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Microcosm and Experimental Pond Evaluation of Microbial Community Response to Synthetic Oil Contamination in Freshwater Sediments

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A multivariate approach was used to evaluate the significance of synthetic oil-induced perturbations in the functional activity of sediment microbial communities. Total viable cell densities, ATP-biomass, alkaline phosphatase and dehydrogenase activity, and mineralization rates of glucose, protein, oleic acid, starch, naphthalene, and phenanthrene were monitored on a periodic basis in microcosms and experimental ponds for 11 months, both before and after exposure to synthetic oil. All variables contributed to significant discrimination between sediment microbial responses in control communities and communities exposed to a gradient of synthetic oil contamination. At high synthetic oil concentrations (4,000 ml/12 m^3), a transient reduction in sediment ATP concentrations and increased rates of oleic acid mineralization were demonstrated within 1 week of exposure. These transient effects were followed within 1 month by a significant increase in rates of naphthalene and phenanthrene mineralization. After initial construction, both control and synthetic oil-exposed microbial communities demonstrated wide variability in community activity. All experimental microbial communities approached equilibrium and demonstrated good replication. However, synthetic oil perturbation was demonstrated by wide transient variability in community activity. This variability was primarily the result of the stimulation of polyaromatic hydrocarbon mineralization rates. In general, microcosms and pond communities demonstrated sufficient resiliency to recover from the effects of synthetic oil exposure within 3 months, although polyaromatic hydrocarbon mineralization rates remained significantly elevated.

In a recent study, microbial community response to a coal coking waste effluent was evaluated as a model for potential environmental contamination by coal-conversion technologies (15). The multivariate, functional community analysis used in that investigation indicated significant stress effects (inhibitory) as well as stimulation and community recovery in response to the presence or absence of the coking effluent. Wastewater derived from coal-conversion processes, which is similar in composition to coal-coking wastes (12), does inhibit methanogenesis in aquatic sediments (10). Although the effects of petroleum and petroleum-derived oils on heterotrophic populations and activity have been studied (6, 7, 17), there is no information on the responses of aquatic microbial communities to synthetic oil exposure. It was hypothesized that community response to a real coal-conversion product, synthetic oil (SO), would approximate that observed for sediments contaminated by coal-coking effluents. The present investigation was designed to test that hypothesis and to validate the multivariate approach. In view of the fact that environmental contamination by SOs is virtually nonexistent, microcosms were chosen to serve as the experimental system to test the hypothesis and the approach.

MATERIALS AND METHODS

Experimental design. The studies described in this investigation were conducted in close collaboration with scientists of the aquatic toxicology group at the Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, and were a component of a larger microcosm evaluation of the effects of SO contamination on aquatic ecosystems.

The investigation was conducted with two groups of experimental microcosms. The first phase of the investigation consisted of a dose-response study in which laboratory aquatic microcosms were exposed to SO. Aquatic microcosms (76 liters) were prepared in glass aquaria containing 13 to 15 cm of pond sediment and 22 to 24 cm (ca. 57 liters) of well water. These aquaria were maintained at 15°C on a 12-h diurnal light cycle.

After an initial 3-month equilibration period, four replicate microcosms were treated with 10 ml of SO and duplicate sets of microcosms were each treated with 2 and 50 ml of SO respectively. Samples for microbiological study were obtained in January and March 1981 before SO exposure and in April and May 1981 after SO exposure (ca. 1 and 5 weeks, respectively). No zero dose control microcosms were used in this study; consequently, the pre-SO treatment period was used as a control for posttreatment evaluation of functional community response to SO contamination.

After the dose-response studies in laboratory microcosms, eight outdoor ponds were constructed to serve as large-scale microcosms to evaluate acute SO contamination effects. The outdoor ponds were lined with a Hypalon (36 mil, Du Pont Co., Wilmington, Del.) permeability barrier which was overlaid with approximately 30 cm of freshwater sediment and 80 cm of pond water. Total volume of the ponds was 12 m^3 (12,000 liters). The ponds were initially constructed in August 1980 and were allowed to equilibrate until 8 July 1981, at which time duplicate ponds received SO treatments of 200, 800, or 4,000 ml. Two ponds were not treated and served as controls. Pond samples were obtained in November 1980 and January and June 1981 during the equilibration period, and in July, August, and September 1982 after SO exposure. During the equilibration state, all ponds developed complex aquatic communities which included both aquatic macrophytes and vertebrates. The design, construction, maintenance, and SO treatment regimes for both groups of microcosms were directed by Jeff Giddings, Environmental Sciences Division, Oak Ridge National Laboratory.

