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Ecology of Soil Arthrobacters in Clarion-Webster Toposequences of Iowa

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Toposequence variations in soil properties were characterized and related to variations in populations of total isolatable bacteria and arthrobacters. Increases in soil NO_3-N, available phosphorous, NO_3-N-producing power, *Arthrobacter* counts, and the percentage of the total counts represented by arthrobacters were correlated with decreases in soil acidity. The total bacterial counts were not correlated with soil acidity but were associated with percentage of soil organic matter and percentage of clay. The percentage of the total counts represented by arthrobacters was lowest at the summit position and increased downslope to the highest value in the toeslope position. Factor analysis of the data revealed that 67 to 81% of the total variance exhibited by all variables per site-sampling period could be accounted for by soil acidity, soil structure, soil fertility, soil moisture, and bacterial factors. A selective medium was developed for soil arthrobacters and tested on a wide variety of central Iowa soils to determine its potential as a medium for enumeration as well as isolation. The medium developed in this study was found to be superior to the other available direct-isolation media for soil arthrobacters.

Various studies have shown that members of the genus *Arthrobacter* are often among the more numerically predominant bacteria routinely isolated from soils (15, 25). These soil arthrobacters are nutritionally very diverse (20, 27), and many isolates can be found that exhibit the ability to degrade various pesticides (9, 14, 24). Very little work has been done, however, to determine possible correlations between variations in soil properties and variations in any particular group of soil bacteria. Soil pseudomonads have been found associated with slightly acid rhizosphere soil samples, whereas arthrobacters have been associated with slightly alkaline non-rhizosphere soil samples (22). Increased numbers of arthrobacters have been associated with soil samples adjusted to higher moisture contents, whereas pseudomonads have been predominant in soil samples adjusted to lower moisture contents (21).

Topography is a very complex soil formation factor that could affect the bacterial populations by influencing certain soil properties through climate or drainage-related functions (11). Proceeding downslope from the shoulder position to the toeslope in Clarion-Webster toposequences of Iowa, the percentage of soil organic matter increases to a maximum while the mean particle size decreases to a minimum. The thickness of the A-horizon and the depth to carbonates or mottles decreases as the slope gradient becomes steeper (29).

The toposequence soils in Clarion-Webster toposequences represent a gradation in textural classes; several studies have associated nematode populations with texture variations (18, 19), but no attempts have been made to do this with bacterial populations. Soil organic matter levels are interrelated with other soil properties, and little is known about the effects of this relatively stable soil property of bacterial populations.

Arthrobacters have normally been isolated from soils by using either enrichment techniques or by randomly picking and identifying isolates from media used to determine total counts (15). Mulder and Antheunisse (16) developed a selective procedure for arthrobacters involving two separate media where the identification was based on observation of a morphological cycle possessed by members of this genus. Their method was not intended to serve as a means of enumerating *Arthrobacter* populations and, because of the lack of a suitable enumeration procedure, one was developed in our study.

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This study investigated variations in total isolatable bacteria and *Arthrobacter* populations in two toposequences in the Clarion-Webster soil association area in Iowa. Toposequence variations in soil properties were characterized in relation to their effects on the total bacterial and *Arthrobacter* populations.

MATERIALS AND METHODS

Site location and description. The two toposequences were located in the Clarion-Webster soil association area in north-central Iowa and are described in Table 1. Site I was in a corn-soybean rotation from 1963 to 1968 and in continuous corn from 1968 to 1973, whereas site II was in a corn-soybean rotation from 1963 to 1973. During this interval site I received no lime treatments, whereas site II received the appropriate amount of lime to maintain the soil pH at 6.9.

Sample collection and sampling periods. Four adjacent rows of corn that extended parallel to the toposequence transect were chosen at each sampling site, and nine core samples were removed, three each from the middle of the furrow between adjacent rows of corn. Three sampling sites were chosen in each of the four soil types comprising the toposequences. The core samples were obtained and processed individually on 30 August at site I (soil temperature, 33 C) and 25 October at sites I and II (soil temperature, 26 C). All core samples were taken from the Ap-horizon at a depth of 10 cm at both sampling sites.

