APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1996, p. 486–493 Vol. 62, No. 2
0099-2240/96/$04.00+0

Anaerobic Microflora of Everglades Sediments: Effects of Nutrients on Population Profiles and Activities

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Received 5 September 1995/Accepted 25 November 1995

Everglades sediments (wetland soils) near sources of agricultural runoff had low redox potentials, were blackened with sulfide, and displayed high porewater phosphorus (total) concentrations and high water column conductivities. These sediments yielded 10^3- to 10^4-fold-higher numbers of culturable anaerobes, including methanogens, sulfate reducers, and acetate producers, than did sediments from Everglades and Lake Okeechobee comparative control sites not as directly associated with agricultural runoff. These observations demonstrated that there was a general, rather than specific, enhancement of the anaerobic microflora in the sediments most likely influenced by agricultural runoff. Despite these differences in microfloral patterns, methylmercury and total mercury levels were similar among these contrasting sediments. Although available sulfate and phosphorus appeared to stimulate the productivity of sulfate reducers in Everglades sediments, the number of culturable sulfate reducers did not directly correspond to the concentration of sulfate and phosphorus in porewaters. Microcosms supplemented with sulfate, nitrate, and phosphate altered the initial capacities of the sediment microflora to produce acetate and methane from endogenous matter. For sediments nearest sources of agricultural runoff, phosphorus temporarily enhanced acetate formation and initially suppressed methane production, sulfate enhanced acetate formation but did not significantly alter the production of methane, and nitrate totally suppressed the initial production of both methane and acetate. In regards to the latter, microbes capable of dissimilating nitrate to ammonium were present in greater culturable numbers than denitrifiers. In microcosms, acetate was a major source of methane, and supplemental hydrogen was directed towards the synthesis of acetate via CO_2-dependent acetogenesis. These findings demonstrate that Everglades sediments nearest agricultural runoff have enhanced anaerobic microbial profiles and that the anaerobic microflora are poised to respond rapidly to phosphate, sulfate, and nitrate input.

The juxtaposition of large human populations with sensitive ecosystems has resulted in complex and often competing natural resource management issues in south Florida (5, 18). Anthropogenic stresses to south Florida's natural systems include nutrient enrichment (particularly phosphorus) from agricultural runoff, altered hydrology due to extensive landscape alterations for flood control and water supply, and the potential occurrence of relatively high levels of mercury in biomass (19, 51, 61, 64). Process-level studies demonstrate that sediments (wetland soils) from this ecosystem emit the greenhouse gases methane (CH_4) and nitrous oxide (N_2O), the productions of which might be influenced by nutrient input (1, 2, 7, 8, 29, 57). Relatively little information is available that directly correlates the sediment conditions of these wetlands, especially those receiving agricultural runoff, with the anaerobic population profiles that might be linked to greenhouse gas production, water quality, phosphorus cycling, and mercury transformation.

Methanogenesis, often viewed as the terminal step in anaerobic decomposition in certain wetlands, may compete with other terminal anaerobic processes such as sulfate reduction (3, 38, 39, 57). Since competing processes might respond differently to nutrient load, it is not surprising that methane fluxes in wetlands and peatlands are highly variable (50, 71, 76). Contrary to thermodynamic considerations, the occurrence of sulfate may not always lessen the competitiveness of methanogens in peatlands (72, 73). Acetogenic and other acetate-forming bacteria have not been evaluated in wetlands but may be competitive with, as well as important trophic partners of, methanogens (3, 10, 20, 37, 43, 56, 60, 76). To evaluate possible structure-function relationships of the Everglades sediment microflora, the main objectives of the present study were to assess (i) the anaerobic microflora of sediments relative to in situ field parameters and (ii) the effects of nutrient input on methanogenesis and acetate turnover in sediment microcosms.

MATERIALS AND METHODS

Site description. Two study sites were located in the remnant Everglades in Water Conservation Areas 2A and 3A (Fig. 1). The latitude and longitude of the sites are as follows: site F1, 26°21′58″N, 80°22′23″W; site 3A, 25°58′50″N, 80°40′16″W. The Water Conservation Areas are Everglades wetlands that were impounded by canal and levee construction during the mid-1900s; these wetlands receive agricultural runoff from sugarcane (*Saccharum* spp.) and winter vegetable crops in the 2.8×10^5-ha Everglades Agricultural Area to the north (19) (Fig. 1). Water flows in a southerly direction, and, via controlled routing of water, site F1 theoretically receives more impact from agricultural runoff than site 3A does. Everglades sediments are characterized by a relatively deep layer (several meters) of peat which continues to be vertically accreted at rates varying between 0.25 cm/year at sawgrass (*Cladium* spp.)-dominated, low-impacted sites (represented by site 3A) and 1.1 cm/year at cattail (*Typha* spp.)-dominated, phosphorus-impacted sites (represented by site F1) (36, 52, 64).

