various depth strata of soil at Stations 2, 18, and 30, and comparison of the sample populations one Station with another.

Summation of analysis of clusters of bacteria having coefficients of association, i.e., relatedness equal to or greater than 80 per cent, is shown in Table 7. These culsters of related organisms occurred within a given soil depth stratum. At Station 2 the top 1 cm (0-1 cm) of soil pro-

duced 2 clusters of related bacteria containing 5 and 3 OTU's (Operational Taxonomic Units, i.e., bacteria) respectively, these 2 clusters accounting for 44 per cent of the 18 OTU's characterized from this stratum. All OTU's were randomly selected for characterization and these results are interpreted as showing a relatively homogeneous bacterial population at this level. Lower intervening levels indicate inhomogeneity or diversity of types until the depth interval 14-21 cm where a reversal trend to homogeneity occurs.

Similar results were obtained at Station 30 with some variation of clustering which extended from the surface to ca. 6 cm and resumed with some consistency as a function of depth in the range of 22-38 cm. It should be noted that primary isolation, cultivation, and characterization of bacteria from Stations 2 and 30 were done in media containing synthetic seawater.

At Station 18 isolation and characterization of bacteria was done in the same media which contained freshwater instead of synthetic seawater. The results (Table 7) of cluster analysis of these bacteria showed high percentages of inclusion in clusters at all soil depth intervals. It is considered significant that the percentages are consistently higher below 12 cm and that the number of groups or clusters are also higher. There is a greater homogeneity of bacterial types throughout the soil column at Station 18, a result not unique to the bacterial flora at this location but one that indicates a possible selective effect of freshwater used in the media instead of seawater. The numbers of bacteria in soil at Station 18 are not indicated to be different from the numbers at Station 2 where synthetic seawater was used; therefore, the taxonomic differences in clustering results

Table 7

CLUSTER ANALYSIS OF SOIL ISOLATES SHOWING GROUPS WITH ≥ 80 PER CENT COEFFICIENTS OF ASSOCIATION

Soil Depth (cm)	Station 2			Station 18			Station 30		
	No. of Groups	No. of OTU/Group		No. of Groups	No. of OTU/Group		No. of Groups	No. of OTU/Group	
0	2	5;3	(44%)*	1	9	(50%)	0	0	
2	0	0		2	5;3	(44%)	2	2;2	(22%)
4	1	2	(11%)	2	7;2	(50%)	1	4	(22%)
6	0	0		1	6	(33%)	1	2	(11%)
8	0	0		2	6;3	(50%)	0	0	
10	1	3	(17%)	2	5;4	(50%)	1	2	(11%)
12	0	0		2	4;2	(33%)	0	0	
14	1	4	(22%)	2	8;7	(83%)	1	2	(11%)
16	2	5;2	(39%)	4	4;3;3;2	(67%)	0	0	
18	2	3;2	(27%)	4	5;4;2;2	(72%)	0	0	
21	2	6;3	(50%)	-	-		-	-	
22	-	-		2	6;2	(67%)	1	2	(11%)
26	-	-		3	7;2;2	(61%)	1	5	(28%)
30	-	-		2	4;4	(57%)	0	0	
34	-	-		4	8;2;2;2	(78%)	3	2;2;2	(33%)
38	-	-		5	6;3;2;2;2	(83%)	2	5;4	(50%)

1) 18 OTU's per soil depth interval, except Station 18 at depths 22 cm (12 OTU), 30 cm (14 OTU), 38 cm (17 OTU).
2) Isolation and cultivation of bacteria from Stations 2 and 30 was done in media containing artificial seawater; media for isolates from Station 18 contained freshwater.

*Parenthetical percentage - proportion of isolates examined which occurred more than once at ≥ 80% level of association.

at Station 18 are qualitative, results that show the presence of a freshwater or freshwater-tolerant bacterial flora mixed with a marine type flora. Detailed data of these numerical taxonomic relationships are found in hierarchical matrices of coefficients of association and dendrograms as graphic representations of these relationships can be found in the Annual Report (1976; pp. 112-197).

