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Methyl-Mercury Degradation Pathways: A Comparison among Three Mercury-Impacted Ecosystems

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We examined microbial methylmercury (MeHg) degradation in sediment of the Florida Everglades, Carson River (NV), and San Carlos Creek (CA), three freshwater environments that differ in the extent and type of mercury contamination and sediment biogeochemistry. Degradation rate constant (k_{deg}) values increased with total mercury (Hg_t) contamination both among and within ecosystems. The highest k_{deg}'s (2.8−5.8 d^{-1}) were observed in San Carlos Creek, at acid mine drainage impacted sites immediately downstream of the former New Idria mercury mine, where Hg_t ranged from 4.5 to 21.3 ppm (dry wt). A reductive degradation pathway (presumably *mer*-detoxification) dominated degradation at these sites, as indicated by the nearly exclusive production of $^{14}CH_4$ from ^{14}C-MeHg, under both aerobic and anaerobic conditions. At the upstream control site, and in the less contaminated ecosystems (e.g. the Everglades), k_{deg}'s were low ($\leq$0.2 d^{-1}) and oxidative demethylation (OD) dominated degradation, as evident from $^{14}CO_2$ production. k_{deg} increased with microbial CH_4 production, organic content, and reduced sulfur in the Carson River system and increased with decreasing pH in San Carlos Creek. OD associated CO_2 production increased with pore-water SO_4^{2-} in Everglades samples but was not attributable to anaerobic methane oxidation, as has been previously proposed. This ecosystem comparison indicates that severely contaminated sediments tend to have microbial populations that actively degrade MeHg via *mer*-detoxification, whereas OD occurs in heavily contaminated sediments as well but dominates in those less contaminated.

Introduction

Methylmercury (MeHg) is a heavy metal organo-toxin formed primarily by sulfate reducing bacteria in anoxic sediments (*1, 2*). Due to concerns regarding its bioaccumulation in aquatic food chains, much attention has been focused on factors controlling MeHg production under various environmental conditions (*3*−*8*). The balance of MeHg production and degradation ultimately controls MeHg concentration, yet comparatively little attention has been given to the degradation process (*9*), which may proceed by a number of abiotic and biotic pathways in the natural environment. Abiotic pathways include photodegradation (*10*) and the reaction with sulfide to form dimethylmercury (Me_2Hg) and HgS (*11*).

Biotic degradation also takes a number of forms. The most thoroughly researched involves mercury resistance in bacteria possessing genes of the *mer*-operon ((*12*−*14*) and references therein). This capacity appears widespread in nature and has been found for both gram negative and gram-positive bacteria and under aerobic and anaerobic conditions (*15*−*17*). The *mer*-operon can be carried on plasmids and other transposable elements and transferred among different bacteria species. "Broad-spectrum" resistance refers to the ability of bacteria to detoxify both inorganic Hg(II) and organomercurials, including MeHg. This contrasts with "narrow-spectrum" resistance, in which only Hg(II) detoxification occurs. The transcription of the specific detoxification and transport proteins is regulated by an organomercurial-responsive MerR protein in the first case and by a Hg(II) responsive regulatory MerR in the second case. Unique to broad-spectrum resistance is the *mer*-B gene that encodes for the organomercurial-lyase enzyme, which cleaves MeHg, forming CH_4 and Hg(II) as end-products. The associated *mer*-A gene, common in both resistance types, produces the enzyme mercuric reductase, which further reduces Hg(II) to volatile elemental Hg^0 (*18*). In this way, broad-spectrum mercury resistant microbes are able to detoxify MeHg by converting it to a form that may readily evade from the immediate environment.

An alternative anaerobic, non-*mer*-mediated, degradation pathway has been demonstrated for the sulfate reducing bacteria *Desulfovibrio desulfuricans* (*19*), where 2 mol of MeHg react with microbially produced sulfide to form an unstable dimethylmercury sulfide $(MeHg)_2S$ intermediate, which decomposes to Me_2Hg and HgS, as in the abiotic pathway above. Me_2Hg is then degraded to MeHg and CH_4. Thus, the production of CH_4 from MeHg is common to both of the above reductive demethylation (RD) pathways. It is unknown if the non-*mer* RD pathway is induced or regulated by ambient MeHg concentrations, as is *mer*-detoxification. However, genes regulating the production of sulfide and the degradation of MeHg were shown to be located on the same plasmid in *Clostridium cochlearium* T-2 (*20*), and it has been proposed that the MeHg degradation pathway in *C. cochlearium* and *D. desulfuricans* is one and the same (*21*).

Reports of CO_2 as a major bacterial end-product of MeHg degradation in anaerobic sediments led to the proposal of an oxidative demethylation (OD) pathway, which has since been demonstrated in freshwater, estuarine, and alkaline-hypersaline sediments (*22*−*24*). OD is thought to represent a cometabolism of MeHg analogous to the metabolism of other small organic substrates (e.g. C_1 compounds) by heterotrophic bacteria and as such does not represent an active detoxification response. Sulfate reducing, methanogenic, and aerobic bacteria have all been implicated in this pathway. While the production of CO_2 from MeHg is what defines OD, the production of both CO_2 and CH_4 via OD is also possible and would be analogous to the production of both end-products in the degradation of methanol or

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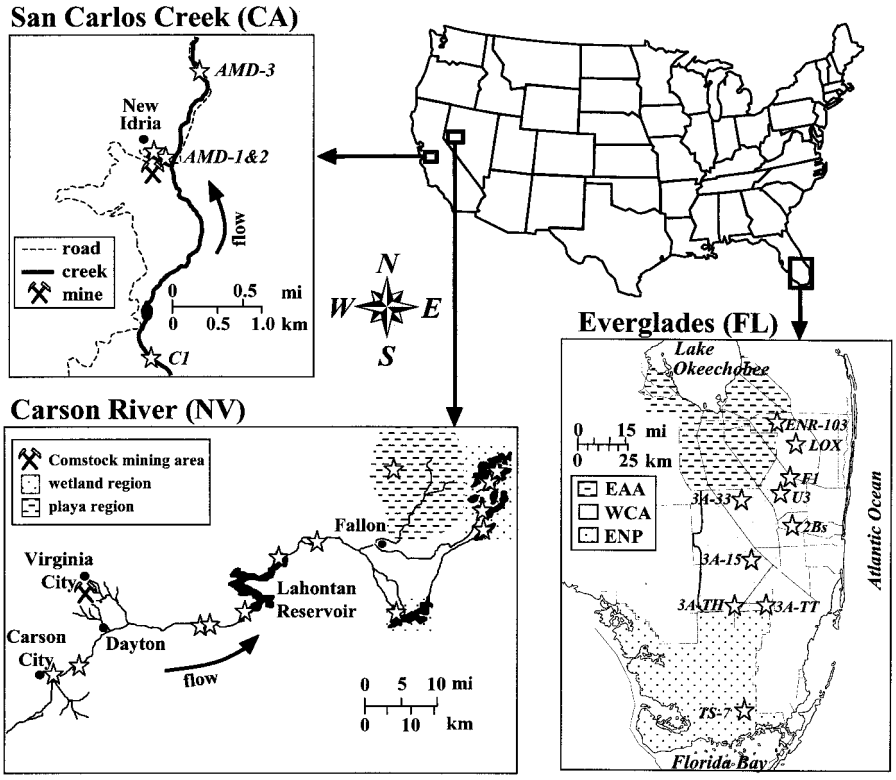