Total bacteria analyses. All core samples were placed in plastic bags and transported to the laboratory, and platings were performed on the same day that each core sample was obtained. A 5.0-g sample was aseptically removed from the previously unexposed center of each core sample and suspended in 495 ml of sterile 0.5% peptone broth. Each sample was then agitated in a Waring blender for 3 min at low speed, serial dilutions in 0.5% peptone broth were made, and 0.1-ml portions of appropriate dilutions were spread over the surface of sterile media in petri plates. Total counts were made from a medium containing 0.1% peptonized milk (Difco), 0.1% yeast extract (Difco), 0.01% Acti-Dione (Upjohn Co.), and 1.5% agar. The pH was adjusted to the pH of the particular soil being plated and plates were incubated at 25 C for 10 days, after which colonies were counted. All platings were done in triplicate.

TABLE 1. *Comparative descriptions of toposequences*

Descriptor	Site I	Site II
Aspect	North	West
Slope shape	Diverging	Converging
Summit-shoulder	Convex	Convex
Backslope-toeslope	Concave	Concave
Curvilinear length (yards)	300	170
Soil series-slope class-erosion class		
Summit	Clarion, 1%, slight	Clarion, 1%, slight
Shoulder	Clarion, 3%, slight	Clarion, 5%, moderate
Backslope	Nicollet, 2%, slight	Nicollet, 2%, slight
Footslope	Webster, 1%, none	Webster, 1%, none
Toeslope	Harps, 0%, none	Harps, 0%, none

Arthrobacter selective medium and analyses. Seventeen *Arthrobacter* named strains from the American Type Culture Collection (ATCC, Rockville, Md.) and 20 *Arthrobacter*, 6 *Bacillus*, 6 *Micrococcus*, 4 *Nocardia*, 4 *Streptomyces*, 4 *Flavobacterium*, and 6 *Pseudomonas* soil isolates were screened on 31 dyes, 13 antibiotics, and 11 assorted compounds to determine possible selective properties for the arthrobacters. The soil isolates were taken from the medium used to determine total counts and were identified to the genus level according to procedures outlined by Buchanan and Gibbons (5). All 67 cultures were tested on a wide range of concentrations of each of the 55 potential selective agents to detect any differential as well as selective properties. The screening was performed by incorporating the various concentrations of the potential selective agents in either Trypticase soy agar (BBL), peptonized milk agar, or nutrient agar (Difco). The three basal media were tested at a variety of concentrations with additions of various amounts of yeast extract as well as with the potential selective agents. Those agents that were heat sensitive were filter-sterilized and added aseptically to the cooled, autoclaved media. The media were adjusted to a variety of pH values ranging from 5.0 to 8.5. The cultures were transferred to the surface of the media with a multipoint inoculation device (7). All plates were incubated at 30 C for 72 h, after which plates were examined.

Those compounds that exhibited either selective or differential properties for the arthrobacters were retested on various concentrations of the three basal media at varying pH values. A total of 720 different variations were examined to determine the best possible combination of a basal medium plus yeast extract plus various concentrations of different selective ingredients.

The best selective medium had the following composition: 0.4% trypticase soy agar, 0.2% yeast extract, 2.0% NaCl, 0.01% Acti-Dione, 150 μg of methyl red (Harleco) per ml, and 1.5% agar. The methyl red was filter-sterilized and added aseptically to the autoclaved, cooled medium (see Results and Discussion). Soil samples were diluted and plated, and plates containing the selective medium were incubated at the temperature used for the total count medium. The pH was adjusted to the pH of the particular soil being plated.

The selective medium was tested, on a variety of soils, to determine what percentage of the isolates were arthrobacters by subculturing and microscopic examination of all the colonies on various randomly selected plates for each soil type. The colonies were transferred to trypticase soy agar plus 0.2% yeast extract and examined microscopically for possession of a rod-to-coccus morphological cycle, snapping divi-

sion, pleomorphism, and V-forms (5). The same procedure was also performed on plates containing the total count medium and the media developed by Mulder and Antheunisse (16). For enumeration of the arthrobacters in the four soils examined in the ecological survey (Table 1), 78% of the counts on the selective medium was taken as the *Arthrobacter* counts.