Further analyses were done to determine the extent to which the bacteria at one depth in a given soil column were found at any other depth in that same column. At Station 2 the clustered group of 5 OTU's (Table 7) was related to a single OTU at the 2 cm depth ($\geq$ 80 per cent level of association), and was not found to be related to any other isolates from the soil column. The single cluster of 4 OTU's at the 14 cm were related to the cluster of 5 OTU's at the 16 cm level. Otherwise, there appears a general discontinuity of relationship between clusters of bacteria occurring at various soil depths. This general absence of relatedness between clusters or individual OTU's was found at Station 30, where ca. 12 per cent of the total of 270 OTU's were found to occur more than once in the column, a number of these occurring at widely separated depths. The higher frequency of clusters of related bacteria at Station 18 showed a greater continuity of isolates with depth, particularly if the discriminant level of relatedness was reduced from $\geq$ 80 per cent to ca. 75 per cent. In nearly all cases, the intra-stratum levels of relatedness among clustered bacteria was greater than the inter-stratum relationships measured by the coefficients of association. These findings stress the importance of the concept that all bacteria are randomly distributed. Comparisons of isolates from all three Stations and at all soil depths showed a broad diversity of types.

3.2.3 Physiological and Metabolic Characteristics of Soil Bacteria - Generally, bacteria can be separated into two large groups on the basis of a staining reaction that places an organism in the Gram-positive or Gram-negative group. Together with morphology of the cell, this staining reaction provides an initial basis of differentiation between two major groups of bacteria.

At Station 2 the bacterial flora was dominated by Gram-positive, spore-forming, rod-shaped bacteria at essentially all soil depths, and a significant number of Gram-negative, rod-shaped bacteria in the 2-12 cm depth interval. At Station 18 the Gram-positive, spore-forming rods dominated the 0-10 cm depth, intermixing with Gram-negative rods in the 12-18 cm depth interval which composed 90-100 per cent of the population in the 22-38 cm depth interval. At Station 30 there was a more general mixture of the Gram-positive and Gram-negative groups with soil depth and fewer spore-forming bacteria than was seen at Station 2.

All bacteria characterized from Stations 2, 18, and 30 were dominated by types able to live under conditions of reduced oxygen, i.e., facultative anaerobes. While facultative anaerobes can grow in the presence of normal atmospheric oxygen, as well as in its absence, this group was further divided into two groups, one growing preferentially under moderately reduced oxygen tension and the second growing under more reduced conditions. These two groups were mixed at Station 2 in the 0-12 cm depth interval. At Stations 18 and 30 the dominant group in the 0-12 cm depth was characterized by growth in the moderately reduced oxygen tension. All three Stations showed dominance of true facultative anaerobes in depths below 12-14 cm. The near absence of soil bacteria growing only in the presence of atmospheric oxygen,

i.e., obligate aerobes, reflects the low oxygen tension usually found in water-saturated soil. It is notable that a predominate facultative anaerobic flora can be discerned below the 12 cm soil depth level, a flora characterized generally by a relatively higher activity as shown by a number of metabolic parameters.

The optimum temperature of growth for the majority of all isolates was 25 C, in a range of 15-35 C. Isolates from Station 18 showed a wider temperature range - 15-45 C - of growth than was found at Stations 2 and 30.

Growth of bacterial isolates as a function of pH, i.e., acidity-alkalinity, is optimal in the range of pH 7 to pH 8, as shown in Figure 2.

The range of pH of the culture medium supporting growth has the effect of separating the bacterial populations in relation to the depth at which they occur. A more acid-tolerant (pH 5.0) occurs at Stations 2, 18, and 30 at depths below 6-10 cm. At Station 18 the lower depth population is also more alkaline-tolerant (pH 8.0), but at Station 30 this is reversed with the more alkaline-tolerant population occurring in the top 6 cm of soil. Station 2 shows a generally less sensitive population capable of growing in the range of pH 6.0 to pH 8.0. These results suggest different bacterial populations at the three Stations as well as differences in populations at intervals of soil depth.