FIGURE 1. Maps of the San Carlos Creek (CA) [a], Carson River (NV) [b], and Everglades (FL) [c] ecosystems, with sampling sites given as (☆) and towns/cities as (●). Part [c] includes the Everglades Agricultural Area (EAA), Water Conservation Areas (WCA), and Everglades National Park (ENP).

monomethylamine by methanogens (*23*, *24*). In contrast, no CO_2 is formed from MeHg by either of the two RD pathways. Recently however, the evidence for OD has come into question. It has been suggested that the formation of $^{14}CO_2$ from ^{14}C-MeHg degradation experiments may simply reflect *mer*-detoxification followed by anaerobic $^{14}CH_4$ oxidation to $^{14}CO_2$ (*25*).

The specific environmental factors that control the relative importance of these biotic pathways in a particular system are largely unknown, although MeHg and/or Hg(II) concentration are likely important. Since *mer*-detoxification of MeHg is induced by the presence of the substrate, some threshold concentration is needed to induce transcription (*26*). A similar threshold concentration might not be necessary for OD to occur if this pathway represents a cometabolism of MeHg. We hypothesize that OD dominates at low in-situ MeHg concentrations, when induction of *mer*-degradation is minimal. It is also unknown if Hg(II) reduction to Hg^0 occurs under conditions favoring OD. If this capacity is lacking, an extended residence time for Hg might be predicted in systems where OD dominates. Thus, our limited understanding of the OD pathway and its potential importance in the global Hg cycle has led us to further investigate this process in various ecosystems throughout the past decade. We have observed OD in all systems investigated to date and focus here on the three most intensively studied. The Florida Everglades represents a moderately contaminated system with a nonpoint-source atmospheric Hg input, whereas San Carlos Creek (CA) and Carson River (NV) exhibit significantly higher Hg levels owing to ongoing point-source and historic regional contamination, respectively. Here we attempt to decipher the relative importance of these various microbial pathways under different environmental conditions by comparing MeHg degradation dynamics, in terms of CH_4 and CO_2 end-products, both within and among these three very different ecosystems. We also directly test the hypothesis that anaerobic CH_4 oxidation can account for the current and previous reports of OD.

Methods

Sites and Field Sampling. Mercury loading to the Florida Everglades is primarily in the form of atmospheric deposition (*27*), with sediment total mercury (Hg_t) concentrations typically 0.1−0.5 ppm (dry wt) (*28*). Surface sediment was collected from ten Everglades wetland sites (Figure 1) during December 1996, July 1997, January 1998, and June 1998, as part of the South Florida Aquatic Cycling of Mercury in the Everglades (ACME) project (*29*, *30*). Sample depth varied from the top 0−4 cm (July 1997 and June 1998) to the complete unconsolidated surface floc layer (top 4−10 cm; Dec 1996 and Jan 1998). These sites represent a 130-km north−south transect along an eutrophication gradient, stemming from high phosphate inputs from the Everglades Agricultural Area (EAA) (*31*). Sites were located in the Water Conservation Areas (WCA), the experimental Everglades Nutrient Removal (ENR) zone, and the more pristine Everglades National Park (ENP). Specific site descriptions have been given elsewhere (*24*, *32*, *33*).

The Carson River U.S. EPA Superfund site, in western Nevada, was originally contaminated during the mid- to late 1800s with elemental Hg^0 used in the processing of gold and silver ores associated with the Comstock load. The river flows northeast and drains into a desert/wetland evaporation basin at the terminus. Historic Hg inputs are from smaller tributaries originating near Virginia City and from a major amalgamation facility near Dayton. Due to the reworking and mobilization of these sediments over the past century, downstream locations currently have benthic Hg_t concentrations as high as several hundred ppm (*34*). Sediment samples (0−4 cm) were collected during October 1998 at 13 sites, over a 100-km stretch from Carson City to the wetlands

TABLE 1. Conditions Used for Methylmercury (MeHg) Degradation Measurement via $^{14}CH_3HgI$ Incubation and CT-LSC Detection of Gaseous ^{14}C End-Products

sample set	date	holding time prior to assay (d)	incubation duration (h)	^{14}C-MeHg added (nCi*cc sed^{-1})	total MeHg added (ng Hg*cc sed $^{-1}$)
Carson R. (NV)	Oct 1998	93−95	24	14	52
Everglades (FL)	Dec 1996	<0.3	22−28	0.5	2
Everglades (FL)	July 1997	<0.3	7−8	2−4	7−15
Everglades (FL)	Jan 1998	<0.3	6−12	2−3	7−11
Everglades (FL)	June 1998	<0.3	6−8	3	11
San Carlos Creek (CA)	Oct 1997	14	20	3	11
San Carlos Creek (CA)	Jan 1999	30	23	2−3	7−11

region. Sites were categorized into five types depending on location and major features (*n* given in [ ]): river [5], Lahontan reservoir [2], agricultural drain [2], wetland [3], and playa [1]. Descriptions of the Carson River and associated wetlands are given elsewhere (*35*, *36*).