Sampling. Vacuum aspiration was used to collect sediment samples from both types of microcosms. One pooled 50- to 100-g random sample was collected from each microcosm on each sampling period for those microcosms originally used in dose-response studies. A 100- to 200-g sample was randomly collected from each of three quadrants within each experimental pond for each sampling period. The samples were collected in sterile 1-liter glass jars and maintained at 5°C overnight before sample processing. Each quadrant sample was processed separately to evaluate within-pond variability. Total sample processing time ranged from 18 to 24 h from the time of sample collection.

Microbial community analysis. The choice of microbial community response measurements was made from information obtained on discriminating variables useful for evaluating the impact of a coal-coking effluent on sediment microbial communities (15). Ten estimates of microbial population density, biomass, and activity were measured to provide the multivariate data base needed to evaluate community response to SO contaminants. These estimates included: (i) total viable cell density (TVC); (ii) acid extractable ATP-biomass; (iii) dehydrogenase (DEHYD); (iv) alkaline phosphatase (PHOS); (v) glucose mineralization rate (GLUMIN); (vi) protein mineralization rate (PROMIN); (vii) starch mineralization rate (STARCH); (viii) oleic acid mineralization rate (OLEIC); (ix) naphthalene mineralization rate (NAPMIN); and (x) phenanthrene mineralization rate (PHEMIN). Organic substrate mineralization rates were calculated from the slope of a linear plot of cumulative substrate mineralized versus time. Methods for these determinations have been previously reported (13–15; R. E. Perkins and G. S. Sayler, submitted for publication). All experimental measurements were performed in triplicate for each variable for each sample collected.

Chemicals. Radiolabeled organic compounds used as mineralization substrates included (all from New England Nuclear Corp., Boston, Mass.): [U-^{14}C]glucose, 329 mCi $mmol^{-1}$; U-^{14}C-labeled *Escherichia coli* B/r protein, 53.2 mCi $mmol^{-1}$; [U-^{14}C]starch, 1.9 mCi mg^{-1}; and [U-^{14}C]oleic acid, 900 and 999.0 mCi $mmol^{-1}$. [4,5,8-^{14}C]naphthalene (5 mCi $mmol^{-1}$) and [9-^{14}C]phenanthrene (19.3 mCi $mmol^{-1}$) (both from Amersham Corp., Arlington Heights, Ill.) were used as polyaromatic hydrocarbon mineralization substrates. Pesticide-grade acetone (Fisher Scientific Co., Puttsburgh, Pa. was used as the solvent for polyaromatic hydrocarbons and in preparation of the glassware. Omnifluor (New England Nuclear) and glass-distilled, pesticide-grade dioxane (Burdick and Jackson Laboratories, Inc., Muskegon, Mich.) were used as the cocktail for scintillation counting in mineralization assessment.

SO from the H-coal direct liquefaction process was obtained from the H-coal process Development Unit, Lawrenceville, N.J., courtesy of William C. Goldman, Hydrocarbon Research Inc. Samples were obtained from run no. 9 in the syncrude mode, September 1979. The product was a 1.7:1 blend of atmospheric separator overheads and atmospheric separator bottoms.

Data analysis. All determinations for each variable were performed in triplicate for each sample collected. All statistical tests of significance were conducted at $P \geq 0.95$. The data were subjected to multiple analysis of variance (MANOVA) to determine whether there were significant SO treatment effects on pond or microcosm microbial communities. Stepwise discriminant analysis was employed to examine the interaction effect between sampling time and SO treatment and to determine which variables provided the greatest information in determining SO effects on the microbial community. Duncan's Multiple Range Analysis was employed to test for significant differences among mean values for individual variables. Programs for MANOVA and Duncan's Multiple Range Analysis were derived from the Statistical Analysis System (1). Discriminant analysis employed computer programs available in *Statistical Package for the Social Sciences* (9). All data analyses were performed by using UTK DEC-10-IBM/360-65 computers. Application of multivariate methods to microbial community analysis, as used in this study, have been previously described (15). Statistical approaches, computational procedures, and tests of significance were derived from Green (5), Pimmentel (11), and Tatsuoka (16).