Since the ground area under study is subject to water intrusion from marine sources, followed by evaporation and resulting deposit of salts, examination was made of the effects of sodium chloride on growth. Sodium chloride concentrations in the range of 1-3 per cent did not significantly suppress growth of bacteria from any soil depth at Stations 2 and 18, as shown in Figure 3, but suppressive growth effects were evident at 5 and 10

Figure 2

MAXIMUM - MINIMUM HYDROGEN-ION CONCENTRATION FOR GROWTH OF SOIL ISOLATES

Stations 2, 18, 30

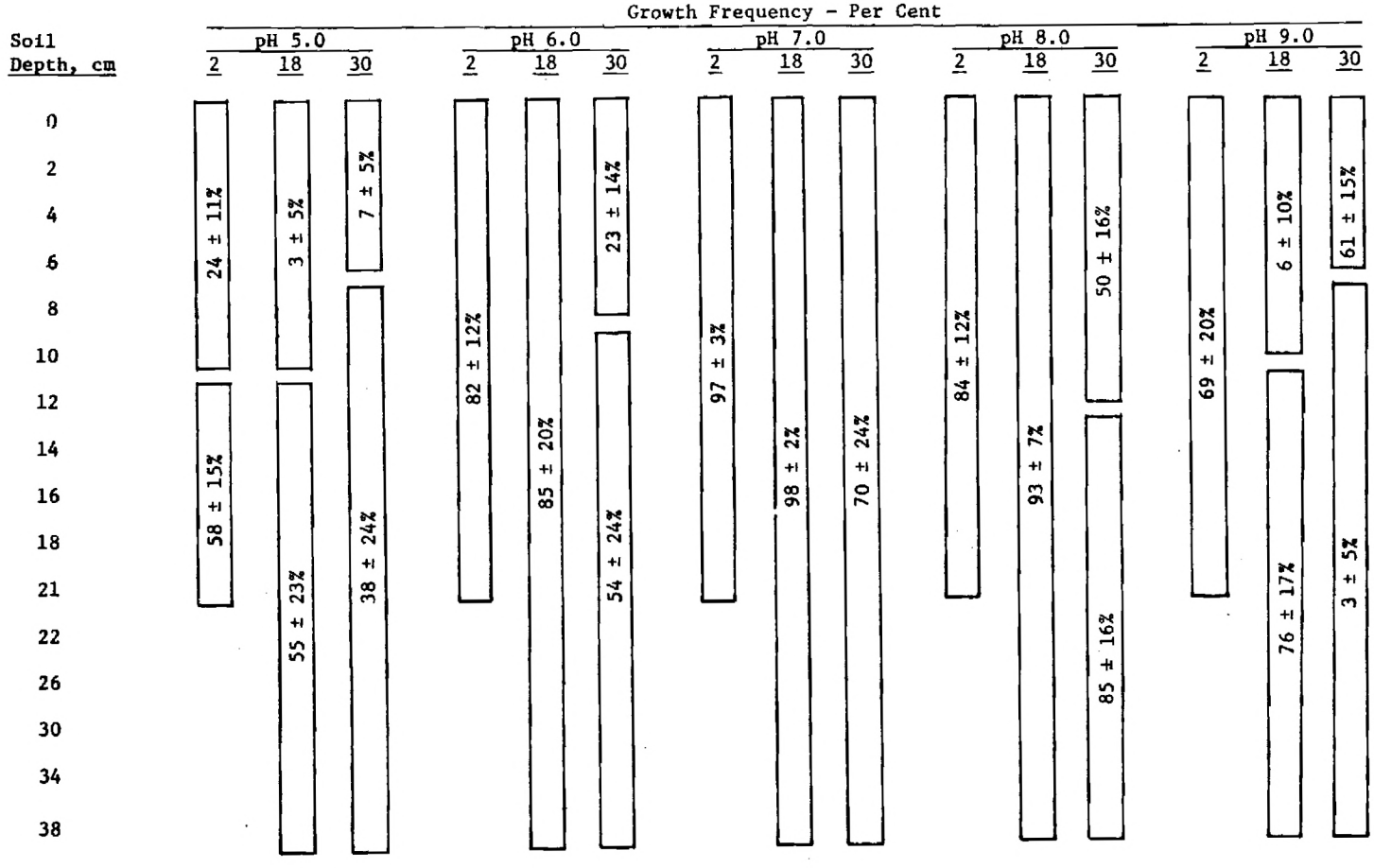
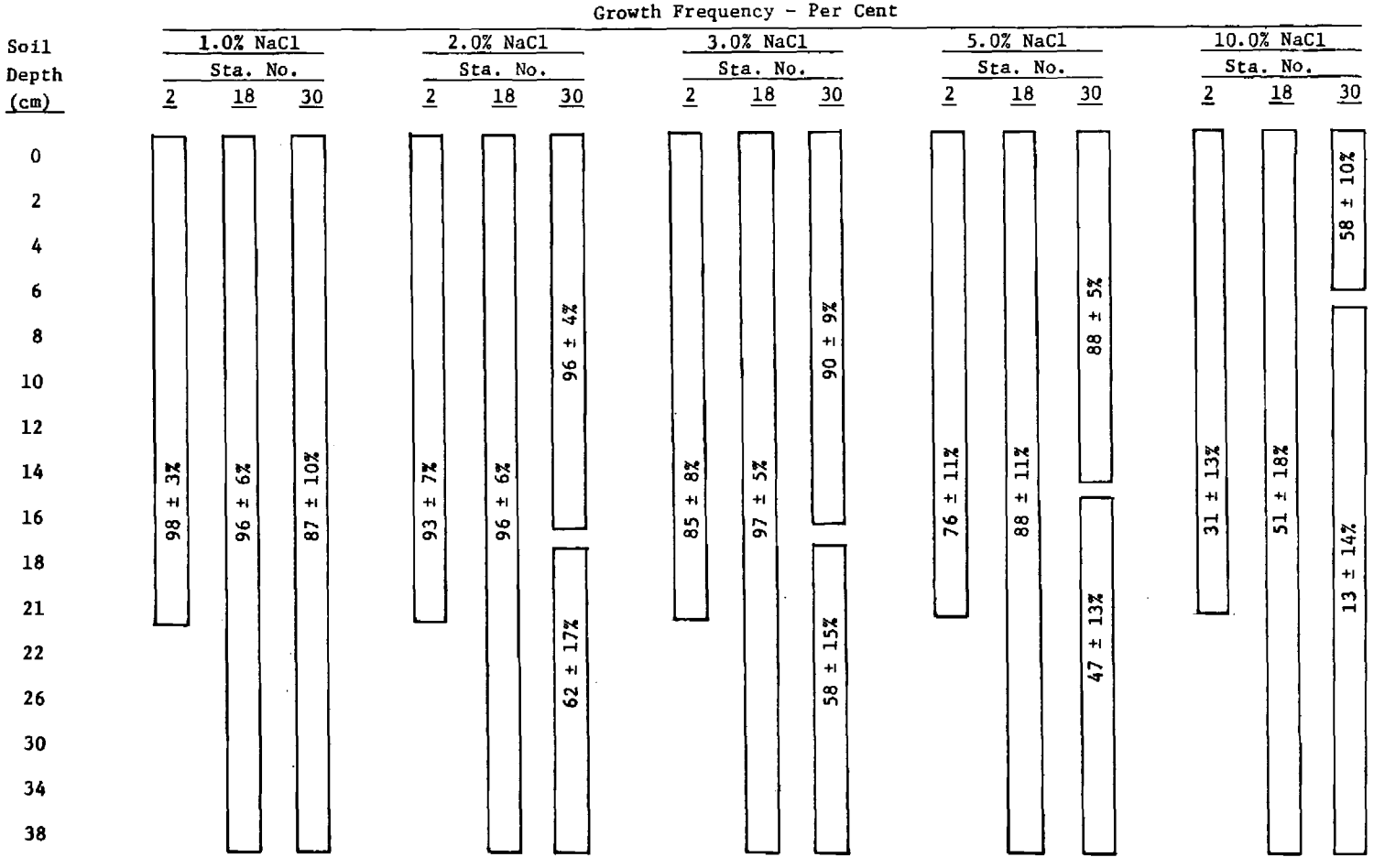