San Carlos Creek (SCC), located in the Diablo Mountain range of central California, intersects the former New Idria mercury mine, which operated for 118 years (1854−1972) and was the second largest producer of elemental Hg^0 in North America (*37*). The creek is impacted both by acid mine drainage (AMD) and mercury contamination from unprocessed cinnabar (HgS) ore and roasted-ore waste. Surface sediment (0−4 cm) was initially sampled in October 1997 at a non-AMD control site (C-1) located 3.2 km upstream of the mine and at an AMD site (AMD-3) located 1.2 km downstream of the mine. A second sampling in January 1999 included the two previous sites plus two additional sites associated with a short (<0.2 km) feeder stream from the New Idria mine flowing into SCC. AMD-1 was located directly in front of the mine, where subsurface AMD emerges as surface flow. AMD-2 was 0.1 km downstream, adjacent to a settling pond at the base of large roasted-ore waste pile. Detailed site descriptions are given elsewhere (*37*, *38*).

Sediment collection for microbial assays was conducted by hand with acid cleaned polycarbonate core tubes in all ecosystems. Holding times prior to the initiation of ^{14}C-MeHg incubations varied from <0.3 to 95 days (Table 1). When incubations were not initiated within a few hours of sample collection, sediment was stored at 5 °C in completely filled acid-cleaned mason jars until further analysis.

Sediment Assays. Sediment was subsampled (3 cm^3) into 13 cm^3 serum vials, which were crimp sealed and flushed with O_2-free N_2 gas. Radiolabeled MeHg (as $^{14}CH_3HgI$) was added (2−42 nCi*100 μL^{-1}) to each. The final ^{14}C-MeHg amendment levels (2−52 ng Hg*cm^{-3} wet sed or 15−2400 ppb dry wt, median = 134 ppb) were higher than in-situ MeHg (<10 ppb dry wt) for these systems (*28*, *39*). Samples were vortexed (30 s) and incubated in the dark at room temperature (17−22 °C) for 6 to 28 h (Table 1). Incubations were arrested with the addition of 1 mL of NaOH (3 N). Each site/depth sample set was replicated ($n = 2-3$) and included one autoclaved killed control. A high specific activity ^{14}C-MeHg stock (54 mCi*$mmol^{-1}$, Amersham Corp., Arlington Heights, IL) was used in all investigations, and ^{14}C-end-products were quantified by a CH_4 combustion and CO_2 trapping technique, followed by liquid scintillation counting (CT-LSC) (*24*). The serum bottle headspace was first flushed with commercial air (35−30 mL*min^{-1} for 15 min), while vortexing, to drive off $^{14}CH_4$. This end-product was combusted to $^{14}CO_2$ in an inline furnace (850 °C, using a CuO catalyst) and subsequently trapped in a solution of 8 mL of methanol and 3 mL of monoethanolamine. Nearly 100% $^{14}CH_4$ extraction efficiency achieved by twice amending the sample with 1 mL of pure unlabeled CH_4 during the flushing period. Samples were subsequently acidified with 1 mL of 6 M HCl to convert base-fixed aqueous $^{14}CO_3^{2-}$ to gaseous $^{14}CO_2$, which was then flushed from the bottle using N_2 and similarly trapped as above in a new CO_2-trap. Pure nonlabeled CO_2 was also twice added (1 mL) to samples during this second flushing step to facilitate the removal of $^{14}CO_2$ from the original sample. Scintillation cocktail (ScintiVerse II, Fisher Scientific) was added to all $^{14}CO_2$ traps, and samples were counted by LSC.

Pseudo-first-order MeHg degradation rate constant (k_{deg}) values were calculated as $k_{deg} = -\ln(1-f)*time^{-1}$, where f was the fraction of added ^{14}C-MeHg degraded to $^{14}CH_4 + {}^{14}CO_2$ (kill corrected). The relative amount of $^{14}CO_2$ produced was expressed as the percentage of total gaseous end-products recovered (henceforth called $\%^{14}CO_2$) and was calculated from kill-corrected data as $\%^{14}CO_2 = [{}^{14}CO_2/({}^{14}CH_4+{}^{14}CO_2)]*100$. While most ^{14}C-MeHg incubations consisted of only one time point (Table 1), multipoint time courses (20−120 h) were also conducted at selected sites from each ecosystem. In these cases, k_{deg} was calculated from the slope of the initial linear portion of each [%MeHg degraded versus time] curve. All determinations of statistically significant relationships were based on the $P < 0.05$ criteria for the slope of a linear model.

Methane ($^{14}CH_4$) oxidation was assessed in parallel with ^{14}C-MeHg degradation in all three ecosystems. Samples from SCC in 1997 were amended with $^{14}CH_4$ (5 nCi*250 μL^{-1}, sp. act. = 56 mCi*$mmol^{-1}$, purity = 97.5%, Amersham Corp.) and incubated 20 h, under both aerobic and anaerobic (static) conditions. Everglades (January 1998) sediment samples (3 cm^3) were amended with $^{14}CH_4$ (6 nCi*100 μL^{-1}, added to the gas phase of sealed vials) and incubated statically (no shaking) for 6−12 h, under both aerobic and anaerobic conditions. Carson River samples were slurried (3 cm^3 sediment plus 1 mL of anoxic DI water), amended with $^{14}CH_4$ (15 nCi*250 μL^{-1}), and incubated on a gyrating shaker table (150 rpm) under anaerobic conditions (only) for 45 h. Radiotracer $^{14}CH_4$ (sp. act. = 21.1 mCi*$mmol^{-1}$), used for Everglades and Carson River experiments, was obtained from and originally produced in the laboratory of B. Ward (UC Santa Cruz, CA) from methanogenic cultures incubated with $H^{14}CO_3^-$ (personal communication). $^{14}CH_4$ and $^{14}CO_2$ was subsequently quantified by the CT-LSC method in all cases.

Sediment Hg_t was quantified using acid digestion, Sn-reduction, gold trapping, and cold vapor atomic fluorescence spectrophotometry (CVAFS) detection (*40, 41*). MeHg was assayed by distillation (*42*), aqueous phase ethylation, G−C separation, and CVAFS detection (*43*). Assays were conducted at the following three institutions: Everglades samples—Academy of Natural Sciences (St. Leonard, MD), Carson River samples—USGS (Madison, WI), and New Idria samples—USGS (Menlo Park, CA). All institutions used similar equipment and assay conditions.

