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Spatial and seasonal variations in mercury methylation and microbial community structure in a historic mercury mining area, Yolo County, California

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A B S T R A C T

The relationships between soil parent lithology, nutrient concentrations, microbial biomass and community structure were evaluated in soils from a small watershed impacted by historic Hg mining. Upland and wetland soils, stream sediments and tailings were collected and analyzed for nutrients (DOC, $SO_4^{=}$, NO_3^{-}), Hg, MeHg, and phospholipid fatty acids (PLFA). Stream sediment was derived from serpentinite, siltstone, volcanic rocks and mineralized serpentine with cinnabar, metacinnabar and other Hg phases. Soils from different parent materials had distinct PLFA biomass and community structures that are related to nutrient concentrations and toxicity effects of trace metals including Hg. The formation of MeHg appears to be most strongly linked to soil moisture, which in turn has a correlative relationship with PLFA biomass in wetland soils. The greatest concentrations of MeHg (>0.5 ng g^{-1} MeHg) were measured in wetland soils and soil with a volcanic parent (9.5–37 μg g^{-1} Hg). Mercury methylation was associated with sulfate-reducing bacteria, including *Desulfobacter* sp. and *Desulfovibrio* sp., although these organisms are not exclusively responsible for Hg methylation. Statistical models of the data demonstrated that soil microbial communities varied more with soil type than with season.

1. Introduction

Mercury (Hg) is a globally significant pollutant (Jackson, 1997; Fitzgerald et al., 1998) associated with both industrial sources, including coal combustion, smelting, paper pulping and waste incineration (Nriagu and Pacyna, 1988), and natural sources, including volcanic gas emissions (Nriagu and Becker, 2003; Pyle and Mather, 2003) and thermal waters (White et al., 1970). Major Hg mineral deposits, including those associated with volcanic centers (e.g., Almaden, Spain), hot springs (e.g., Alaska, Nevada, California Coast Range), and silica-carbonate deposits (e.g., California Coast Range) are derived from these thermal waters (Rytuba, 2003).

Weathering of Hg mineral deposits releases sediments and colloids to stream water, flood plains and estuaries, contributing to a regionally elevated background concentration in sediment and soil. Mining accelerates sediment and colloid transport of Hg by preferentially breaking down rocks that contain Hg and disturbing soil with naturally elevated Hg through vegetation removal and road construction. Historic Hg mining in the northern California Coast Range has had a regional-scale impact on the San Francisco Bay estuary, the Sacramento River and its tributaries. Approximately 97,000 metric tons of Hg were produced in the Coast Range between 1852 and 1972 (Bailey et al., 1973; Cargill et al., 1980), with a significant loss to the atmosphere, approximately 34,500 metric tons (Churchill, 2000), through the roasting and retorting processes that extracted Hg metal from HgS minerals. The resulting atmospheric deposition of Hg had local, regional and global impacts.

Sediment and colloids from the Coast Range and Sierra Nevada were transported downstream through the Sacramento River and into San Francisco Bay. Sediment cores from San Francisco Bay indicate pre-mining sediment Hg was around 0.06 mg Hg kg^{-1} (Hornberger et al., 1999), consistent with the mean soil Hg concentration for the conterminous United States (0.06 mg Hg kg^{-1}) (Smith et al., 2005). Sediment core Hg corresponding to the mid-20th century reached a maximum concentration of 0.9 mg Hg kg^{-1} in Grizzly Bay at the north end of the San Francisco Bay, with maximum concentrations from 0.5 to 0.7 mg Hg kg^{-1} elsewhere in the bay in the mid-20th century (Hornberger et al., 1999). Concentrations in post-mining sediment core concentrations are between 0.2 and 0.5 mg Hg kg^{-1}, well above pre-mining background concentrations (Hornberger et al., 1999; Conaway et al., 2004).

Methylmercury (CH_3Hg^+, or MeHg), a neurotoxin readily bioaccumulated in aquatic ecosystems (Rudd, 1995), is formed from Hg(II) in anoxic sediments. Sulfate-reducing bacteria (Compeau and Bartha, 1985; Choi et al., 1994; Devereux et al., 1996; Pak and Bartha, 1998) and iron-reducing bacteria (Fleming et al., 2006; Kerin et al., 2006) have been demonstrated to methylate Hg in flooded, anoxic sediments.