RESULTS

The data gathered in this investigation are too extensive for complete reporting in the format of this report. The raw data are stored in a DEC-10 system file and are available on request as either a correlation matrix or as a raw data output, 80-column format card deck.

Laboratory microcosms. An initial MANOVA demonstrated significant microbial community

response to SO treatments during the posttreatment period as compared with the pretreatment period (Table 1). This response was identical for all individual variables except TVC. A significant multivariate SO treatment-related effect was observed. Univariate responses to SO were limited to TVC, DEHYD, GLUMIN, and OLEIC. There was no significant interaction between sampling time (prior and posttreatment) and treatment level (dose), except for TVC. These combined results are interpreted as a significant SO-induced variation in microbial community response, with specific SO treatment-level effects demonstrated only for TVC, DEHYD, GLUMIN, and OLEIC. This interpretation is supported by the results of Perkins and Sayler (submitted for publication), who demonstrated that SO caused elevated mineralization rates of simple organic substrates as a result of selective growth stimulation of specific physiological populations.

Discriminant analysis was employed to more precisely define those variables contributing to significant differences between microcosms after SO treatment and to more fully describe the response of the microcosm community after treatment. The individual variables used to characterize the microbial community were entered into a stepwise discriminant analysis to determine which variables provided the greatest information in detecting differences between microcosms due to the SO treatment. The stepwise selection of discriminating variables sequentially enters those variables into the analysis based upon their greatest individual explanation of total variation among the microcosms. Consequently, only those variables that provide significant information are included in the analysis. All variables used in this investigation were utilized to discriminate between microcosms (Table 2). Each variable is given an absolute weight (discriminant function coefficient, Z) relevant to a discriminant function (linear multivariate mathematical relationship) to which each sample is related. Consequently, the order of entry into the analysis and the Z scores of the variables for each discriminant function determine the information content of the variable (explanation of variation) and the importance of that variable in defining that discriminant function. A graphic representation of this analysis is developed by plotting the group centroids (equivalent to a multivariate grand mean; each centroid represents 30 data points, 10 variables examined in triplicate) for each microcosm sample relative to the discriminant functions derived (Fig. 1). The first three discriminant functions derived in this analysis provide adequate separation between microcosms to evaluate both variation among microcosms before SO treatment

TABLE 1. MANOVA for experimental microcosms treated with SO

Effects examined	Multivariate F approximation		Univariate F statistic									
	df	Wilk's criterion	TVC	ATP	DEHYD	PHOS	GLUMIN	STARCH	OLEIC	PROMIN	NAPMIN	PHEMIN
Sampling time	7, 84	11.01**[a]	3.87	7.14*	6.90*	13.93*	69.41*	27.43*	17.54*	28.29*	29.28*	18.17*
SO treatments	14, 168	3.78**[b]	4.07**	0.02	5.73**	0.52	5.65**	0.20	3.37**	1.01	1.72	0.65
Time × treatment interaction	14, 168	0.29	5.68[c]	0.48	0.10	1.24	0.47	0.42	0.04	0.47	2.28	1.02

[a] *, Significant variability at $\alpha = 0.05$; reject the null hypothesis that there is no difference in microbial community function or structure before and after SO treatment.

[b] **, Significant variability at $\alpha = 0.05$; reject the null hypothesis that there is no SO dose response effect.

[c] A significant time × treatment interaction at $\alpha = 0.05$.

TABLE 2. Standardized discriminant function coefficient of microbial variables discriminating between control and SO-contaminated microcosms

Discriminating variable[a]	Discriminant function coefficient (Z)[b]		
	Function 1	Function 2	Function 3
PROMIN	−0.37	−0.71	−0.0
NAPMIN	0.52	−0.37	0.33
TVC	0.32	−0.10	−0.90
DEHYD	−0.03	0.30	−0.46
GLUMIN	−0.09	−0.13	−0.32
STARCH	−0.08	−0.09	0.22
PHEMIN	−0.10	0.02	−0.08
PHOS	0.01	0.07	0.06
ATP	0.06	0.05	−0.02
OLEIC	−0.01	−0.01	−0.09

[a] In order of entry into stepwise discriminant analysis. Those entered first contribute the most to explaining overall variation among experimental microcosms.