As measures of organic content, dry sediment samples were subject to weight loss on ignition (LOI) analysis (*44*) (all three systems) and to particulate carbon (PC) analysis (Carson River only) measured with a Carlo Erba elemental analyzer (Model 1500). Sediment pH was measured (Carson River and SCC only) by inserting a pH electrode (Cole-Parmer, model 59002-72) directly into homogenized sediment. Acid-volatile-

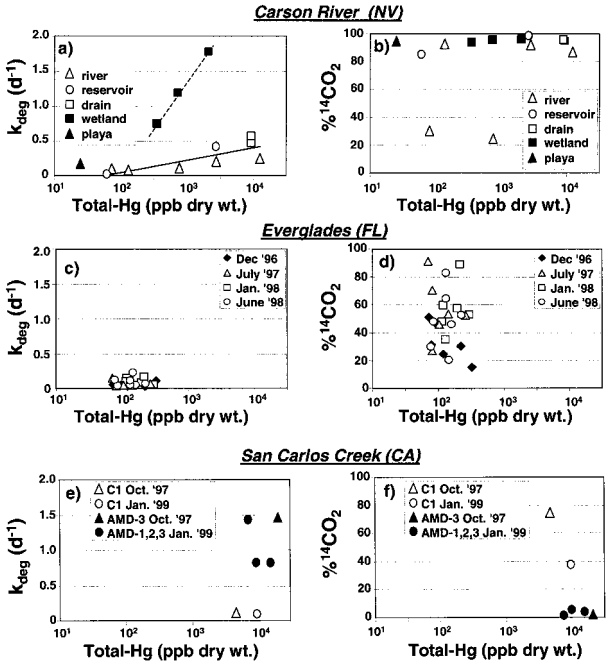

FIGURE 2. Log-linear plots of MeHg degradation rate constant (k_{deg}) [a,c,e] and $\%^{14}CO_2$ end-product [b,d,f] versus Hg_t concentrations in sediment from the Carson River (NV) 1998 [a,b], Everglades (FL) 1996−1998 [c,d], and San Carlos Creek (CA) 1997/1999 [e,f]. Carson River data is grouped by ecosystem zone, Everglades data by sampling date, and San Carlos Creek data by region and sampling date. Least-squares regression line and associated r^2 is given in [a] for data grouped by either wetlands or all other sites (excluding playa).

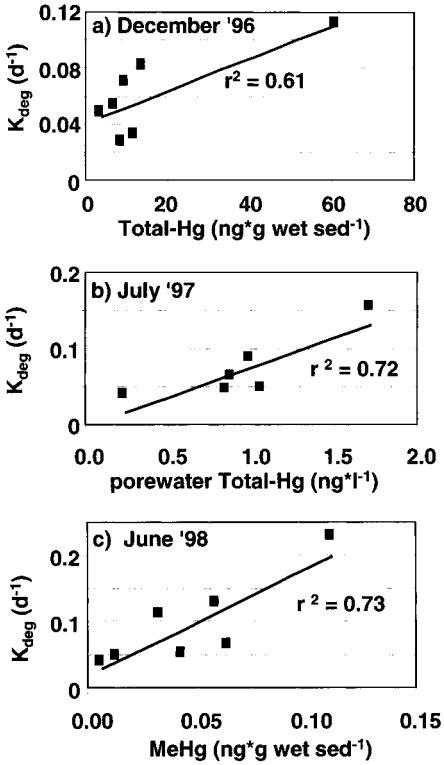

FIGURE 3. Significant linear regressions of MeHg degradation rate constant (k_{deg}) values versus various mercury pools in the Everglades data set. Depth intervals for k_{deg} data were variable (top 4 to 10 cm) during December 1996 and 0−4 cm during both July 1997 and June 1998. The corresponding mercury pool concentrations represent the 0−4 cm average depth interval in all cases.

sulfur (AVS) in Carson River samples was determined spectrophotometrically (*45*) after zinc-acetate trapping of H_2S from acidified whole sediment (*46*). AVS was determined similarly (*47*) in Everglades samples. Pore-water from Carson River sediment was collected under anaerobic conditions via centrifugation and was assayed for SO_4^{-2} via ion-chromatography (*48*) and for free sulfide (*45*). Everglades pore-water was collected by direct filtration of whole sediment or by using an in-situ interstitial pore-water sampler, with free sulfide analyzed using an ion-specific electrode (*28*). Methanogenesis in Carson River samples was measured as the net CH_4 production, over 7 days, quantified by gas chromatography with flame ionization detection. Methanogenesis in Everglades (1997) samples was measured as the conversion of radiolabeled $H^{14}CO_3^-$ (spec. act. = 54.4 mCi*mmol^{-1}; ICN Biomedicals, Irvine, CA) to $^{14}CH_4$, quantified via gas chromotography with gas proportional counting detection (*22*).

Results

Values of k_{deg} increased with increasing Hg_t (20−12 700 ppb dry wt) in the Carson River (Figure 2a). Two distinct regional groupings were observed, with the wetland sites exhibiting a stronger MeHg degradation response to Hg_t than river, reservoir, and agricultural drain sites (combined). All sites exhibited >80% $^{14}CO_2$, except two river sites which were 20−30% (Figure 2b). Everglades Hg_t concentrations (Figure 2c−d) fell into a low and narrow range (70−320 ppb dry wt) compared to the Carson River. Everglades k_{deg}'s were consistently low (0.03−0.23 d^{-1}) and similar to previously measured values (*24*). The $\%^{14}CO_2$ ranged from 15 to 92%. Neither k_{deg} nor $\%^{14}CO_2$ varied as a function of log[Hg_t] in the complete Everglades data set. However, significant relationships between k_{deg} and specific mercury fractions (e.g. bulk sediment Hg_t and MeHg, pore-water Hg_t) were found when individual sampling dates were analyzed (Figure 3a−c), although, specific results were not consistent among all dates.

SCC Hg_t levels were very high at both the control site (4800−9600 ppb dry wt) upstream and AMD sites (4500−21 300 ppb dry wt) downstream of the New Idria mercury mine (Figure 2e−f). A consistent spatial trend of low k_{deg} (≤0.1 d^{-1}) and high $\%^{14}CO_2$ (37−74%) upstream of the mine, and high k_{deg} (0.8−1.5 d^{-1}) and minimal $\%^{14}CO_2$ (1−4%) below the mine, was observed for both sampling dates.