Figure 3

GROWTH TOLERANCE OF SOIL ISOLATES FOR VARIOUS CONCENTRATIONS OF SODIUM CHLORIDE

Stations 2, 18, 30



per cent sodium chloride concentrations. At Station 30 some depression of growth occurred in the presence of 1 per cent sodium chloride and a rather marked suppression of growth of the soil populations at different depths as the concentration of sodium chloride was increased. For example, the population most resistant (halotolerant) to 10 per cent salt occurs in the top 6 cm of soil. This result might indicate that Station 30, particularly at lower depths, is less subject to the 'salt-pan effect' which is caused by evaporative loss of water with resulting aggregation of salt deposit possibly occurring at Stations 2 and 18. It also should be pointed out that isolation and culture of bacteria from Station 18 was done in freshwater, yet these organisms are evidently tolerant of sodium chloride.

Separation of the bacterial populations at Stations 2, 18, and 30 into freshwater and marine groups is indicated by ability of the bacteria to grow in a salts-free medium or by a dependence on the salts of sodium, calcium, potassium, or magnesium. Bacteria (ca. 80 per cent) from Station 2 were not dependent upon salts for growth. In growth systems where 3 of the 4 elements (sodium, calcium, potassium, magnesium) were present, only bacteria from Station 30 showed a significant dependence on sodium with dependency of some isolates on calcium or magnesium. Isolates from Station 18 were intermediate in that no dependency on a single element existed, growth occurring on any three elements in combination. It is known that magnesium and calcium will reduce the requirement for sodium by some marine and halophilic bacteria. It thus appears that there are three population zones of bacteria: Station 2, freshwater but halotolerant; Station 18, intermediate with dependency on sodium chloride relieved by a combination of selected elements; and Station 30, marine forms significantly dependent on sodium

which can not be substituted by calcium, potassium, or magnesium. No variation was evident in these findings to indicate that depth of soil influenced the distribution of sodium-dependent bacteria, except in the 1 cm surface of Station 30 where ca. 45 per cent of the bacteria were not dependent on sodium.

Carbon metabolism - Isolates from Stations 2, 18, and 30 were examined for their ability to utilize glucose, lactose, and sucrose by oxidative or fermentative pathways of metabolism. Lactose was utilized by a negligible number of isolates from all three Stations with the exception of isolates in the top 1 cm of soil at Station 2. Glucose was utilized oxidatively by a large number of isolates from all soil depths at Station 2; the highest frequency of similar isolates occurred at depths below 10 cm at Stations 18 and 30. Fermentative reactors on glucose were predominately from the lower depths. Only isolates from lower soil depths at Stations 18 and 30 produce gas in glucose fermentative metabolism. Metabolism of glucose showed a different pattern relative to the source of soil isolates. None from Station 2 used sucrose oxidatively, and, excepting isolates from the top 1 cm of soil, the isolates fermenting sucrose were found below 8 cm, increasing in frequency with depth. Oxidative and fermentative patterns of glucose metabolism were found among isolates at all depths of Station 30. A few such isolates were found at Station 18, the majority occurring below the 14 cm depth. These different frequency patterns among isolates from the three Stations and the evident association of isolates with soil depth further underlines apparent differences in bacterial populations at these locations. An additional group of 15 carbohydrates, i.e., sugars and alcohols, were examined for utilization by fermentative metabolism with similar

patterns of divergency between Stations and with differences associated with soil depth.

Another group of 17 carbon compounds were examined as sole sources of carbon supporting growth. This is a more exacting condition determining the ability of the isolates to utilize and therefore be active in cycling of carbon compounds in nature. Isolates from Station 2 utilized the widest range of carbon substrates, which included acetate, citrate, gluconate, glucose, lactate, malonate, succinate, alanine, arginine, proline, and tryptophane. These isolates were generally distributed throughout the soil column except at soil depths of 2, 4, and 6 centimeters where there was an absence or reduction in the numbers of isolates utilizing acetate, gluconate, glucose, lactate, malonate, arginine, and tryptophane. The trend toward a reduction in active isolates at the 2-6 cm levels was followed by a generally consistent high frequency of active isolates in the 8-21 cm section of the soil column.