[b] Z scores are weight coefficients given to each variable for each discriminant function. Standardized score limits are ±1.0. The variables having the highest absolute Z score are most important in defining the left (−) or right (+) end of a discriminant axis (see Fig. 1). Discriminant functions 1, 2, and 3 mathematically describe 85% of the total multivariate community variation—45, 23, and 17%, respectively.

and effects of the SO treatments on community response (Fig. 1). During the initial sampling period (January) the microcosms can be clearly differentiated with respect to discriminant function 1 (horizontal axis); this axis is defined by NAPMIN and TVC (Z = 0.52 and 0.32, respectively) and PROMIN (Z = −0.37). Further separation among microcosms is obtained relative to discriminant function 2, defined by DEHYD (Z = 0.30) and PROMIN and NAPMIN (Z = −0.71 and −0.37, respectively). Minor discrimination between microcosms was achieved relative to discriminant function 3, defined by NAPMIN and STARCH (Z = 0.33 and 0.22, respectively) and TVC, DEHYD, and GLUMIN (Z = −0.90, −0.46, and −0.32, respectively). During the second sampling period (March 1981), all microcosms were separated from the previous sampling period relative to DEHYD on discriminant function 2 (Fig. 1). In addition, group centroids of all microcosms demonstrated less variability between microcosms than the previous sampling period. These results indicate that during the later stage of microcosm equilibration, all microcosms tended to demonstrate a similar community response.

Samples collected April 1981, immediately after SO treatment, demonstrated a community response similar to that of the previous, before-treatment sampling period (Fig. 1). The positions of group centroids relative to discriminant function 3 indicated greater variability between microcosms than the previous sampling period, yet significantly less variability than the initial microcosm sampling.

The effects of SO treatment on microbial community responses were clearly demonstrated in samples collected during the fourth sampling period (May) (Fig. 1). All microcosms were readily distinguished from any of the previous sampling period by the position of group cen-

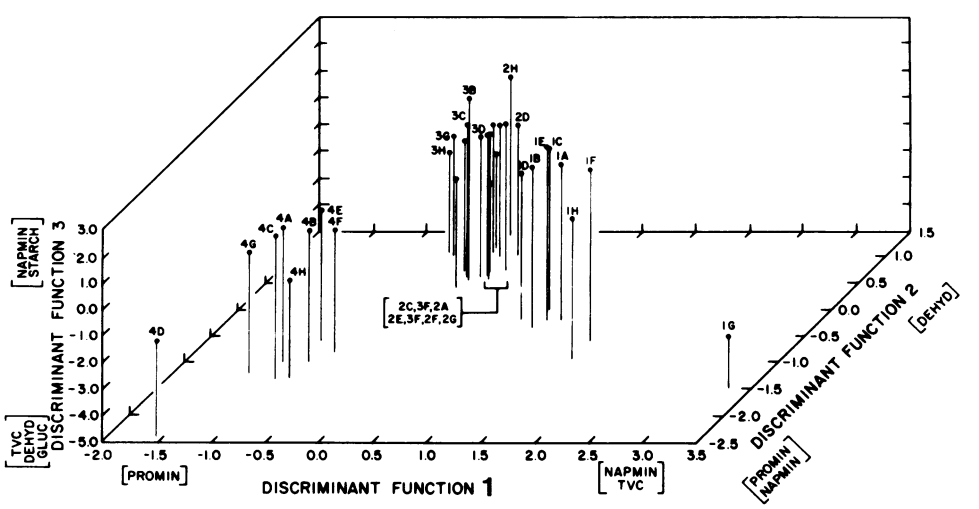

FIG. 1. Discrimination among experimental microcosms treated with SO. Microcosm code: sampling periods 1 and 2, January and March, before treatment; sampling periods 3 and 4, April and May, posttreatment; A and B microcosms, 2-ml SO treatment; C, D, E, and F microcosms, 10-ml SO treatment; G and H microcosms, 50-ml SO treatment. Each microcosm centroid (●) represents 30 data points (10 variables by 3 replications). See Table 2 for discriminating variables.

troids relative to discriminant functions 1 and 2. Furthermore, variation between microcosms during sampling period 4 was greater than any previous sampling period. The results of discriminant analysis indicated that the primary effect of SO treatment was manifested in the microbial community within 2 months of treatment. Primary SO treatment effects resulting in differences among microbial community responses were reflected by general increases in PROMIN and NAPMIN and a net decrease in DEHYD. Increased mineralization rates of polyaromatic hydrocarbons, such as naphthalene (data not shown), have also been observed in natural sediments contaminated by wastewaters rich in aromatic hydrocarbons (8, 15). Such increases were, in part, responsible for the significantly different community response observed in Fig. 1.