Time course k_{deg}'s (Figure 4a−g) ranged from 0.017 d^{-1} for the modestly contaminated Everglades ENR-103 site, to 5.8 d^{-1} for the severely contaminated SCC AMD-3 site. This latter value was significantly larger than 1.5 d^{-1} depicted in Figure 2e for the same site and date. The lower value was calculated using the single 20-h data point so as to be comparable with the k_{deg} for January 1999 SCC, which was based on a single-point (22 h) incubation. The nonlinear time courses (Figure 4a,e,g) point out the potential for the underestimation of k_{deg} when calculated from a single time point, particularly from a prolonged incubation. MeHg degradation was slow (<0.1 d^{-1}) and increased linearly with time for both Everglades sites and SCC site C1. In contrast, after an initial rapid rate (0.45 d^{-1}), MeHg degradation slowed over time at Carson River site F1. A similar, but more pronounced, rapid initial degradation followed by a much slower rate was observed for SCC AMD-3 sediment, under both oxic and anoxic incubation conditions. The $\%^{14}CO_2$ was high (≥40%) and remained relatively constant over time for Carson River F1, Everglades ENR-103, and SCC C1 (anaerobic). There was a distinct decrease in $\%^{14}CO_2$ with time at both Everglades 3A-15 and SCC C1 (aerobic). Very little (<0.05%) $^{14}CO_2$ was produced in both aerobic and anaerobic SCC AMD-3 time courses.

Carson River k_{deg}'s increased with a number of biogeochemical parameters associated with the transition from

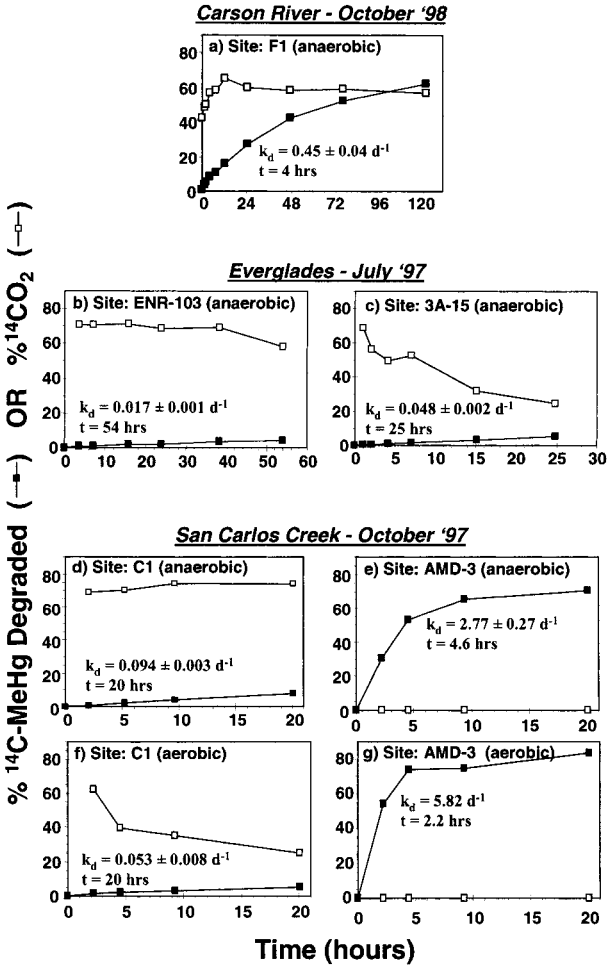

FIGURE 4. Time course experiments: percent MeHg degraded (closed square) and percent $^{14}CO_2$ end-product (open square) versus time for Carson River (NV), site F1 [a]; Everglades (FL), sites ENR-103 and 3A-15 [b,c]; and San Carlos Creek (CA), sites C1 and AMD-3 [d−g]. Degradation rate constants (k_{deg}'s) are calculated from the initial linear portion of each % degradation versus time plot. The maximum time point used for each regression is noted in each case, as are aerobic or anaerobic incubation conditions.

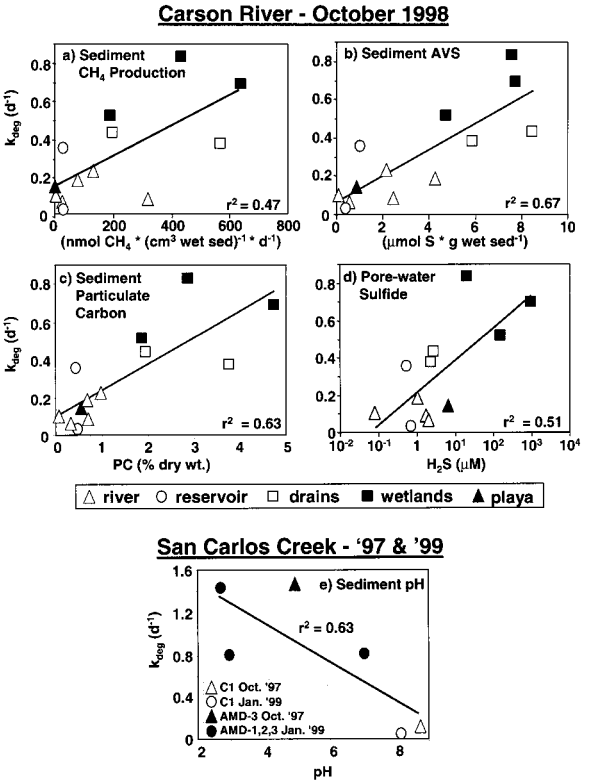

FIGURE 5. Significant linear regressions of biogeochemical variables (CH_4 production, acid-volatile-sulfur (AVS), particulate carbon (PC), pore-water sulfide (H_2S), and sediment pH) versus MeHg degradation rate constants (k_{deg}) for 0−4 cm depth interval sediment in the Carson River (NV) 1998 [a−d], and San Carlos Creek (NV) 1997/1999 [e].

low-organic river to comparatively high-organic wetland sediments, including methanogenesis rate, sediment PC and AVS, pore-water sulfide (Figure 5a−d), and LOI (not shown, similar to PC graph 5c). These parameters did not covary with Hg_t (data not shown). In contrast, no significant relationships were found between k_{deg} and methanogenesis, AVS, or sulfide in the Everglades data (no PC data). A weak negative relationship with LOI was seen for July 1997 but was heavily weighted by a single data point (not shown). No relationship between k_{deg} and LOI was seen for SCC (not shown), although degradation increased with decreasing sediment pH, which ranged from 8.1 to 8.7 at C1 and from to 2.6 to 7.1 at the AMD sites (Figure 5e). No significant relationship between pH and k_{deg} was observed for the Carson River data, where pH varied over a much narrower range (6.9−8.2).