A pattern of sole carbon source utilization similar to Station 2 was observed among isolates from Station 30, although the frequency of isolates using the various substrates tended to be lower, particularly the substrate malonate. A few isolates from Station 30 utilized benzoate, formate, and methionine, a capability essentially absent from Station 2 isolates. The influence of soil depth was not as marked among Station 30 isolates but the pattern of higher frequency of isolates from lower depths was evident with some substrates.

Isolates from Station 18 showed fewer substrates used as the sole source of carbon for bacterial growth and energy. Only acetate, gluconate, lactate, and succinate were utilized by a significant number of isolates,

and practically all of these isolates were recovered from soil depths below 10 cm.

Studies were also made to determine the frequency of specific enzymes called decarboxylases which remove the carboxyl group (-COOH) from the amino acids arginine, lysine, and ornithine. Isolates obtained from Station 18 on primary culture medium containing distilled water showed significantly higher frequencies of decarboxylation of arginine, lysine, and ornithine as compared to isolates from Stations 2 and 30. Primary isolation and examination for decarboxylase activities of these isolates was done in growth systems containing artificial seawater. Station 2 produced a relatively high frequency of arginine decarboxylase but an insignificant number of isolates showing decarboxylation of lysine and ornithine. Station 30 produced the lowest frequencies of decarboxylation of the three amino acids. The clustered groups of these organisms at Station 30 were restricted to a comparatively narrow depth interval (22-38 cm) for arginine decarboxylase and for ornithine decarboxylase (8-16 cm). These results suggest that the bacterial populations at Stations 2, 18, and 30 are different under the conditions of primary isolation and testing for decarboxylase production.

Further activities of soil isolates in their possible activity in cycling of carbon compounds were examined by determining the ability of the isolates to break down (hydrolyze) a number (9) of more complex carbon substrates. A summary of these results expressed as averages for isolates throughout the soil column is shown in Table 8. Generally, the isolates capable of hydrolyzing these substrates were distributed throughout the soil column, although some isolates, e.g., chitinolytic isolates from Station 30 and gelatinolytic isolates from Station 18 were predominant in the

Table 8

SUMMARY OF HYDROLYSIS OF VARIOUS SUBSTRATES BY ALL ISOLATES IN A SOIL COLUMN

Stations 2, 18, 30

Substrate	Average Frequency (Per Cent) of Hydrolysis					
	Station 2		Station 18		Station 30	
	(%)	Total OTU Tested	(%)	Total OTU Tested	(%)	Total OTU Tested
Aesculin	31	198	33	252	52	270
Araban	32	136	13	248	16	165
Casein	53	192	72	242	79	244
Cellulose	21	198	0	0	3	270
Chitin	17	195	3	194	28	201
Gelatin	16	197	33	252	2	270
Starch	59	193	62	244	74	244
Tributyrin	69	179	61	215	60	238
Xylan	2	193	1	171	7	213
Depth of Soil Column (cm):	21		38		38	

lower soil strata. Chitin is of interest as a material in the exoskeletons of invertebrate animals, e.g., crabs, and in the cell walls of fungi. The ability to degrade cellulose was rather limited but most frequent among isolates throughout the soil column at Station 2. Cellobiose, a constituent of cellulose was metabolized by an average of 16-30 per cent of isolates from the three Stations with the majority of such isolates occurring at the lower soil depths.

Details of carbon metabolism of the isolates from Stations 2, 18, and 30 have been previously reported (Annual Report, 1976, pp. 222-226; pp. 234-251).

Nitrogen Metabolism - A number of compounds were examined as sole sources of nitrogen required for growth of soil isolates. These included ammonium sulfate, aspartic acid, asparagine, cysteine, glutamic acid, glucosamine, sodium nitrate, and atmospheric nitrogen, nitrogen sources expected to be found in natural soil systems.

Isolates from Station 2 showed the highest frequency in using most of these compounds as nitrogen sources. More than an average of 72 per cent of the isolates utilized these sources with a range of 22 per cent using aspartic acid to 95 per cent using glutamic acid. Only 14 per cent were able to utilize atmospheric nitrogen.