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Elevated MeHg concentrations have been documented in water and sediment of the Sacramento River and Cache Creek (Domagalski, 2001). Cache Creek was determined to be a significant source of Hg to the San Francisco Bay-Delta, with annual loads of 12 kg Hg yr^{-1} (Water Year 2000) and 4 kg Hg yr^{-1} (Water Year 2001), with loads of 1.1 to 6.7 g MgHg day^{-1} during storm events (Domagalski et al., 2004). A significant proportion of the Hg load in Cache Creek appears to be related to resuspension of bed sediments and colloids during storm events (Domagalski et al., 2004).

Macalady et al. (2000) and Batten and Scow (2003) measured MeHg concentrations in sediments and flocs from Hg mine sites in the headwaters of Cache Creek, and also evaluated microbial communities using phospholipid fatty acids (PLFA). A major structural component of microbial cell membranes, PLFAs provide information on microbial biomass and community composition (Federle et al., 1983). PLFA biomarkers analyzed in sediments collected from Clear Lake, located approximately 60 km from the study site, showed a dominance of *Desulfobacter*-like bacteria amongst sulfate-reducing bacteria, with an increasing proportion of the microbial biomass represented by these bacteria where MeHg potential was greater (Macalady et al., 2000). Similarly, PLFA biomarkers indicated an association between sulfate-reducing bacteria *Desulfobacter* and *Desulfovibrio* and MeHg in flocs and sediments associated with Cache Creek Hg mines including the Reed Mine in the Davis Creek watershed (Batten and Scow, 2003).

The purpose of this study is to evaluate MeHg concentrations and accompanying microbial community structures in soils from a small watershed impacted by historic Hg mining at the headwaters of Cache Creek. Seasonal and spatial variations of MeHg and the soil microbial community facilitating methylation were examined, building upon previous work by addressing the extent to which Hg methylation occurs in upland and wetland soils where cinnabar is a primary source of Hg.

1.1. Site description

The study area is located in the Davis Creek watershed upstream from Davis Reservoir (drainage area ~1 km^2) on the University of California McLaughlin Preserve in the northern California Coast (Fig. 1). Davis Creek is a tributary to Cache Creek, which flows eastward from the Coast Range into the Sacramento River, draining an area of approximately 2950 km^2. Elevation ranges from 370 to 730 m above mean sea level. The region has a Mediterranean climate, with hot, dry summers and cool, wet winters. Mean annual precipitation is 62 cm, with an average temperature of 25 °C in July and 8 °C in January. Vegetation includes mixed serpentine and nonserpentine chaparral in the upland. The riparian zone along the banks of Davis Creek is vegetated with willow, forbs and horsetails. The stream enters Davis Creek Reservoir, forming a delta of sediment with willow and cattails along the edge of the reservoir.

The study area includes the historic Reed and Andalucia Hg mines, part of the historic Knoxville Hg mining district. Mercury in the Knoxville district occurred primarily as cinnabar (HgS hexagonal) and metacinnabar (HgS cubic) with minor montroydite (HgO) and native Hg (Kim et al., 2004). The roasting and condensing process used to extract Hg from the ore materials left behind calcines (roasted ore), with fine-grained secondary Hg phases, including metacinnabar, cinnabar, and mercuric chloride ($HgCl_2$) (Kim et al., 2004), which are readily transported downstream as colloids (Rytuba, 2003).