Methane oxidation was investigated to determine if this microbial process could account for any of the $^{14}CO_2$ production routinely observed for ^{14}C-MeHg degradation experiments. In four Everglades sediments, 48−69% $^{14}CO_2$ was produced from ^{14}C-MeHg under anaerobic conditions, whereas only 0−4% $^{14}CO_2$ was produced from $^{14}CH_4$ during contemporaneous incubations (Table 2). In contrast, 9−23% $^{14}CH_4$ oxidation was observed in aerobic samples from the two sites. A corresponding increase in the $\%^{14}CO_2$ from ^{14}C-MeHg was also observed under aerobic conditions, although the total amount of MeHg degraded decreased slightly in both cases. In a similar set of parallel incubations (anaerobic only), no $^{14}CO_2$ was produced from $^{14}CH_4$ (detection limit ca. 0.1%) at any of 13 Carson River sites (not shown), while end-product $\%^{14}CO_2$ from ^{14}C-MeHg ranged from 24 to 98% (Figure 2b). Finally, no $^{14}CH_4$ oxidation was observed at either SCC (1998) site under either aerobic or anaerobic conditions, during a 20-h incubation (not shown). A positive relationship between $\%^{14}CO_2$ and pore-water SO_4^{2-} was observed in three of the four Everglades sampling dates (Figure 6), although, a similar relationship was not evident in the Carson River data.

TABLE 2. Parallel ^{14}C-MeHg Degradation and $^{14}CH_4$ Oxidation Experiments Conducted under Both Anaerobic and Aerobic Conditions with Florida Everglades Whole Sediment (Jan 1998)[a]

site	MeHg degradation k_{deg} (d^{-1})	MeHg end-product $\%^{14}CO_2$	$^{14}CH_4$ oxidation to $^{14}CO_2$ (%)
	Anaerobic Incubations		
LOX	0.06 (0.01)	53 (7)	0.8 (0.8)
TS-7	0.11 (0.01)	48 (5)	0.2 (0.1)
2Bs	0.09 (0.02)	57 (23)	4.1 (1.6)
ENR-103	0.07 (0.01)	69 (8)	0.0
	Aerobic Incubations		
LOX	0.04 (0.00)	75 (9)	23 (3)
TS-7	0.05 (0.01)	102 (14)	9 (1)

[a] Standard deviations are given in parentheses. Replication was n = 3 and n = 2 for ^{14}C-MeHg and $^{14}CH_4$ amended samples, respectively. Incubation time was 6−12 h.

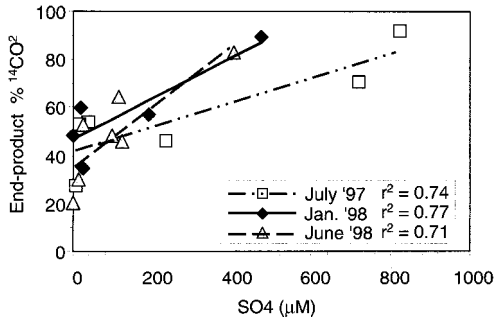

FIGURE 6. Significant linear regressions of the percent $^{14}CO_2$ end-product versus pore-water SO_4^{2-} concentrations (0−4 cm depth interval) from the Everglades data set.

Discussion

The positive relationship between k_{deg} and log[Hg_t] in the Carson River data set (Figure 2a) reconfirms earlier findings for this system in which the demethylation rate increased among three sites with increasing Hg contamination (*23*). The two distinct spatial groupings in the current data indicate that MeHg degrading bacteria in the wetlands were more responsive to Hg contamination than bacteria in other regions. This may reflect differences in the composition, abundance or activity of the respective microbial communities, and/or differences in the MeHg availability. The $\%^{14}CO_2$ data suggests that OD dominated MeHg degradation even at the most contaminated sites. This appears in contrast to results from the earlier investigation noted above, in which $\%^{14}CO_2$ decreased with increasing Hg contamination and demethylation rate. Such a trend would suggested a shift in microbial populations, from those invoking OD at low Hg levels to those invoking RD at higher contamination levels. The natural selection of bacteria, able to invoke *mer*-detoxification of both inorganic and organic mercury, has been shown in other Hg contaminated sediments (*18*, *49*). The lack of any clear relationship between $\%^{14}CO_2$ and Hg_t in the current study may indicate that the long sediment holding time (>90 days), prior to ^{14}C-MeHg incubation, impacted the original community composition so as to obscure this relationship. Alternatively, the apparent trend in the earlier report may have been spurious due to the limited number of observations ($n = 3$) or because *mer*-detoxification was inadvertently stimulated in contaminated sediments due to the high ^{14}C-MeHg amendment levels used (1800 ng Hg*cm^{-3}) compared to the current study (2−52 ng Hg*cm^{-3}).

The positive relationship between k_{deg} and various mercury pools evident in the Everglades data (Figure 3) further illustrates the potential for increased degradation with increasing contamination within an ecosystem. The inconsistency in the types of significant regressions observed among sampling dates partially reflects the fact that while sediment for both MeHg degradation assays and Hg-speciation analysis was collected at the same site and date, these samples were collected by two different groups of researchers, often tens of meters apart and not always at the same depth intervals (see Figure 3 legend). Thus, the analysis of k_{deg} and Hg-speciation relationships is less than optimal for this data set. However, since within-site variation in k_{deg} and Hg-species concentrations was presumably smaller than regional (among-site) variations (not directly tested), significant trends were detected in some cases. Further, since the range of Hg_t concentrations is much smaller in the Everglades compared to the Carson River, the expected response of the microbial community to increasing Hg contamination in the Everglades is expected to be more subtle and significant relationships more difficult to decipher. Finally, other geochemical factors assuredly influence k_{deg} values and thus partially obscure the direct influence of increased contamination in the Everglades.

