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

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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 cultivable numbers than denitrifiers. In microcosms, acetate was a major source of methane, and supplemental hydrogen was directed towards the synthesis of acetate via CO2-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 (CH4) and nitrous oxide (N2O), the products 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 \(2.8 \times 10^3\)-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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Salix Rhynchospora tracyi), beakrush (wetlands subject to similar seasonaland atmospheric influences. The emer-
depth, 2.7 m) subtropical lake centrally located in peninsular Florida (Fig. 1), and
persite.

gases were obtained by funnel trapping and overpressure injection into argon-
are the average of triplicate measurements per site depth. Emitted sediment
measured in the field with an Orion Research (Boston, Mass.) 250A meter and
with a Hydrolab (Austin, Tex.) Surveyor III. Sediment redox potential was
estimated by assessment of CFU on tryptc 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 H2-CO2 (30:70) gas phase (17); the final pH was approxi-
amately 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. Incu-
lation 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 produc-
tion of acetate in UM; MPN tubes forming acetate in excess of uninoculated
tubes were scored positive. (ii) General H2 consumers were determined by
assessing the consumption of H2 in UM; MPN tubes that consumed H2 in excess
of uninoculated controls were scored positive. (iii) H2-consuming "acetogens"
were determined by supplementing UM with 20 mM bromothymol blue (an
inhibitor of methanogens) (59) and assessing the simultaneous consumption of
H2 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 over-
pressure CO2 tubes that consumed vanillate or CO in excess of uninoculated
controls were scored positive. (v) Methanogens were determined by the produc-
tion of CH4 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 H2 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, Dieti-
kon, Switzerland).

(i) Protocol A. To assess the effects of single nutrient input on the anaerobic microflora of sediments, 125 ml microcosms containing 1 mg (wet weight) of
sediment (0 to 10 cm) and 25 ml of sterile anaerobic distilled water were
supplemented with Na2SO4, NaNO3, or Na3PO4 as indicated to a final concen-
tration of 5 mM (shown to not appreciably alter the initial pH of the micro-
ocosms). A headspace of 30% CO2-70% N2 was provided for both anaerobic
bacteria (i.e., reducers, and a H2-CO2 (30:70) gas phase (17); the final pH was approxi-
mately 6.8. This medium is referred to as UM. Crimp-sealed, serum-stoppered
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prepared and inoculated anaerobically, were used in all MPN analyses. Incu-
lation 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.

(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 Na2SO4, NaNO3, or Na3PO4
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 CO2 on the capacity of sediments to con-
sume H2.

(iii) Protocol C. To assess the turnover of H2 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
H2-CO2 (1:2) gas phase at approximately 50 kPa of overpressure. For all proto-
cols, microcosms were incubated horizontally at 30°C without shaking.

Characterization of the culturable microflora. To minimize the exposure of
the anaerobic microflora to O2, sediments were handled inside a Mecaplex
(Grenchen, Switzerland) anaerobic chamber (100% N2 gas phase), and the
dilution series for all population estimates were performed by anaerobic tech-
niques (35). The sterile, anaerobic mineral solution used for the dilution series
contained the following (in grams per liter): K2HPO4, 0.225; KH2PO4, 0.225;
(NH4)2SO4, 0.45; NaCl, 0.45; MgSO4·7H2O, 0.045; Na2CO3, 4.0; and cysteine-
HCl, 1.0 g (reducing agent). The headspace was 100% argon from a 0.1% stock solution. The mineral solution was prepared and dispersed
under a 100% CO2 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 manipula-
tions were performed anaerobically at room temperature. The cultivation tem-
perature for all enumerations was 30°C.

Microcosms were incubated under a 95% N2-5% CO2 mixture for 20 days and
anaerobic microorganisms were enumerated from the microcosms. A 250 ml of
each microcosm was added to a 500 ml serum-stoppered bottle and incubated as
indicated. Titrations were performed anaerobically at room temperature. The cultivation tem-
perature for all enumerations was 30°C.

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 O2 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.
The counterion chloride were higher in pore waters from site F1.

Concentrations of phosphorus (both total and dissolved) and all sites, redox potential were lower in upper sediments. The comparison with that at site 3A (Table 1).

Water column conductivity was relatively high at site F1 in Lake Okeechobee littoral zone (less than 2 mg/liter) in Lake Okeechobee littoral zonesite two Everglades sites (Table 1) and were below detectable levels. However, levels of porewater sulfate were not dissimilar at 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. The porewater sulfates of porewaters at sites 3A and L14, the upper layers of peat at 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 0- 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 microorganisms 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 0- to 40-cm sediments approximated 0.5 µg/kg (dry weight) of sediment.