A third site (L14) was located in the Lake Okeechobee littoral zone at

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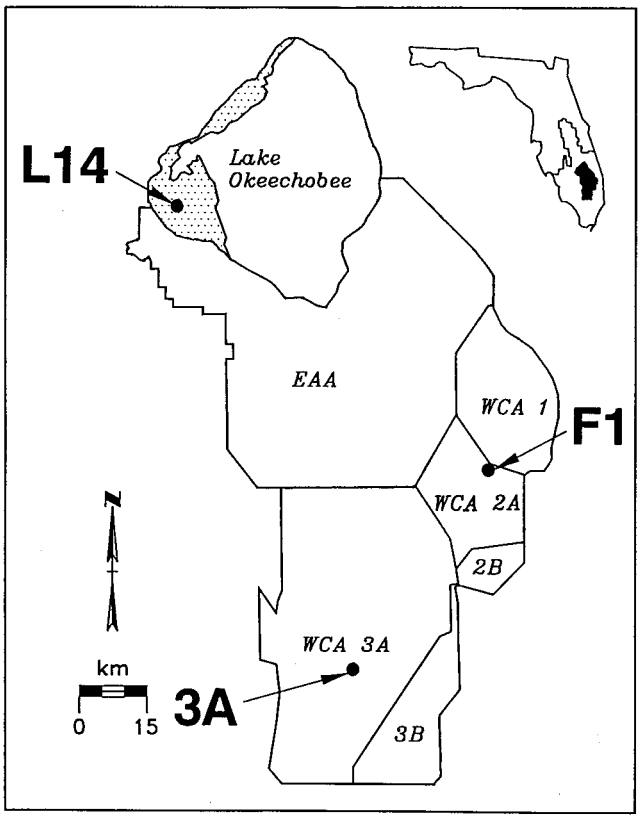

FIG. 1. Location of study sites in the Everglades Water Conservation Areas (WCA, sites F1 and 3A) and the Lake Okeechobee littoral zone (shaded area, site L14). EAA, Everglades Agricultural Area.

26°52′25″N, 81°03′17″W. Lake Okeechobee is a large (1,732-km^2), shallow (mean depth, 2.7 m) subtropical lake centrally located in peninsular Florida (Fig. 1), and its littoral zone was selected for comparative purposes as a non-Everglades, wetland site subject to similar seasonal and atmospheric influences. The emergent vegetation in the littoral zone is dominated by bulrush (*Scirpus californicus*), spikerush (*Eleocharis cellulosa*), beakrush (*Rhynchospora tracyi*), willow (*Salix caroliniana*), and cattail (*Typha* spp.) (5). The littoral zone (represented by site L14) has sandy sediments overlaid with a shallow layer of organic matter (51, 55). Phosphorus control practices in recent years have led to decreases in phosphorus loading of the lake (5, 6, 32).

Field measurements and sediment collection. Sediment (wetland soil) samples and field measurements were collected in August 1994 (rainy season) under high water (water depth, approximately 1 m) conditions. Sediment samples were obtained with a 7.6-cm-diameter coring device constructed of polyvinylchloride pipe. The coring device was inserted into sediments and driven down at least 50 cm with an impact sleeve. Upon placement, the top of the coring device was sealed, and the device was extracted. Consolidated sediment samples from two depths, 0 to 10 and 30 to 40 cm below the floc-enriched interface, were collected for chemical and microbiological analyses. (It is important to note that the upper floc-enriched sediment layer [up to approximately 5 cm thick] was removed to minimize inclusion of the major O_2 interface zone of the sediment.) Extruded sediment samples for microbiological analyses were placed in sterile, wide-mouth serum-stoppered bottles. The bottles were filled to capacity, sealed to minimize aeration, and stored at ambient temperatures. Microbiological analyses were initiated within 3 days of sample collection. Extruded sediment samples for porewater analyses were placed in plastic bags and stored on ice. Porewater sample processing was initiated within 8 h of sampling.

Water column temperature, pH, and conductivity were determined in the field with a Hydrolab (Austin, Tex.) Surveyor III. Sediment redox potential was measured in the field with an Orion Research (Boston, Mass.) 250A meter and platinum-tipped redox probes. The redox probes were allowed to equilibrate in sediments for at least 20 min before measurements were taken; values reported are the average of triplicate measurements per site depth. Emitted sediment gases were obtained by funnel trapping and overpressure injection into argon-filled, serum-stoppered bottles; values are the average of triplicate gas samplings per site.

Characterization of the culturable microflora. To minimize the exposure of the anaerobic microflora to O_2, sediments were handled inside a Mecaplex (Grenchen, Switzerland) anaerobic chamber (100% N_2 gas phase), and the dilution series for all population estimates were performed by anaerobic techniques (35). The sterile, anaerobic mineral solution used for the dilution series contained the following (in grams per liter): K_2HPO_4, 0.225; KH_2PO_4, 0.225; $(NH_4)_2SO_4$, 0.45; NaCl, 0.45; $MgSO_4 \cdot 7H_2O$, 0.045; Na_2CO_3, 4.0; and cysteine-HCl–H_2O (reducing agent), 0.5; plus resazurin (redox indicator) at 1 ml/liter from a 0.1% stock solution. The mineral solution was prepared and dispensed under a 100% CO_2 gas phase; the pH of the mineral solution approximated 6.7. After making the original 1:10 dilution (25 g [wet weight] of sediment plus 225 ml of mineral solution), sediments were preincubated for 1 h on an end-over-end shaker prior to completion of the serial dilution (1:10) series. These manipulations were performed anaerobically at room temperature. The cultivation temperature for all enumerations was 30°C.