Reclamation work has removed or immobilized most tailings from the Reed Mine. There is an exposed scarp and tailings in the vicinity of the Andalucia mine. Used to concentrate Au during the Mother Lode era

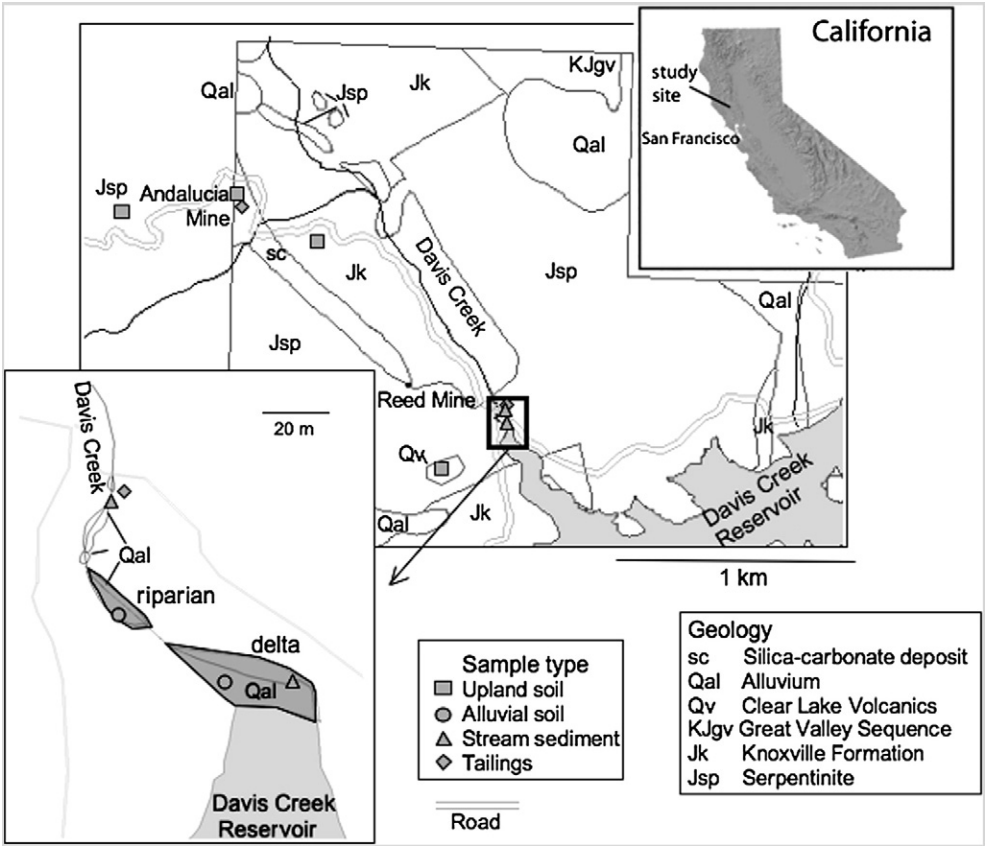

Fig. 1. Map of study site, showing sample locations for upland and alluvial soil, stream sediments and tailings collected June 2005. Replicate wetland samples from December 2004 and March 2005 were collected within the dark areas labeled "riparian" and "delta". The riparian area is a seasonal wetland, flooded during winter and spring. The delta where Davis Creek enters the reservoir is a permanent wetland, remaining flooded throughout the year. Hg-mineralized silica-carbonate deposits are hydrothermally altered serpentinites. The geology base map was provided by the University of California McLaughlin Reserve.

in the eastern Sierra Nevada and Ag during mining of the Comstock Lode in western Nevada, Hg was intermittently mined in the Coast Range from the 1850s until the 1970s (Bailey et al., 1973) with peak production (2776 metric tons Hg) in 1877 (Bradley, 1918). The Knoxville district produced an estimated 4170 metric tons of Hg (Rytuba et al., 1993) through 1948.

Geology in the Davis Creek watershed includes the Knoxville Formation, a siltstone basal unit of the Late Jurassic to Middle Cretaceous Great Valley Sequence (Blake and Jones, 1981). The Coast Range Ophiolite is a 600–1500 m thick sequence of metamorphosed mafic and ultramafic rocks, including serpentinite (Carlson, 1984). Thermal activity associated with the emplacement of the Clear Lake Volcanics approximately 2.1 Ma (Hearn et al., 1988) resulted in extensive serpentinite-hosted silica-carbonate type Hg deposits (Rytuba, 2003).