Soil analyses. After the bacterial analyses were performed, eight portions were taken from each core sample, two each for the following determinations: soil NO_3-N (4), soil NH_4-N (2), NO_3N-producing power (26), and soil moisture (28). One particle size analysis was performed on each core sample by using a modified pipette method (28). The remainder of each core sample was air-dried and screened through a 4.0-mm sieve, and two replicate determinations for all procedures were made on each core sample. Soil pH, total exchangeable bases, exchangeable hydrogen (10), soil organic matter (6), available phosphorous (17), and soluble salts and saturation percentage (3) determinations were then performed.

Statistical analyses. Simple correlation matrices were computed, and a preliminary set of factor-loading values for the factor analysis was computed from these matrices by using the principal components method (8). These factor-loading values were subjected to a varimax rotation (12) to maximize the factor loadings without changing the specific variance of each variable.

The linear factor analysis model (23) used for each of the 16 variables was $z_1 = a_1F_1 + a_2F_2 + a_3F_3 + c_1E_1$. This model equation expresses each variable, z, in terms of three factors, F_1 to F_3, and an error factor E. The factor loadings, a and c, indicate the extent to which each factor participates in the test. The specific variance of the error factor for each variable indicates how much of the variation exhibited by the variables is not explained by the three factors.

This particular factor analysis model was used because the results of a test of significance for the total number of factors indicated that there were not more than three factors involved at any one site-sampling period (13). In using this model we assumed that the sample size of 108 toposequence samples per site-sampling period was large enough to avoid sampling error. To insure this, only factor-loading values larger than 0.50 or smaller than −0.50 were considered significant correlational values.

RESULTS AND DISCUSSION

Of all the compounds tested as possible selective agents, only a few exhibited any selective properties for the arthrobacters. The combination of Acti-Dione at 0.01% and NaCl at 2.0% effectively inhibited all fungi and most streptomycetes, nocardia, and gram-negative bacteria. The methyl red at 150 μg/ml inhibited other gram-positive bacteria (bacilli and micrococci) but did not affect the arthrobacters. The pH of the medium, between 5.0 and 8.5, did not affect its selectivity, and the combination of trypticase soy agar at 0.4% and yeast extract at 0.2% gave the highest yield of arthrobacters with the addition of the selective ingredients over the other basal media (data not shown).

In testing the selective medium (Table 2), the percentage of the colonies identified as arthrobacters was much higher (74%) than that of either the total count medium (14%) or the nutritionally poor medium (24%). From the soils tested, approximately 25% of the colonies on the selective medium were not arthrobacters, and microscopic examination was necessary to distinguish them. In examining the selective medium for enumeration potential (Table 3) the percentages of arthrobacters from the selective medium were close to or slightly higher

TABLE 2. *Counts and percentage of the counts represented by arthrobacters from nine soil types from the total count medium, nutritionally poor medium, and selective medium*

Soil type	PMA[a]		NPM[b]		SM[c]	
	Counts ($\times 10^6$)	Percentage of A[d]	Counts ($\times 10^6$)	Percentage of A	Counts ($\times 10^6$)	Percentage of A
Clarion sandy loam	87	13	98	27	158	78
Nicollet loam	125	12	80	25	167	73
Webster clay loam	63	16	75	27	186	80
Harps clay loam	95	17	123	36	232	82
Ames loam	52	12	50	18	113	75
Hayden loam	63	15	74	23	135	66
Okoboji clay loam	73	19	76	31	214	83
Nicollet clay loam	76	14	84	19	157	78
Storden sandy loam	47	11	62	12	118	55
Average percentage of A		14		24		74

[a] PMA, Peptonized milk agar medium used for total counts.
[b] NPM, Nutritionally poor medium of Mulder and Antheunisse (16).
[c] SM, Selective medium.
[d] Percentage of A, Percentage of arthrobacters found on each of the respective media.

than the percentages from the total count medium. Because of this close agreement, it was decided to use the selective medium for enumeration purposes by taking a percentage of the colonies growing on the plates as being the arthrobacter counts and comparing these with the counts from the total count medium to arrive at the percentage of arthrobacters contained in any one sample. The nutritionally poor medium (Table 3) was not suitable for enumeration purposes. Further tests on the four soils used in the ecological survey (data not shown) indicated that 78% of the counts on the selective medium was a suitable figure for determining the arthrobacter counts from the respective soils.