Experimental ponds. The results of the preliminary microcosm study indicated that microbial community response to SO treatment could be detected, using the a priori list of discriminating varibles, at the approximate concentration of SO employed. Using the identical experimental and analysis format, we evaluated the microbial community response to SO contamination in experimental ponds.

Multiple analysis of various results indicated a significant interaction between sampling time and SO treatments. This result supports the hypothesis that SO-treated ponds demonstrate significant SO treatment-induced variation (Table 3). Since a significant sampling time × SO treatment interaction effect was observed, discriminant analysis was employed to clarify the individual multivariate effects of sampling time and treatment.

Two discriminant analyses were performed. One used all microbiological variables except PHEMIN, NAPMIN, and PROMIN to discriminate among pond samples for all sampling periods. The second analysis was conducted for the posttreatment period and included all microbiological variables except PROMIN. The reason for this dichotomy was the lack of sufficient pretreatment observations of PHEMIN, NAPMIN, and PROMIN due to the transient unavailability of the commerical ^{14}C-labeled substrate.

In the first analysis (Table 4), all microbiological variables were utilized with discriminant analysis to separate ponds during the course of sampling. In the second analysis, after SO treatment, OLEIC and NAPMIN superseded STARCH and DEHYD as dominant discriminating variables (Table 5). These results are important in that they indicate induction of polyaromatic hydrocarbon degradative capacity and, perhaps, aliphatic hydrocarbons as principle characteristics of microbial communities ex-

TABLE 3. MANOVA for experimental ponds treated with SO

Effects examined	Multivariate F approximation		Univariate F statistic								
	df	Wilk's criterion	TVC	ATP	DEHYD	PHOS	GLUMIN	STARCH	OLEIC	PROMIN	NAPMIN	PHEMIN
Sampling time	35, 1,760	50.10*[a]	81.03*	9.47*	119.54*	31.38*	53.47*	159.08*	108.28*	165.92*	9.05*	5.64*
SO treatments	21, 1,050	10.51*	12.81*	2.35	1.54	7.20*	25.58*	8.61*	50.21*	20.44*	4.00*	6.43*
Time × treatment interaction	105, 2,478	5.15*	10.88*	4.71*	3.81*	5.46*	3.73*	2.02*	7.94*	3.75*	5.41*	9.25*

[a] *, Significant variability at $\alpha = 0.5$; reject null hypothesis that there is no difference in microbial community structure or function between SO-treated and control ponds. Since there is significant interaction, analysis of specific time or treatment level effects cannot be made.

TABLE 4. Microbiological variables utilized by stepwise discriminant analysis to discriminate between experimental ponds before and after SO treatment

Discriminating variable[a]	Discriminant function coefficient (Z)[b]		
	Function 1	Function 2	Function 3
STARCH	−0.62	0.13	−0.07
DEHYD	0.10	0.61	0.12
OLEIC	−0.22	0.07	1.00
TVC	−0.31	−0.12	−0.07
PHOS	0.07	0.11	−0.25
GLUMIN	0.08	0.43	−0.29
ATP	−0.10	−0.07	−0.14

[a] See Table 2, footnote *a*.
[b] See Table 2, footnote *b*. Discriminant functions 1, 2, and 3 mathematically describe 75% of the total multivariate community variation—46, 20, and 9%, respectively.