General aerobes were estimated by assessment of CFU on tryptic soy agar (containing 2.5 g of glucose per liter) plates (pH 7.0). General anaerobes were estimated by most probable number (MPN) analysis with an undefined medium containing yeast extract, vitamins, mineral salts, trace metals, cysteine and sulfide as reducers, and a H_2-CO_2 (30:70) gas phase (17); the final pH was approximately 6.8. This medium is referred to as UM. Crimp-sealed, serum-stoppered tubes (approximately 7.5-ml liquid phase and 20.5-ml gas phase), which were prepared and inoculated anaerobically, were used in all MPN analyses. Inoculation was 0.5 ml (from dilution series) per 7 ml of medium. MPN values were calculated from standard MPN tables and were within 95% certainty.

MPN analyses based on substrate consumption or product formation were as follows. (i) General acetate producers were determined by assessing the production of acetate in UM; MPN tubes forming acetate in excess of uninoculated controls were scored positive. (ii) General H_2 consumers were determined by assessing the consumption of H_2 in UM; MPN tubes that consumed H_2 in excess of uninoculated controls were scored positive. (iii) H_2-consuming "acetogens" were determined by supplementing UM with 20 mM bromoethanesulfonate (an inhibitor of methanogens) (59) and assessing the simultaneous consumption of H_2 and production of acetate; tubes positive (relative to uninoculated controls) for both were scored positive (note that the term acetogens in quotation marks indicates that the method for their determination, although sometimes used, is not absolute proof of acetogens [20]). (iv) Vanillate and CO consumers were determined with UM supplemented with 5 mM vanillate and 30 kPa of overpressure CO; tubes that consumed vanillate or CO in excess of uninoculated controls were scored positive. (v) Methanogens were determined by the production of CH_4 in either UM or UM supplemented with 2.5 mM acetate, 100 mg of streptomycin per liter, and 100 mg of penicillin per liter (to inhibit eubacteria); tubes that produced methane in excess of uninoculated controls were scored positive. (vi) Sulfate reducers were determined with lactate medium B (48) containing 0.1 g of cysteine per liter and 25 kPa of overpressure H_2; tubes yielding an intense blackening due to the production of sulfide were scored positive.

Anaerobic microcosm studies. Microcosm studies were conducted with either 125-ml or 500-ml screw-cap, serum-stoppered infusion flasks (Merck ABS, Dietikon, Switzerland).

(i) Protocol A. To assess the effects of single nutrient input on the anaerobic microflora of sediments, 125-ml microcosms containing 10 g (wet weight) of sediment (0 to 10 cm) and 25 ml of sterile anaerobic distilled water were supplemented with Na_2SO_4, $NaNO_3$, or Na_3PO_4 as indicated to a final concentration of 5 mM (shown to not appreciably alter the initial pH of the microcosms); the headspace was 100% argon at approximately 50 kPa of overpressure.

(ii) Protocol B. To assess the effects of combined nutrient input (and to provide a greater number of samplings during incubation), 500-ml microcosms containing 20 g (wet weight) of sediment (0 to 10 cm) and 50 ml of sterile anaerobic distilled water were supplemented with Na_2SO_4, $NaNO_3$, or Na_3PO_4 as indicated to a final concentration of 5 mM each; the headspace was 100% argon at approximately 50 kPa of overpressure. Microcosms from protocols A and B had the same sediment-to-liquid volume ratio. Protocol B was also used to evaluate the effects of supplemental CO_2 on the capacity of sediments to consume H_2.

(iii) Protocol C. To assess the turnover of H_2 under enriched conditions, 500-ml microcosms containing 10 g (wet weight) of sediment (0 to 10 cm) plus 90 ml of a yeast-extract-enriched undefined medium (17) were incubated with an H_2-CO_2 (1:2) gas phase at approximately 50 kPa of overpressure. For all protocols, microcosms were incubated horizontally at 30°C without shaking.