TABLE 3. *Total counts on peptonized milk agar (PMA) and percentage of the total counts represented by arthrobacters on PMA, the nutritionally poor medium (NPM), and selective medium (SM) using counts on PMA as the base figure for determining percentage of arthrobacters*

Soil type	PMA counts ($\times 10^6$)	Percentage of arthrobacters[a]		
		PMA	NPM	SM
Clarion sandy loam ..	87	13.4	2.9	14.2
Nicollet loam	125	12.0	2.0	12.0
Webster clay loam ...	63	16.6	3.2	23.6
Harps clay loam	95	17.3	4.6	20.0
Ames loam	52	12.4	1.7	16.4
Hayden loam	63	13.0	2.7	14.1
Okoboji clay loam	73	19.2	3.3	24.3
Nicollet clay loam	76	14.0	2.1	16.2
Storden sandy loam ..	47	11.5	1.2	13.8

[a] Percentage of arthrobacters for the NPM and SM were obtained by using the numbers of arthrobacters from both of these media as determined from the data in Table 2. These figures were then compared with the total counts for each soil type to obtain the percentage of the total counts represented by arthrobacters for each of the media.

The largest total bacterial and *Arthrobacter* populations occurred at the toeslope position of both toposequences during each sampling period. The smallest total bacterial and *Arthrobacter* populations occurred at the backslope position and increased down to the toeslope and up to the summit position (Table 4). The percentage of the total counts represented by arthrobacters was lowest at the summit and increased downslope to the highest percentage in the toeslope position.

Pronounced changes in soil variables accompanied these variations in bacterial populations at each toposequence (Table 4). However, due to the higher pH caused by the limed conditions in the soils at site II, the variation in most of the variables was not as great as for either sampling period at site I. Proceeding from the summit to the toeslope position, there were increases in soil pH, percentage of clay, percentage of silt plus percentage of clay, soluble salt levels, percentage of organic matter, soil NO_3-N,

TABLE 4. *Toposequence-related soil and bacterial population data for site I during the 25 October sampling period*[a]

Variable	Summit	Shoulder	Backslope	Footslope	Toeslope
Clay (%)	27.25	20.92	21.72	34.08	40.13
Silt (%) plus clay (%)	68.80	54.05	53.17	71.24	76.00
Organic matter (%)	5.13	4.26	4.07	5.90	6.17
NO_3-N (μg/g)	0.93	2.30	10.10	20.20	35.00
Available phosphorous	1.55	1.36	2.04	2.09	2.32
NH_4-N (μg/g)	7.63	4.73	3.40	2.17	1.43
NO_3-N-producing power[b]	36.65	37.93	54.57	88.64	102.83
Soil pH	5.70	6.12	6.24	6.06	7.42
Exchangeable bases	16.70	18.05	20.93	24.43	29.95
Exchangeable H^+[c]	5.83	4.87	4.03	3.45	2.57
Soluble salts at percent saturation	0.24	0.29	0.40	0.76	0.94
Moisture (%) relative to field capacity	82.00	73.50	74.50	93.70	102.00
Moisture (%) relative to percent saturation	42.31	44.88	44.98	47.11	49.48
Total bacterial counts	1.6×10^8	6.6×10^7	3.0×10^7	2.1×10^8	2.4×10^8
Arthrobacter counts	7.2×10^6	4.0×10^6	2.6×10^6	3.6×10^7	5.6×10^7
Arthrobacters (%)	4.68	6.26	8.30	16.48	23.39

[a] Each figure represents the average value of the variable for the toposequence position.
[b] Expressed as micrograms of NO_3-N per gram of soil.
[c] Exchangeable hydrogen expressed as milliequivalents of H^+/100 g of soil.

NO_3-N-producing power, available phosphorous, total exchangeable bases, percentage of moisture relative to field capacity, and percentage of moisture relative to percentage saturation. There were decreases downslope in the exchangeable hydrogen and soil NH_4-N (Table 4).

Due to the interpretational method of factor analysis, each of the factors was arbitrarily named, depending upon which variables appeared to be consistently interrelated (Table 5). The soil fertility factor name was chosen because NO_3-N is the end product of nitrification and therefore is a useful indicator of the ability of the soil to supply plant-available NO_3-N. The other factors were named according to the obvious combinations of variables composing the various factors.