posed to SO. The first discriminant analysis demonstrated both inherent pond variability as well as the effect of SO treatment (Fig. 2). After initial construction, the ponds demonstrated extreme variability with respect to discriminant axis 1 defined by DEHYD (Z = 0.10) and STARCH and TVC (Z = −0.62 and −0.32, respectively), with additional variability relative to discriminant functions 2 and 3 (Fig. 3). Variability between ponds was reduced during both sampling periods 2 and 3, with sampling period 3 differentiated by discriminant function 2 defined by DEHYD and GLUMIN (Z = 0.61 and 0.43, respectively) and TVC (Z = −0.12) (Fig. 3A). During the first sampling after SO treatment (sampling period 4), the ponds demonstrated increased variability relative to discriminant function 3 defined by OLEIC (Z = 1.00), and GLUMIN and PHOS (Z = −0.29, and −0.25, respectively) (Fig. 3B). Additional variability was observed relative to discriminant function 1. Ponds C and I, which received the highest SO treatment, demonstrated similar responses, different than the remaining ponds. During sampling period 5, variability between ponds decreased to that observed before SO treatment. And, during sampling period 6, pond variability was reduced to the point that only ponds C and I demonstrated major differences from the control and other SO-treated ponds.

When NAPMIN and PHEMIN were included in the analysis, greater definition between ponds exposed to SO treatment was obtained (Fig. 3). During sampling period 4 (1 week after SO treatment), variability among all ponds was observed (Fig. 3). The magnitude of this variability was as great as that observed during initial construction of the pond (comparison of Fig. 2 and 3). Discrimination among ponds after SO treatment was achieved relative to discriminant axis 1 defined by OLEIC and DEHYD (Z = 0.54 and 0.35, respectively) and NAPMIN, PHEMIN, and ATP (Z = −0.45, −0.42, and −0.30, respectively; Table 5). Ponds C and I, which received the highest level of SO treatment (4,000 ml), were clearly separated by this axis, primarily as a result of twofold higher rates of OLEIC than any of the other ponds (data not shown). A similar SO-induced elevation of OLEIC, a function of enrichment for lipolytic populations, was observed by Perkins and Sayler (submitted for publication).

During sampling period 5 (ca. 1 month after SO treatment), overall variability among ponds was reduced and separation among ponds was achieved on discriminant axis 2 (Fig. 3). This axis is defined by PHOS, STARCH, ATP, and NAPMIN. Although the high-treatment-level ponds C and I demonstrated similar responses, the control ponds B and Q demonstrated wide variability in community response. However, this control pond variability and overall pond variability decreased during the sampling period 6 (Fig. 3). Discrimination among ponds was again achieved relative to discriminant axis 1, and all ponds were oriented toward the left on this axis. This discrimination, achieved relative to NAPMIN and PHEMIN, is readily apparent for ponds I (4,000 ml of SO) and P (800 ml of SO), which, along with pond C (4,000 ml of SO) demonstrated significantly higher rates of aromatic hydrocarbon mineralization than the remaining ponds (data not shown). This analysis demonstrates both the relative importance of NAPMIN and PHEMIN as response variables to SO treatment and the SO-induced variability

TABLE 5. Influence of NAPMIN and PHEMIN on microbial variables utilized by stepwise discriminant analysis to discriminate between experimental ponds after treatment with SO

Discriminating variable[a]	Discriminant function coefficient (Z)[b]		
	Function 1	Function 2	Function 3
OLEIC	0.54	0.20	−0.16
NAPMIN	−0.45	0.35	0.50
STARCH	0.19	0.73	−0.15
PHOS	0.15	−0.52	0.61
ATP	−0.30	0.39	0.31
DEHYD	0.35	−0.07	0.35
PHEMIN	−0.42	0.16	−0.08
GLUC	0.17	0.03	0.47
TVC	0.01	0.16	−0.08

[a] See Table 2, footnote *a*.
[b] See Table 2, footnote *b*. Discriminant functions 1, 2, and 3 mathematically describe 75% of the total multivariate community variation—46, 20, and 9%, respectively.