Isolates from Station 30 produced fewer isolates utilizing these nitrogen sources. Asparagine was the most frequently utilized (49 per cent) with the other compounds being available as sole nitrogen sources for an average of ca. 18 per cent of isolates with a range of 3-27 per cent. Atmospheric nitrogen was utilized by 18 per cent of the isolates. No particular pattern of distribution of these isolates with depth of soil was evi-

dent at either Station 2 or 30, the frequencies being more randomly distributed with depth.

The isolates from Station 18 were essentially unable to utilize these nitrogen sources, ca. 10 per cent of 249 isolates examined could do so but with no apparent preference for a particular nitrogen source or relationship to soil depth.

Reduction of nitrate to nitrite was accomplished by isolates from all soil depths at Stations 2 and 30 with the highest frequencies of such isolates occurring at the lower depths. Such isolates were found principally at the 16 cm depth and below at Station 18. Many bacteria can utilize the oxygen of nitrate when grown under reduced tension of atmospheric oxygen. This reaction has significant implications in the depletion of nitrates in the soil, a nitrogen form available for plant metabolism. In such an area as the study transect where the soil tends to be water logged a greater part of the time with accompanying low oxygen tension, the reduction of nitrates by such bacteria in the soil has significant implications for soil fertility.

IV. CONCLUSIONS

Numerical taxonomic analysis of the morphological, physiological, and biochemical characteristics of ca. 870 bacteria from soil samples taken at three selected intervals of 200, 1800, and 3000 meters along the ecological study transect at Turkey Point showed a wide diversity of bacterial types relative to sampling sites. Clusters of related taxonomic groups were evident among bacteria isolated from the same depth of soil at a particular location. This clustering was significantly high in numbers of bacteria show-

ing similar characteristics which reflected the more homogeneous conditions of localized soil areas and the importance of micro-ecosystems. These micro-ecosystems are evidence that bacterial populations do not occur randomly throughout a column of soil but certain related groups will become prominent in relationship to localized environmental factors of chemical and physical nature as well as the undefined influence of other microbial forms.

Results of this study were principally those related to chemoorganotrophic, mesophilic, and predominately facultative anaerobes. The ability to live either in the presence of oxygen or under reduced concentrations of oxygen, which is definitive of facultative anaerobes, is a characteristic compatible with soil conditions of poor aeration associated with wetlands. The numerical size of this population decreases exponentially with soil depth. A significant characteristic of the population as a function of soil depth is that the most metabolically diverse segments of this population is found in the surface layer of soil and in the lower depths, i.e., below the 10-14 cm (4-6 inches) level. Thus, a smaller proportion of the vertically distributed bacterial population is found in the lower depths but this population contains more metabolically diverse members as measured by their ability to utilize a variety of carbon substrates. Location of these metabolically diverse bacteria is associated with various concentrations of organic carbon in the soil.

Several bacterial populations are identifiable by characteristics which associate them with a predominantely marine environment under the influence of Card Sound at Station 30, an intermediate environmental zone at Station 18, and an 'upland' zone at Station 2 where the population is most characteristically non-marine or freshwater oriented yet is tolerant of con-

ditions simulating a marine environment. Many biochemical activities are shared by the non-marine and marine-oriented populations which indicate their functional roles in the microbial ecosystem might be similar but performed under different environmental conditions. The degree to which these microbial systems contribute to the cycling of nutrients, e.g., carbon, in the macro-ecosystem of the area is postulated to depend to an important degree on the vertical exchange of water in the soil system and the characteristics of flow of surface water. Such a mechanism would seem to be required if the more metabolically active deep soil populations comprise an important input source of metabolites to the larger surface system. The higher concentration of organic carbon in lower soil depths suggests that this postulated process occurs at a low rate of activity.

The bacterial population decreases with depth in association with a corresponding decrease in total biomass as measured by the adenosine triphosphate (ATP) content of soil. Bacteria account for a small part of this total biomass, the remaining life forms remain unidentified. The highest measures of biomass were found in the hammock and associated areas, followed by the non-hammock saw grass areas distal to Card Sound, i.e., the 'upland' areas. The non-hammock areas are considered to be decreasingly productive, as measured by biomass, in progression from the 'upland' zones toward Card Sound to the beginning boundary of the dwarf mangroves (Station 30), which was the limit of the transect studied.

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