TABLE 5. *Composition of factors used in factor analysis*

Factor name	Correlated variables
Soil structure	Silt (%) plus clay (%)
	Clay (%)
	Soil organic matter (%)
Soil acidity	Soil pH
	Total exchangeable bases
	Exchangeable hydrogen
	Soluble salts at percent saturation
Soil fertility	Soil NO_3-N
	Soil NH_4-N
	Available phosphorous
	NO_3-N-producing power
Soil moisture	Moisture relative to field capacity
	Moisture relative to percent saturation
Bacterial	Total bacterial counts
	Arthrobacter counts
	Arthrobacters (%)

More of the variation in the soil fertility, acidity, structure, and bacterial variables was accounted for in factor analysis than variation soil moisture variables, which was indicated by higher specific variance values of the soil moisture variables compared with the other variables (Tables 6–8). At site I during both sampling periods (Table 6, 7), the soil acidity factor was positively correlated with soil NO_3-N, NO_3-N-producing power, available phosphorous, *Arthrobacter* counts, and the percentage of the total counts represented by arthrobacters, and negatively correlated with soil NH_4-N. The soil structure factor was negatively correlated with percentage of moisture relative to field capacity, total bacterial counts, and *Arthrobacter* counts. The absence of a soil fertility factor at site I on 30 August (Table 6) was probably due to interference by the roots of the corn plants with the soil fertility variables (uptake of available P and NO_3-N). By 25 October (Table 7) the roots were dead, the interference was removed, and a soil fertility factor, which was positively correlated with percentage of moisture relative to percentage of saturation, was generated.

At site II (Table 8) the soil acidity factor was positively correlated with the same variables as at site I, but the degrees of correlation were not

TABLE 6. *Factor analysis of the data for site I during the 30 August sampling period*

Variable	Rotated factor-loading values		
	Soil acidity factor	Soil structure factor	Specific variance of variables
Silt (%) + clay (%)	NS[a]	−0.92	0.23
Clay (%)	0.59	0.77	0.08
Organic matter (%)	NS	−0.97	0.14
Soil pH	0.98	NS	0.06
Exchangeable bases	0.96	NS	0.13
Exchangeable hydrogen	−0.96	NS	0.08
Soluble salts	0.87	NS	0.28
NO_3-N (μg/g)	0.90	NS	0.07
NH_4-N (μg/g)	−0.99	NS	0.15
Available phosphorous	0.90	NS	0.16
NO_3-N-producing power	0.96	NS	0.19
Moisture (%) relative to field capacity	0.59	−0.78	0.64
Moisture (%) relative to percent saturation	NS	NS	0.35
Total bacterial counts	NS	−0.96	0.27
Arthrobacter counts	0.67	−0.97	0.21
Arthrobacters (%)	0.88	NS	0.14

[a] NS, Nonsignificant correlational values.

TABLE 7. *Factor analysis of the data for site I during the 25 October sampling period*

Variable	Rotated factor-loading values			
	Soil acidity factor	Soil structure factor	Soil fertility factor	Specific variance of variables
Silt (%) + clay (%)	NS[a]	−0.97	NS	0.22
Clay (%)	NS	0.84	NS	0.06
Organic matter (%)	NS	−0.97	NS	0.16
Soil pH	0.87	NS	NS	0.07
Exchangeable bases	0.86	NS	NS	0.10
Exchangeable hydrogen	−0.93	NS	NS	0.14
Soluble salts	0.80	−0.54	NS	0.25
NO_3-N (μg/g)	0.96	NS	0.77	0.04
NH_4-N (μg/g)	−0.96	NS	NS	0.13
Available phosphorous	0.79	NS	NS	0.36
NO_3-N-producing power	0.81	−0.53	0.65	0.08
Moisture (%) relative to field capacity	NS	−0.77	NS	0.52
Moisture (%) relative to percent saturation	NS	NS	0.88	0.30
Total bacterial counts	NS	−0.96	NS	0.12
Arthrobacter counts	0.72	−0.91	NS	0.26
Arthrobacters (%)	0.97	NS	NS	0.05

[a] NS, Nonsignificant correlational value.