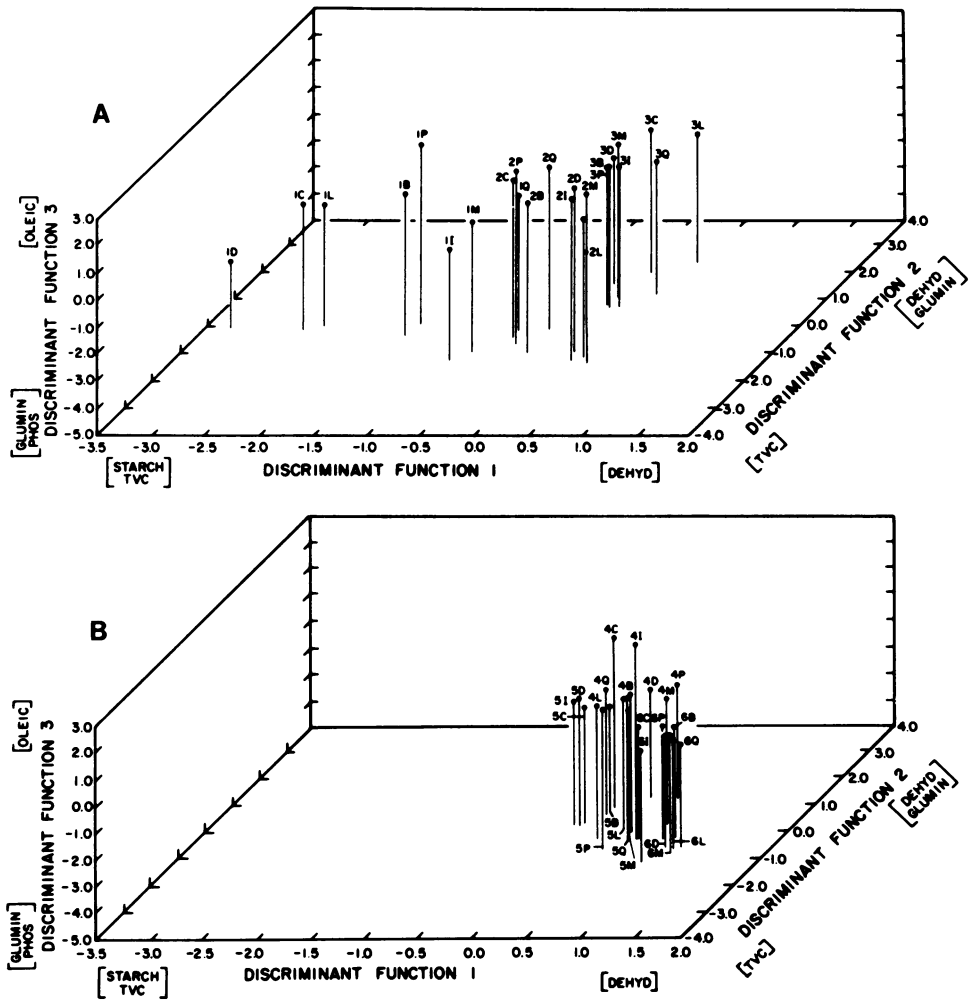

FIG. 2. Discrimination among experimental ponds before and after treatment with SO. NAPMIN, PHEMIN, and PROMIN data were deleted due to insufficient numbers of observations; see the text and Table 4 for discriminating variables. Variables in brackets define the ends of a discriminant axis as a result of high Z scores for that discriminant function. Sample code: part A, sampling periods 1 (November), 2 (January), and 3, (June); part B, posttreatment sampling periods 4 (July), 5 (August), and 6 (September). SO treatment levels: ponds B and Q, control; D and L, 200 ml of SO; M and P, 800 ml of SO; C and I, 4,000 ml of SO.

in microbial community response immediately after treatment and reduced variability over time (Fig. 2).

DISCUSSION

The results of this investigation contribute to two important aspects of assessing the fate and effects of environmental contaminants in aquatic microcosms. These aspects are: comparative microbial respone variation in large- and small-scale microcosms, and the effect of SO contamination on the functional activity of complex microbial communities.

As observed in both microcosms and experimental ponds, initial construction results in a predictable non-equilibrium among replicate microcosms followed by an equlibration period of several months. Equilibration results in stabilization and decreased variability among replicate microcosms in which functional microbial community activity estimates and community responses tend to approximate some general average condition. These interpretations are derived from both multivariate community response measures (Fig. 1–3) and individual variables describing aspects of microbial population density, biomass, and activity. The microbial com-