Sediments from Water Conservation Area sites F1 and 3A also yielded similar numbers of culturable vanillate-consuming microorganisms. In the vanillate-CPN analysis, CO was provided to

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 (N2O) was not detected in site F1 sediment gas; it was present at levels approximating 80 ppb at site 3A (atmospheric N2O was 324 ppb). Carbon dioxide (CO2) 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% O2. Nitrogen (N2) 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.

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). F1, approximated 50 to 100% of the total dissolved phosphorus of porewaters (data not shown) (47a). The levels of organic carbon and dissolved ammonium and nitrate were relatively uniform and approximated 6.55 and 8.0, respectively. NO3 − is nitrate plus nitrite.

C org, organic carbon.

### Results

#### Physical and chemical characteristics of sites.

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### Table 1. Analysis of water columns, sediments, and porewaters of study sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Water column conductivity (µS cm−1)</th>
<th>Sediment</th>
<th>Porewater (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Depth (cm)</td>
<td>Redox (mV)</td>
<td>Dry wt (%)</td>
</tr>
<tr>
<td>F1</td>
<td>1,212</td>
<td>0−10</td>
<td>−302</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30−40</td>
<td>−220</td>
</tr>
<tr>
<td>3A</td>
<td>402</td>
<td>0−10</td>
<td>−94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30−40</td>
<td>−64</td>
</tr>
</tbody>
</table>

* The pH values of the water columns and porewaters were relatively uniform and approximated 6.55 and 8.0, respectively. NO3− is nitrate plus nitrite.

* Corg, organic carbon.

### Table 2. Enumeration of the culturable microflora of Everglades sediments

<table>
<thead>
<tr>
<th>Site</th>
<th>Sediment depth (cm)</th>
<th>General aerobic²</th>
<th>General anaerobes⁴</th>
<th>Acetate producers</th>
<th>H2-consuming microbes</th>
<th>H2-consuming “acetogens” (BES)⁴</th>
<th>Vanillate-consuming microbes</th>
<th>CO-consuming microbes</th>
<th>Methanogens</th>
<th>Methanogens (antibiotics)</th>
<th>Sulfate-reducing bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0−10</td>
<td>4 × 10⁷</td>
<td>4 × 10¹⁰</td>
<td>4 × 10⁷</td>
<td>1 × 10⁸</td>
<td>5 × 10⁵</td>
<td>2 × 10⁵</td>
<td>3 × 10¹⁰</td>
<td>&gt;10¹²</td>
<td>4 × 10¹¹</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30−40</td>
<td>5 × 10⁶</td>
<td>2 × 10⁷</td>
<td>1 × 10⁶</td>
<td>1 × 10⁶</td>
<td>1 × 10⁵</td>
<td>2 × 10⁸</td>
<td>2 × 10⁸</td>
<td>5 × 10⁶</td>
<td>5 × 10⁸</td>
<td></td>
</tr>
<tr>
<td>3A</td>
<td>0−10</td>
<td>5 × 10⁶</td>
<td>4 × 10⁷</td>
<td>5 × 10⁶</td>
<td>4 × 10⁶</td>
<td>2 × 10⁷</td>
<td>2 × 10⁸</td>
<td>2 × 10⁸</td>
<td>5 × 10⁶</td>
<td>5 × 10⁸</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30−40</td>
<td>1 × 10⁶</td>
<td>2 × 10⁷</td>
<td>5 × 10⁶</td>
<td>1 × 10⁷</td>
<td>1 × 10⁵</td>
<td>2 × 10⁸</td>
<td>1 × 10⁵</td>
<td>&lt;10³</td>
<td>5 × 10⁸</td>
<td></td>
</tr>
</tbody>
</table>

* 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.

* Values are expressed in CFU per gram (dry weight) of sediment and are the average of two replicates.

* BES, bromoethanesulfonate.

* 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 H2-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 N2 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 107 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 H2 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 (H2) inhibits acetoclastic methanogenesis [24], and acetate accumulated in H2-supplemented, argon-gassed microcosms not supplemented with CO2 and containing only sterile water (Fig. 6). Under these conditions, H2 was not consumed until supplemental CO2 was added; the subsequent, CO2-dependent consumption of H2 was coupled primarily to acetate production (Fig. 6). In the initial absence of supplemental CO2 and in the presence of H2 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...
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 10³ to 10⁴. (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 H₂ 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-
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 CO₂ 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 microbiologically mediated biotransformations (e.g., biomethylation) of this element (21, 54, 61). Although anaerobic conditions, sulfate-reducing bacteria, and cobaltammediated 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.

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REFERENCES