TABLE 8. Factor analysis of the data for site II during the 25 October sampling period

Variable	Rotated factor-loading values			
	Soil acidity factor	Soil structure factor	Soil fertility factor	Specific variance of variables
Silt (%) + clay (%)	NS[a]	NS	NS	0.18
Clay (%)	NS	NS	0.84	0.12
Organic matter (%)	NS	−0.76	NS	0.35
Soil pH	0.88	NS	NS	0.17
Exchangeable bases	0.77	NS	−0.59	0.26
Exchangeable hydrogen	−0.99	NS	NS	0.03
Soluble salts	0.90	NS	NS	0.15
NO_3-N (μg/g)	0.83	0.61	NS	0.14
NH_4-N (μg/g)	−0.83	0.52	NS	0.45
Available phosphorous	0.75	−0.51	NS	0.33
NO_3-N-producing power	0.64	0.67	NS	0.16
Moisture (%) relative to field capacity	NS	−0.81	NS	0.85
Moisture (%) relative to percent saturation	NS	0.72	NS	0.37
Total bacterial counts	NS	0.91	NS	0.24
Arthrobacter counts	0.55	0.88	NS	0.29
Arthrobacters (%)	0.74	NS	NS	0.33

[a] NS, Nonsignificant correlational value.

as great. The soil structure factor was not correlated with any variables, whereas the soil fertility factor was negatively correlated with the percentage of moisture relative to field capacity and positively correlated with the percentage of moisture relative to percentage of saturation, total counts, and *Arthrobacter* counts.

At site I, increased soil acidity resulted in decreased soil NO_3-N and increased soil NH_4-N (Table 5). This was a measure of the lessened activity, due to acid sensitivity, of the nitrifying bacteria *Nitrosomonas* and *Nitrobacter* (1). The increased soil acidity was responsible for the decreased *Arthrobacter* counts and percentages of the total counts represented by arthrobacters. The total bacterial counts were influenced strongly by the soil structure factor (percentages of clay and organic matter) and, to a lesser degree, by the soil acidity factor. The

Arthrobacter counts were positively correlated with acidity, but the degree of correlation was not as great (Tables 6–8) as was that of the percentage of the total counts represented by arthrobacters. This was due to the effects of the soil organic matter and clay content on the *Arthrobacter* counts, especially at the shoulder position (Table 4), whereas these variables did not significantly affect the percentage of the total counts represented by arthrobacters. The same relationships were found at site II, but the significant correlations were not as great due to the decreased variation in many of the variables caused by the limed conditions.

During the 30 August sampling period at site I, 76.80% of the total variance removed by all factors was accounted for, whereas 80.92% was accounted for in the 25 October sampling period site I (Table 9). At site II 67.84% of the total variance was accounted for. The increased contribution of the soil acidity factor at site I accounted for the greater total percentage of the variance removed as compared with site II. The 20 to 30% of the total variation unaccounted for represented other unmeasured and/or unknown environmental factors.

Factor analysis of the data from the toposequence soils examined in this study indicated that the arthrobacters in these soils were acid sensitive and their numbers decreased in a cause-and-effect relationship with increasing acidity. At site I on 25 October (Table 4), the percentage of the total counts represented by arthrobacters was 4.68% at the summit (pH 5.79) and increased to 23.39% at the toeslope as the acidity decreased (pH 7.42). At site II on 25 October (data not shown), the variation was less due to the limed conditions since, at the summit, 14.81% of the total counts were arthrobacters (pH 6.91) and increased to 20.26% at the toeslope as the acidity decreased (pH 7.38). The total bacterial counts were not correlated with soil acidity during any of the site-sampling periods, which probably indicated that as the acidity increased and the *Arthrobacter* counts decreased, the numbers of some type of acid-tolerant bacterium were increasing.

TABLE 9. *Total variance removed by factors in factor analysis*

Site	Sampling period	Factor	Percentage of total variance removed by factor	Total variance removed by all factors (%)
I	August 30	Soil acidity	53.72	
		Soil structure	23.08	76.80
	October 25	Soil acidity	39.96	
		Soil structure	31.00	
		Soil fertility	9.96	80.92
II	October 25	Soil acidity	35.49	
		Soil fertility	17.09	
		Soil structure	15.26	67.84

This study demonstrated that the distribution and abundance of certain types of bacteria (in this case, arthrobacters) were, to a large extent, determined by certain ecological variables. If the assumption is valid that microbial populations are selected by their environments, then the methodology applied in this study might find additional uses in determining which environmental variables most strongly influence the distribution of any of a wide range of microorganisms.

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