Analytical determinations. Sediment dry weights were determined by weighing the sediments before and after drying at 105°C (18 h). Porewater samples were obtained by centrifugation of sediments. Supernatant fluids were filtered through 0.45-μm-pore-size filters (47-mm diameter), and the resulting filtrates were analyzed for total dissolved phosphorus, ammonium, nitrate, and sulfate and used for analysis of total organic carbon. Ammonium, nitrate (NO_x^-, including nitrite), and total phosphorus were determined by the automated phenate, cadmium reduction, and ascorbic acid methods, respectively (4), with an Alpchem rapid flow analyzer (Perstorp Analytical Inc., Silver Spring, Md.). Sulfate was determined by ion chromatography with a Dionex (Sunnyvale, Calif.) model 4500i ion chromatograph equipped with a conductivity detector (64). Total organic carbon was determined with a Dhormann DC-190 (Rosemont Analytical Inc., Santa Clara, Calif.) carbon analyzer (65). Total mercury of sediments was extracted and analyzed with a Perkin-Elmer (Norwalk, Conn.) flame ionization

TABLE 1. Analysis of water columns, sediments, and porewaters of study sites[a]

Site	Water column conductivity ($\mu S\ cm^{-1}$)	Sediment				Porewater (mg/liter)					
		Depth (cm)	Redox (mV)	Dry wt (%)	CH_4 (vol %)	NH_4^+	NO_x^-	SO_4^{2-}	P[b]	Cl^-	C_{org}[c]
F1	1,212	0–10	−302	9.4	58	1.07	0.16	10.0	3.97 (0.28)	326	100
		30–40	−220	10.6		0.38	0.11	<2	0.52 (0.33)	439	98
3A	402	0–10	−94	8.4	43	0.25	0.23	9.4	0.44 (0.17)	38	106
		30–40	−64	9.3		0.77	0.25	<2	0.18 (0.03)	30	61

[a] The pH values of the water columns and porewaters were relatively uniform and approximated 6.55 and 8.0, respectively. NO_x^- is nitrate plus nitrite.
[b] Total phosphorus. Values in parentheses are for total dissolved phosphorus.
[c] C_{org}, organic carbon.

atomic absorption spectrometer model AA 4100 (66). Methylmercury was determined by high-performance liquid chromatography-coupled UV-postcolumn oxidation–cold vapor atomic absorption spectrometry with an LDC (Riviera Beach, Fla.) analytical mercury monitor (22, 34).

Sediment and culture gases were measured with Hewlett-Packard (Palo Alto, Calif.) 5980 Series II gas chromatographs. The detectors and column packing materials used for each gas were as follows: CH_4, flame ionization and molecular sieve; H_2, CO, O_2, and N_2, thermal conductivity and molecular sieve (Alltech, Unterhaching, Germany); CO_2, thermal conductivity and Chromosorb (Alltech); N_2O, electron capture and Porapak Q (Supelco, Bellefonte, Pa.). Acetate and vanillate were measured with a Hewlett-Packard 1090 Series II high-performance liquid chromatograph, an HP 1050 UV detector, and a fermentation monitoring column (Bio-Rad Laboratories, Richmond, Calif.). Gas pressures were measured with a Ballmoos (Horgen, Switzerland) DMG 2120 needle manometer. The analytical methods used for both gas chromatography and high-performance liquid chromatography were described previously (30, 40, 41, 44).

RESULTS

Physical and chemical characteristics of sites. Water column temperatures at the time of sampling ranged from an average of 29°C in the Water Conservation Areas to 33°C in the Lake Okeechobee littoral zone. In contrast to the sediments at sites 3A and L14, the upper layers of peat at site F1 were dark brown to charcoal gray-black and smelled of sulfide. However, levels of porewater sulfate were not dissimilar at the two Everglades sites (Table 1) and were below detectable levels (less than 2 mg/liter) in Lake Okeechobee littoral zone site L14. Water column conductivity was relatively high at site F1 in comparison with that at site 3A (Table 1).

Site F1 exhibited significantly lower sediment redox potentials than did site 3A (Table 1). The redox potentials of site L14 were very similar to those of site 3A (data not shown). At all sites, redox potentials were lower in upper sediments. The concentrations of phosphorus (both total and dissolved) and the counterion chloride were higher in porewaters from site F1 than in those from site 3A (Table 1). P_i approximated 50 to 100% of the total dissolved phosphorus of porewaters (data not shown) (47a). The levels of organic carbon and dissolved ammonium and nitrates were similar among porewaters (Table 1).

Methane was a major component of sediment gases (Table 1); methane at site L14 was similar to that at site 3A. Nitrous oxide (N_2O) was not detected in site F1 sediment gas; it was present at levels approximating 80 ppb at site 3A (atmospheric N_2O was 324 ppb). Carbon dioxide (CO_2) constituted 9% of the sediment gas at site F1 and 6% at site 3A. Sediment gases at sites F1 and 3A both contained approximately 0.6% O_2. Nitrogen (N_2) constituted the remaining gas detected, which was 38 and 52% of the sediment gases at site F1 and 3A, respectively.

The amounts of total mercury and methylmercury detected in 0- to 10-cm Everglades sediments were similar to those amounts detected in Lake Okeechobee sediments and approximated 100 and 5 μg/kg (dry weight) of sediment, respectively. Methylmercury values obtained for 30- to 40-cm sediments approximated 0.5 μg/kg (dry weight) of sediment.