Comparisons among systems further confirm the positive relationship between k_{deg} and Hg_t. In the Everglades, where Hg_t values were comparatively low, k_{deg}'s were likewise consistently low **(**Figure 2c**)**. In SCC, where Hg_t was very high both above and below the New Idria mine, k_{deg}'s were high at all AMD sites and low at the control site (Figure 2e). Mercury bioavailability likely accounted for this among-site difference within SCC. The source of Hg upstream is primarily recalcitrant and insoluble weathered cinnabar (HgS) abundant throughout the local area, whereas the downstream source includes particle-bound Hg(II) liberated by acidic conditions within the mine and the leaching of roasted-ore waste adjacent to the mine (*37*, *38*). Thus, the higher levels of bioavailable Hg_t at sites downstream of the mine would be more prone to select for bacterial populations that actively degrade MeHg.

As with the 1998 Carson River data, the lack of any clear relationship between $\%^{14}CO_2$ and log[Hg_t] in the Everglades (Figure 2d) indicates that something other than Hg_t alone influences MeHg degradation pathways or stoichiometric end-product ratios. The positive relationship between $\%^{14}CO_2$ and pore-water SO_4^{2-} (Figure 6) suggests that this anion plays a role in MeHg degradation pathway, although it is unclear if this role is biological (e.g. mediating sulfate reduction) or abiotic (e.g. affecting MeHg-complex formation). In either case, the consistent production of $^{14}CO_2$ at all sites demonstrates that OD was active, if not dominant. We infer that in-situ Hg was not high enough to induce a strong RD response in the Everglades sites. The situation appears altogether different in SCC sediments, where the lack of significant $^{14}CO_2$ production in AMD sites indicates that RD dominated degradation, as might be predicted under severely contaminated conditions. Abundant $^{14}CO_2$ production observed upstream of the mine (at C-1) supports the hypothesis that a strong RD response is invoked when Hg is not only very high in concentration but also bioavailable in form.

Time course experiment results (Figure 4) for Carson River site F1 and SCC site AMD-3 indicate that the ^{14}C-MeHg amendment was sequestered into at least two pools; one readily available to the resident microbial community and one less available. This was apparent from the initial rapid degradation rates followed by a slowing or cessation of degradation and was in contrast to the slow linear degradation seen in both Everglades sites and SCC site C1. Variations in substrate availability, due to refractory MeHg-complex formation with dissolved and/or particulate phases, may partially account for these spatial differences. Carson River site F1 had low organic content (4% LOI) compared to the two Everglades sites (80−91% LOI), suggesting that very organic-rich sediments may sequester a larger fraction of MeHg into slowly degrading refractory pools. We conclude that sediment organic content was not responsible for the large spatial differences in k_{deg} observed for SCC because LOI percentages were similar for SCC sites C1 and AMD-3 (11% and 17% LOI, respectively) and no significant relationship between k_{deg} and LOI was observed for SCC data. However, large differences in solid-phase composition were evident, with the AMD sites primarily composed of orange colored flocculent material, presumably iron(III)-oxy-hydroxy sulfate precipitate, typical of acid mine drainage (*50*). The distribution of ^{14}C-MeHg between organic and inoganic solid phases was not directly assessed. However, since 55−75% of the ^{14}C-MeHg amendment was readily degraded within 5 h at AMD-3 (Figure 4e,g), we speculate that much of the substrate was associated with the iron(III)-oxy-hydroxy sulfate fraction and that this portion was readily available to the MeHg degrading microbial population.

The nearly constant $\%^{14}CO_2$ produced in five of seven sites (Figure 4) indicates that one pathway dominated MeHg degradation in most cases. Specifically, OD is implicated in the case of Carson River F1, Everglades ENR-103, and SCC C1 (anaerobic), and *mer*-detoxification is implicated in the case of SCC AMD-3. We emphasize *mer*-detoxification in the latter case and not the alternative RD pathway (via reaction with H_2S), which would have been inhibited under aerobic conditions. Similarly for SCC C1, the change in the $\%^{14}CO_2$ trend, from constant and high under anaerobic conditions to decreasing with time under aerobic conditions, also suggests *mer*-detoxification may have been preferentially stimulated under aerobic conditions. The clear decrease in $\%^{14}CO_2$ with time at Everglades 3A-15 [anaerobic] and SCC C1 [aerobic] (Figure 4c,f) indicate that OD and RD were simultaneously active with RD dominating, since $\%^{14}CO_2$ would be expected to increase if OD dominated. An alternative explanation for decreasing $^{14}CO_2$ with time is that different microbial groups are capable of OD but with different stoichiometric end-product $^{14}CO_2/^{14}CH_4$ ratios and/or at different rates. It is important to emphasize that $\%^{14}CO_2$ end-product measurements alone do not indicate the relative importance of OD verses RD, particularly with single time point incubations, as some $^{14}CH_4$ may also be an OD end-product (*23, 24*). Only in cases where either $^{14}CH_4$ or $^{14}CO_2$ is the exclusive end-product can either RD or OD, respectively, be solely inferred. Then, only under aerobic conditions can the non-*mer* Me_2Hg intermediate pathway be ruled out and *mer*-detoxification surmised (e.g. SCC AMD-3).

Amendments with ^{14}C-MeHg, above ambient MeHg levels, may have stimulated RD to varying degrees at some sites (e.g. Figure 4c,f), which may partially account for the wide range of $\%^{14}CO_2$ values observed in Everglades samples (Figure 2d). It is noteworthy that when observed, the decrease in $\%^{14}CO_2$ was immediate and did not involve a lag time, suggesting that the bacterial community was preacclimated to MeHg (*18, 51*). Alternatively, the addition of ^{14}C-MeHg to organic-poor sediments may have stimulated heterotrophic bacteria capable of using MeHg as an organic substrate. If so, this would cause us to overestimate the importance of OD, as the rate of $^{14}CO_2$ produced would presumably be higher than that of nonlabeled CO_2 produced from in-situ MeHg levels. Previous experiments with Everglades sediment did show a significant increase in $\%^{14}CO_2$ produced with increasing ^{14}C-MeHg over a large amendment range (50−4000 ng MeHg*g dry sed^{-1}) but no significant increase in $\%^{14}CO_2$ over a much smaller range (2−18 ng MeHg*g dry sed^{-1}) (*24*). Amendment concentrations in the current work were varied over a wide range (in ng MeHg*g dry sed^{-1}: Everglades, 16-2600; Carson River, 68-270; SCC, 16-61), due to large variations in sediment porosity. While the corresponding amount of carbon added from ^{14}C-MeHg was small on a volumetric basis (0.01−0.25 nmol C*cm^{-3}), and only a fraction of the added radiolabel may be available for degradation, it is uncertain if the ^{14}C-MeHg amendment levels used in the current experiments resulted in a significant stimulation of heterotrophic activity. This possibility cannot be ruled, especially for some of the low organic sediments of the Carson River systems.