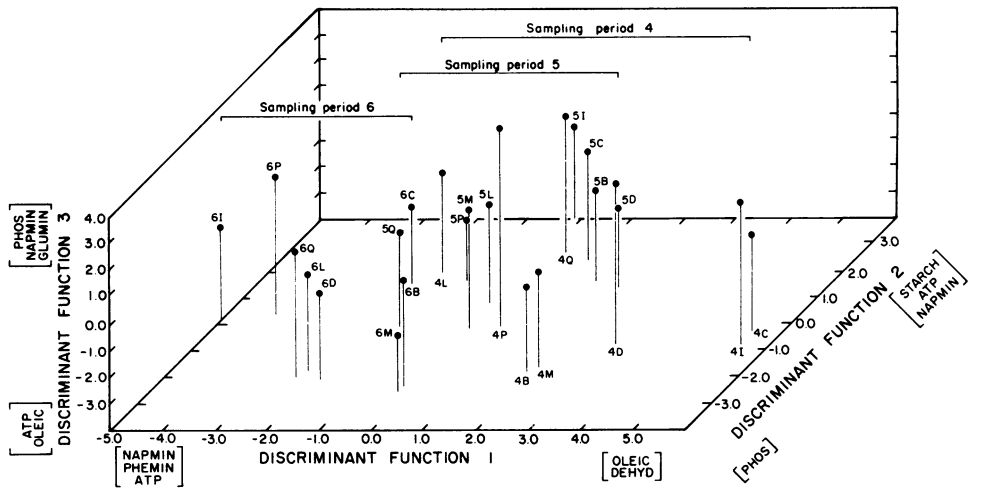

FIG. 3. Effect of NAPMIN and PHEMIN on discrimination among experimental ponds after treatment with SO. Variables in brackets define the ends of a discriminant axis; see the text and Table 5 for discriminating variables. Sample code: sampling period 4, July; 5, August; 6, September. Treatment code: ponds B and D, control; D and L, 200 ml of SO; M and P, 800 ml of SO; C and I. 4,000 ml of SO.

munity response patterns observed in this study were similar to those described for a natural community recovering from the effect of a coal-coking effluent (15) and further reinforce the value and utility of microcosms in assessing microbial community interactions with environmental contaminants.

The response of the microbial community to SO contamination was manifested within 1 week of exposure in experimental ponds (Fig. 2 and 3). This SO-induced perturbation was similar to the response observed in laboratory microcosms (Fig. 1). Howver, laboratory-scale microcosms demonstrated less resiliency (ability to recover from contaminant stress) compared with the pond microcosms. This effect was due partly to the shorter length of time over which SO effects were monitored and the fact that the ponds were more complex and subject to environmental gradients (i.e, temperature, sunlight, evaporation, etc.) that may have affected the residence time of specific SO components in the artifical ecosystem. It should be noted that the absence of posttreatment control microcosms in the SO dose-response study limits the conclusions that can be drawn from these results. Since the pretreatment period served as the control for posttreatment SO exposure, the possibility exists that variability observed after SO exposure may have been random temporal variability also present in non-SO-exposed microcosms.

The individual variables used to monitor microbial community response to SO contamination were chosen based on the results of a previous investigation (15). Discriminant analysis techniques used in this study further supported the choice of these variables in that they all provided information needed to assess SO exposure effects. It should be pointed out that other variables not examined may have contributed to greater definition of SO exposure effects. An example of this point is the inclusion of PHEMIN and NAPMIN data in discriminating among ponds after exposure to SO (Fig. 3). Although interpretation of the overall community response to SO contamination does not change, these variables demonstrate a greater magnitude of community perturbation after SO exposure and indicate more selective effects on specific physiological activities. SO exposure tended to cause an immediate increase in mineralization rates of simple organic substrates followed by a longer time enhancement in the rates of polyaromatic hydrocarbon mineralization. This effect is analogous to that observed for coal-coking effluent perturbation of natural sediment microbial communities (15) and is likely a result of both acclimation and enzymatic induction, resulting in selective enrichment or stimulation of degradative populations (Perkins and Sayler, submitted for publication).

It can be concluded from the results of this investigation that acute SO contamination causes a transient perturbation of sediment microbial communities which results in both inhibition and stimulation of physiological functions. At the SO treatment levels employed in this study, these effects were dampened by the microbial community within 4 months and the system approximated equilibrium conditions.

these observations support the conclusion that microbial communities of artificial ecosystems respond to and recover from effects of environmental contamination in a manner similar to the expected response observed during perturbation of natural ecosystems (2, 3, 4, 15).

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