Characterization of culturable microflora. The numbers of the aerobically culturable microflora of sediments from Water Conservation Area sites F1 and 3A were similar (Table 2). In contrast, culturable microbes capable of anaerobic growth were distinctly more numerous in 0- to 10-cm sediments from site F1 than in the other sediments sampled (Table 2). Acetate producers were similar in number to or more numerous than methane producers (Table 2). Sediment at a depth of 0 to 10 cm from Water Conservation Area site F1 was particularly rich in culturable acetate and methane producers as well as sulfate reducers. The MPN values obtained for these bacteriological groups from site L14 were similar to those obtained from site 3A (data not shown).

All sediments displayed similar numbers of microbes capable of consuming H_2 under anaerobic conditions (Table 2). Sediments from Water Conservation Area sites F1 and 3A also yielded similar numbers of culturable vanillate-consuming microbes. In the vanillate-MPN analysis, CO was provided to

TABLE 2. Enumeration of the culturable microflora of Everglades sediments[a]

Site	Sediment depth (cm)	General aerobes[b]	Anaerobic growth and activities								
			General anaerobes	Acetate producers	H_2-consuming microbes	H_2-consuming "acetogens" (+ BES)[c]	Vanillate-consuming microbes	CO-consuming microbes	Methanogens	Methanogens (+ antibiotics)	Sulfate-reducing bacteria
F1	0–10	4×10^7	4×10^{11}	4×10^{11}	1×10^8	5×10^5	2×10^5	2×10^5	3×10^{11}	$>10^{12}$	4×10^{11}
	30–40	5×10^6	2×10^6	1×10^6	1×10^6	1×10^6	2×10^3	2×10^3	2×10^6	5×10^6	5×10^6
3A	0–10	5×10^7	4×10^8	5×10^7	4×10^4	2×10^6	2×10^5	2×10^3	2×10^5	5×10^5	5×10^7
	30–40	1×10^6	2×10^6	1×10^6	5×10^7	1×10^5	1×10^4	$<10^3$	—[d]	5×10^3	4×10^6

[a] Unless otherwise indicated, values are expressed in units of most probable number (MPN) per gram (dry weight) of sediment and are the average of three replicates.
[b] Values are expressed in CFU per gram (dry weight) of sediment and are the average of two replicates.
[c] BES, bromoethanesulfonate.
[d] —, methane was not detected in 10^{-5} dilution MPN tubes; lower dilutions were not evaluated.

assess the potential of culturable organisms to concomitantly consume CO (theoretically a favorable combination of substrates for acetogens [20]). Microbes capable of consuming CO were similar in number to vanillate consumers in site F1 but not site 3A (Table 2). The numbers of vanillate and CO consumers were generally less than the values obtained for H_2-consuming "acetogens" (Table 2).

With an undefined medium enriched with tryptic soy broth, glucose, and nitrate, the highest-dilution MPN tubes (of all sediments) positive for both nitrate consumption and growth did not produce N_2 but rather produced large amounts of ammonium (data not shown). This response suggested that denitrifying organisms were less culturable than nitrate-dissimilating microbes. Estimates on the culturable numbers of nitrate dissimilators yielded values (averaging 10^7 per g [dry weight] of sediment) similar to those obtained for other anaerobic groups (data not shown).

Anaerobic microcosm studies. Microcosm studies were implemented to assess process-level behavior relative to nutrient input. In these studies, methane and acetate production were used as indexes of process-level response relative to carbon and energy flow. Supplemental sulfate did not significantly alter the initial capacity of sediments to produce methane (Fig. 2) but decreased the amount of methane formed by all sediments over a 50-day incubation period (data not shown). Sulfate initially stimulated acetate production by site F1 sediment (Fig. 3B); in contrast, acetate production by site 3A and L14 sediments was not stimulated by sulfate (data not shown). Supplemental nitrate totally inhibited the initial onset of methane formation by all sediments (Fig. 2); acetate formation and accumulation were not apparent in nitrate-supplemented microcosms (Fig. 3C and data not shown). Phosphate caused a decrease in the initial amount of methane formed by all sediments but appeared to stimulate the final amount of methane formed by sediments from site 3A (Fig. 2). Phosphate also caused a stimulation in the initial production and accumulation of acetate by all sediments; acetate was subsequently consumed (Fig. 3D and data not shown).

Multiple nutrient input likely occurs from agricultural runoff, and the addition of nutrients in combination augmented the effects observed with the addition of nutrients singly (Fig. 4 and data not shown). When site F1 sediment microcosms were supplemented with sulfate in combination with nitrate or phosphate, methane production was almost completely eliminated. In contrast, although nitrate plus phosphate caused a delay in the onset of methanogenesis, significant amounts of methane were formed in the latter stages of incubation. In this regard, all combinations with nitrate had the same initial effect, i.e., the onset of methane formation was delayed. With site F1 sediments, acetate was not appreciably formed (detected) in microcosms with combined supplemental nutrients, except when sulfate was combined with phosphate (data not shown).