It is not surprising that a clear relationship between Hg_t and k_{deg} was not evident in all cases, as other environmental factors undoubtedly also impact observed MeHg degradation rates. The increase in k_{deg} with methanogenesis, sediment organic content (PC), and reduced S suggest that within-system regional differences in benthic microbiology and/or geochemistry are important in the Carson River (Figure 5a−d). It is difficult to assess the relative contribution and mechanism of each of these covarying parameters, as a control on microbial MeHg degradation, without conducting controlled experiments. Taken together, however, they depict a shift to higher k_{deg}'s going from organic-poor (river) to comparatively organic-rich and sulfidic (wetland and agricultural drain) sites with higher rates of anaerobic metabolism. These relationships were statistically independent ($P > 0.05$) of increases in k_{deg} due to increasing Hg_t. The increase in k_{deg} with increasing pore-water sulfide might suggest the non-*mer* RD pathway (via Me_2Hg formation), although the major end-product (>80%) was $^{14}CO_2$ and not $^{14}CH_4$ in most cases (Figure 3b). Thus, it would appear that it was OD, not RD, which dominated degradation. It is unknown if OD can also be carried out on $(MeHg)_2S$ or Me_2Hg, but such reactions could explain the spatial variation in the Carson River system.

The lack of positive relationships in the Everglades data set, similar to those noted above for the Carson River, may be due to the difficulty in detecting such relationships with such a comparatively low and narrow range of k_{deg}'s values. Alternatively, the relative influences of individual environmental controls on MeHg degradation may differ among systems. Specifically, the large difference in sediment organic content (as assessed by LOI) between the Everglades (33−91%, median = 80%, $n = 25$) and the Carson River (1−12%, median = 2%, $n = 13$) may partially account for the contrast in k_{deg} values among these ecosystems, for different reasons. The consistently low k_{deg} values in the organic-rich Everglades could reflect a high degree of MeHg-organic (or MeHg-reduced-S) complex formation, thereby decreasing MeHg availability to bacteria. While benthic anaerobic metabolism is presumably not carbon limited in the Everglades, organic substrate appears to limit microbial rates in the Carson River system, as evident from the increase in both methanogenesis and SR along a transect from organic-poor river sites to comparatively organic-rich wetland sites (data not shown). This increase in microbial rates parallels the increase in MeHg degradation for the Carson. Additional unpublished sequential extraction experiments conducted with ^{14}C-MeHg amended Carson River sediment indicates decreasing dissolved (water-extractable) and readily exchangeable (acid-extractable) MeHg pool size and an increase in MeHg-organic complex (base-extractable) pool size, with increasing organic content (data not shown). Assuming that the dissolved and readily exchangeable MeHg pools are more available for degradation than the MeHg-organic complex pool, then the increase in the overall activity of the MeHg degrading community more than compensates for the decrease in bioavailable MeHg pool size along the Carson River organic gradient.

The apparent increase in k_{deg} with decreasing pH in SCC sediments (Figure 5e) was not due to abiotic acid cleavage of the methyl group from MeHg, since the k_{deg}'s presented were kill corrected and represent microbial degradation only. Acidophilic bacteria were thus clearly involved in MeHg degradation at the AMD sites. To our knowledge, this is the first time that a significant MeHg degradation capacity has been suggested for this general bacterial group.

The lack of significant anaerobic $^{14}CH_4$ oxidation, in any of the three ecosystems, demonstrates that this process could not explain the $^{14}CO_2$ produced from anaerobic ^{14}C-MeHg degradation, as has been recently proposed (*25*). The fact that CH_4 oxidation was readily observed in Everglades samples incubated aerobically demonstrates our ability to detect this process. The corresponding increase in $\%^{14}CO_2$ from ^{14}C-MeHg, under aerobic conditions, indicates that some of this $^{14}CO_2$ could have been due to aerobic oxidation of $^{14}CH_4$ produced from either RD or OD.

A clear demonstration of OD in pure culture remains outstanding to date. The methylotrophic methanogen GS-16 (*22*), subsequently named *Methanolobus taylorii* sp. nov. (*52*), produced $^{14}CO_2$ from ^{14}C-MeHg ($\%^{14}CO_2$ = 29−46%) when grown on trimethylamine, although the total amount of ^{14}C-MeHg degraded was low (<3%). No anaerobic $^{14}CO_2$

production from ^{14}C-MeHg was detected for two sulfate reducing strains (*Desulfovibrio desulfuricans* LS and ND 132) and one methanogen (*Methanococcus maripaludis*), in a subsequent study (*25*). However, the high ^{14}C-MeHg amendment level used (500 ng/cm^3) was far in excess of typical environmental contamination levels and in excess of the levels used in the current study (2−52 ng/cm^3). Subsequently, *mer*-detoxification may have been induced, giving rise to detection of $^{14}CH_4$ only. It was not noted whether these bacteria were screened for the *mer*-operon. Further, previous work by Baldi et al. (*19*) demonstrated that *D. desulfuricans* degrades MeHg by the non-*mer* RD pathway (via reaction with H_2S), even under SO_4^{2-} limited conditions. While the above study (*25*) cites low SO_4^{2-} conditions for the culture media, it is also possible that low sulfide levels also existed in the sulfate reducing cultures and that the non-*mer* RD pathway was subsequently responsible for the detection of $^{14}CH_4$ as the sole gaseous end-product.

The current study demonstrates strong within-system and among-system differences in MeHg degradation rates and pathways. Systems or regions with low Hg contamination exhibited low k_{deg}'s and OD dominated the degradation pathway. A much wider range of k_{deg}'s was observed at higher contamination levels as other environmental factors become important, such as overall metabolic rates, sediment reduced S, organic matter concentrations, and substrate availability. The sequestering of MeHg by various solid phase fractions, as a key factor in mediating MeHg availability to bacteria, should be more fully investigated. Only under conditions of extreme contamination with bioavailable Hg was a strong RD pathway clearly dominant, and only then under aerobic conditions was *mer*-detoxification specifically implicated. While OD appears widespread in natural systems, its unambiguous demonstration in pure culture remains elusive, and the pathway specifics remain unknown. More work is needed to reconcile the results from the limited number of pure culture experiments with those from whole sediment field measurements.

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