Origin of methane in Everglades sediments. Sediment from site F1 initially formed acetate, rather than methane, in response to H_2 when incubated at 30°C in microcosms supplemented with undefined medium (Fig. 5); the subsequent turnover of acetate appeared to yield methane. To further assess the possible relationship between acetate and methane, sediments were incubated in microcosms at reduced temperature to slow the reactions that were potentially linked to methane production (75). At 10°C, acetate accumulated and methane was not formed (data not shown). Hydrogen (H_2) inhibits acetoclastic methanogenesis (24), and acetate accumulated in H_2-supplemented, argon-gassed microcosms not supplemented with CO_2 and containing only sterile water (Fig. 6). Under these conditions, H_2 was not consumed until supplemental

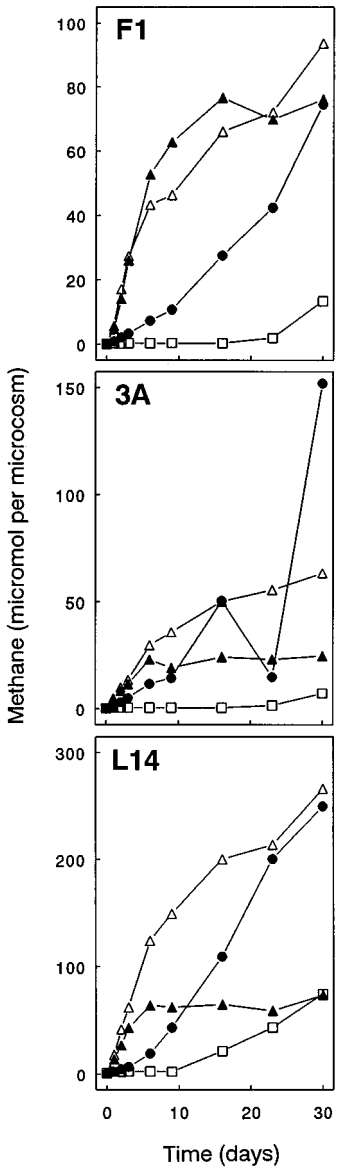

FIG. 2. Effects of supplemental sulfate (▲), nitrate (□), or phosphate (●) (5 mM each) on the production of methane by microcosms of sediments (depth, 0 to 10 cm) from field sites F1, 3A, and L14. The control (△) was unamended. For details, see description of microcosm protocol A in Materials and Methods.

CO_2 was added; the subsequent, CO_2-dependent consumption of H_2 was coupled primarily to acetate production (Fig. 6). In the initial absence of supplemental CO_2 and in the presence of H_2 to preclude acetate turnover, site F1 and 3A sediments did not display equivalent capacities to form and accumulate acetate from the turnover of endogenous matter. Collectively, these results demonstrated that (i) acetate was a major precursor of methane in Everglades sediment microcosms and (ii) site F1 sediments were dissimilar to site 3A sediments relative to the capacity to form (accumulate) acetate from endogenous matter.

DISCUSSION

The culturability of the resident microflora was used to comparatively evaluate sediments (wetland soils) of the Everglades

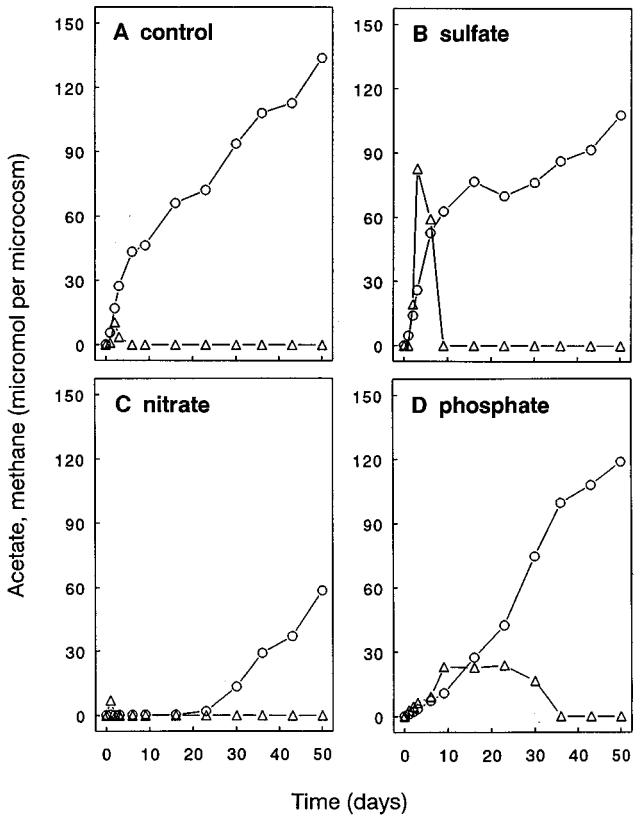

FIG. 3. Effects of supplemental sulfate, nitrate, or phosphate (5 mM each) on the production and turnover of acetate by microcosms of site F1 sediments (depth, 0 to 10 cm). Symbols: △, acetate; ○, methane. The control was unamended. For details, see description of microcosm protocol A in Materials and Methods.

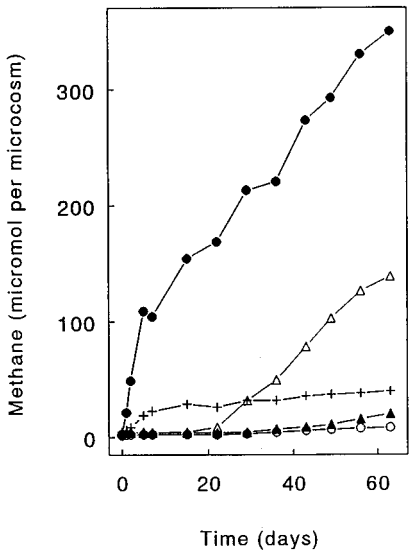

FIG. 4. Effects of combined supplemental sulfate, nitrate, and phosphate (5 mM each) on the production of methane by microcosms of site F1 sediments (depth, 0 to 10 cm). Symbols: ●, unamended (control); +, sulfate plus phosphate; ▲, sulfate plus nitrate; △, nitrate plus phosphate; ○, sulfate plus nitrate plus phosphate. For details, see description of microcosm protocol B in Materials and Methods.

Water Conservation Areas and the Lake Okeechobee littoral zone relative to in situ field parameters. Although such evaluations cannot resolve all structural and functional aspects of the microbial community, the following four general patterns were observed from the anaerobic enumerations (Table 2). (i) The highly reduced upper (0- to 10-cm) sediments of Water Conservation Area site F1 contained higher numbers of culturable anaerobes than the other sediments assessed. (ii) Values for culturable acetate producers, methanogens, and sulfate-reducing bacteria from 0- to 10-cm sediments from Water Conservation Area site F1 exceeded those of the other 0- to 10-cm sediments by a factor of 10^3 to 10^4. (iii) Sediments from a depth of 0 to 10 cm contained more culturable microbes than did sediments from a depth of 30 to 40 cm at each site. The general enrichment of many culturable bacteriological groups in the upper sediments of site F1 demonstrated that there was a general, rather than specific, enhancement of the anaerobic microflora in this sediment.

Both the Water Conservation Area and Lake Okeechobee sediments emit methane (2, 57) (Table 1); consistent with this emission, all microcosm sediments not supplemented with nutrients formed methane without apparent delay (Fig. 2 to 4). In contrast to microcosms of site F1 sediments, which formed essentially no acetate in unsupplemented microcosms, unsupplemented microcosms of site L14 sediments formed approximately 45 μmol of acetate per microcosm during the initial 10-day incubation period (data not shown). Although the underlying activities responsible for acetate production remain unresolved, acetate was a major trophic link to methane in microcosm studies. Similar observations have been made with diverse sediments and terrestrial soils incubated under anaerobic conditions (3, 37, 41–43, 48). However, such studies do not effectively account for the interspecies transfer of H_2 that might be involved in methane production in situ (60).

Sulfate concentrations were negligible in the surface sediments of site L14, and the smell of sulfide was absent from site L14 sediment cores. In contrast, site F1 cores were blackened with sulfide. Despite relatively uniform porewater sulfate con-

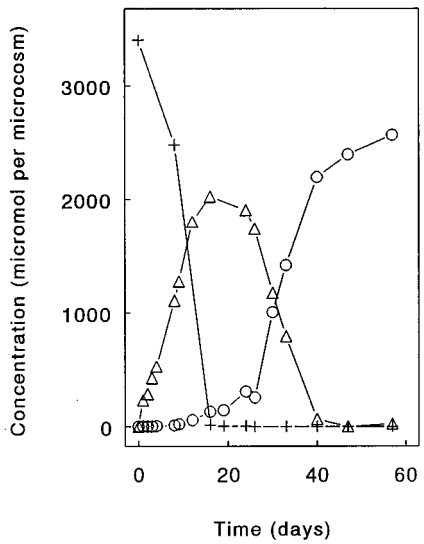

FIG. 5. Consumption of H_2 and sequential formation of acetate and methane by site F1 sediment (depth, 0 to 10 cm) in undefined medium. Symbols: +, H_2; △, acetate; ○, methane. For details, see description of microcosm protocol C in Materials and Methods.

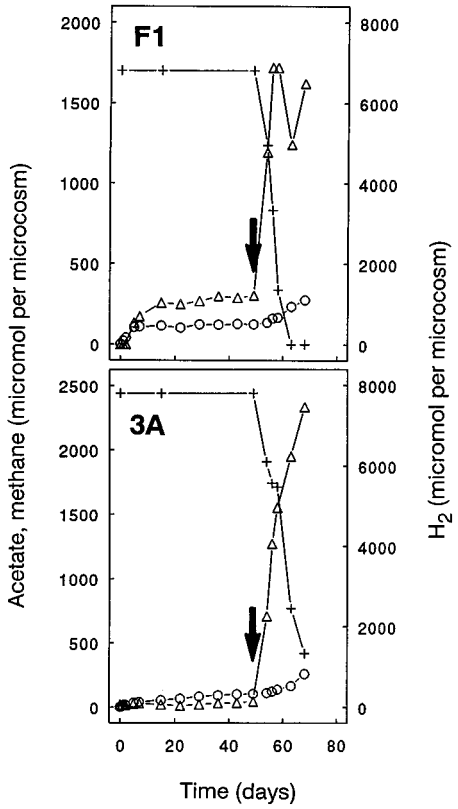

FIG. 6. Effect of CO_2 on the consumption of supplemental H_2 by microcosms of site F1 and 3A sediments. Microcosms received 35 kPa (approximately 7,000 μmol) of CO_2 at the time indicated by the arrow. For details, see description of microcosm protocol A in Materials and Methods (microcosms were not supplemented with nutrients). Symbols are defined in the legend to Fig. 5.

centrations at Water Conservation Area sites F1 and 3A, site F1 sediments contained a significantly higher number of culturable sulfate reducers. Incoming sulfate might be readily reduced in site F1 sediments and kept at low concentrations. Acetate can be a major product of certain sulfate-reducing bacteria (49); sulfate-dependent stimulation of acetate synthesis with site F1 microcosms was consistent with the possible enrichment of such sulfate-reducing bacteria. The total dissolved phosphorus concentration in porewaters of 0- to 10-cm site F1 sediment was markedly higher than that in the other sediments. Although these observations suggest that phosphorus and sulfate stimulated the productivity of sulfate reducers at site F1, further studies would be required to evaluate the extent of acetate-consuming sulfate reducers (not directly evaluated in the present study) and sulfur cycling that might influence the population distributions obtained from these sediments.

Supplemental nitrate had a very marked effect on the production of methane by all sediments tested (Fig. 2C), indicating that the resident microflora was poised to respond quickly to nitrate input. Thermodynamically, nitrate is more easily reduced than CO_2 or sulfate and is thus considered a favored electron acceptor in many anaerobic habitats (11, 74). MPN analyses demonstrated that these sediments harbored nitrate-dissimilating microbes. Denitrification is catalyzed by facultative microbes, while dissimilatory nitrate reduction is catalyzed mostly by obligate anaerobes (62, 63); thus, the anaerobicity of these low-redox-potential sediments may have selected for a predominance of dissimilatory nitrate utilizers. In this regard, the dissimilation of nitrate to ammonium accounts for the majority of nitrate turnover in Everglades sediments (29). Although similar observations have been made with other anaerobic habitats (14), recent findings indicate that manganese can uncouple assimilatory nitrate reductase activity of soil microbes, resulting in the production of ammonium (45).

Previous studies indicate that supplemental phosphate (10 mM) in Everglades peat-soil microcosms can cause trophic-level shifts that favor anaerobic processes (2). Because of the variability in physical and chemical properties of Everglades sediments as well as potentially different microfloras, supplemental phosphate does not always influence the production of methane or CO_2 by Everglades sediments in microcosm studies (1, 2, 7). In the present study, supplemental phosphate decreased the initial production of methane by all sediments evaluated. In addition, phosphate influenced the formation and turnover pattern of acetate, demonstrating that phosphorus enrichment affected not only methane production but also the underlying activities that were directly or indirectly linked to methanogenesis. Phosphorus levels and MPN values for various anaerobic groups were highest in the 0- to 10-cm porewaters of site F1 (Tables 1 and 2). The selective stimulation of certain microbial processes by either single or combined nutrient inputs may alter phosphorus cycling and, thus, the assumed phosphorus removal capacity of constructed wetlands planned for treatment of agricultural runoff (25).

The occurrence and bioavailability of mercury in sediments may be linked to the microbially mediated biotransformations (e.g., biomethylation) of this element (21, 54, 61). Although anaerobic conditions, sulfate-reducing bacteria, and cobalamin-mediated reactions are implicated in the methylation of mercury (9, 12, 13, 15, 27, 28, 33, 53), these factors are not consistently correlated to the formation of methylmercury (12, 13, 15, 54, 67). Diverse microflora, including methanogens, clostridia, and yeasts, also mediate the methylation of mercury (31, 67–70). Because both Everglades and Lake Okeechobee sediments were found to contain equivalent levels of methylmercury, the occurrence of methylmercury in sediments was not directly dependent upon either the apparent activity of sulfate reducers or low redox potentials of sediments. Certain sulfate-reducing bacteria and acetogens are capable of the reductive dissimilation of nitrate and other diverse electron acceptors (16, 20, 23, 26, 46, 47, 58), suggesting that these obligate anaerobes might engage alternative metabolic potentials under conditions that preempt their primary functions. Such alternative activities would be subject to regulation by nutrient input and may influence the capacity of these or other anaerobes to transform mercury.

ACKNOWLEDGMENTS

We thank G. Ilgen, R. Falter, J. Laing, R. Meeker, C. Hanlon, S. Newman, P. McCormick, D. Muszick, and A. Kruczynski for excellent technical and analytical assistance and S. Daniel, C. Matthies, P. McCormick, S. Newman, and A. Steinman for review of the manuscript.

This study was supported in part by the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (0339476A0) and by the South Florida Water Management District.

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