Upland soils have parent materials derived from these rocks. In addition to material weathered from upland soils, mine tailings contribute to the overall sediment load in Davis Creek. Stream sediment is the parent material for wetland soils in both riparian and delta settings. A seasonal wetland, saturated in winter, was found associated with a riparian area (Fig. 1), with a permanently saturated wetland associated with the delta. Soils range in degree of development from Entisols (e.g., Soboba) to Vertisols (e.g., Millsholm) to Inceptisols (e.g., Climara) (Table 1).

2. Methods

Triplicate soil samples were collected from the riparian and delta wetlands to evaluate variations in soil chemistry and microbial communities between plant senescence (December 2004) and active growth (March 2005). Additional upland soils, sediment and tailings were collected from Davis Creek watershed in June 2005 to evaluate variations in methylmercury and microbial communities. Samples were collected using a stainless steel auger and separated into splits to analyze for soluble nutrients, THg, MeHg, and PLFA.

2.1. Soil–water leachates and pH

Material used for soil–water leachates were shipped and stored chilled in glass jars and leached within one week of collection. Soil–water leachates were extracted using 10 g soil agitated in sterile centrifuge tubes for 60 min in 30 mL distilled deionized water. This period of time was selected to minimize dissolution of soil organic matter while extracting pore waters with sufficient volume for multiple analyses. Following centrifugation at 5000 rpm for an hour, the solute was filtered through 0.45 μM Metricel filters, diluted for analyses by quantitatively adding 20 mL distilled deionized water and transferred to baked glass vials. Inorganic carbon was removed with phosphoric acid and the remaining organic carbon was oxidized with ammonium persulfate and quantified by wet oxidation (Aiken, 1992). Nitrate was quantified by chemoluminescence (Sievers 280 Nitric Oxide Analyzer) after the conversion of NO_3^- to NO in a VCl_3 reagent followed by ozonation to produce NO*. Sulfate was measured by ion chromatography. A 1:1 soil–water paste was used to measure pH.

2.2. Total Hg and MeHg

Sample splits were collected in I-Chem jars with Teflon-lined lids, pre-cleaned for organic compounds and trace metals. Samples were frozen in the field on dry ice and shipped frozen to the Battelle/Marine Sciences Laboratory in Sequim, Washington for analyses. Samples analyzed for Hg and MeHg were mixed by hand but not ground prior to analysis to minimize loss of MeHg through volatilization. Because of the inhomogeneity inherent to natural samples, the average value between duplicate samples was reported for Hg.

Samples were completely digested for total Hg using aqua regia. Hg in the digestate was reduced by acidic $SnCl_2$ to elemental Hg, purged from the sample with argon, and detected by cold vapor atomic absorption (EPA, 1996). Samples were extracted for MeHg with an ethylating agent to form a volatile methyl-ethylmercury derivative, and then purged onto graphitized carbon traps to preconcentration and remove potential interferences (Bloom et al., 1997). The extracts were pyrolitically reduced to elemental Hg and analyzed by cold vapor fluorescence (Bloom, 1989).

The average detection limit was 0.005 μg g^{-1} for total Hg and 0.002 ng g^{-1} for MeHg. Total Hg was not detected above the achieved detection limit in analytic blanks and the blank MeHg concentration was <0.015 ng g^{-1}, well below sample concentrations. Matrix spikes and duplicates were analyzed for each analyte. Standard reference materials NIST 2704 and MESS-3 were analyzed for total Hg with recoveries from 99 to 103%. Standard reference material IAEA 405 was analyzed for MeHg with recoveries of 75 to 81%. The IAEA 405 standard was certified using the distillation method, which has since been found to exhibit a methylation artifact. The lower results analyzed by the extraction method used for this study fall within the range of 70 to 95% recovery.

2.3. Phospholipid fatty acids

Triplicate subsamples of soil were extracted for 2 h in a chloroform/methanol/phosphate buffer (1:2:0.8 v/v/v/) with the amount of phosphate buffer adjusted for existing soil–water content. Following centrifugation, the supernatant was decanted into separatory funnel, vortexed and re-extracted for 30 min with an additional extractant.

Table 1
Upland soils with bedrock parent material and wetland soils with stream sediment parent material.

Geomorphology	Parent	Soil series[a]	Soil order	General description	Horizons and depth[b]
Upland	Serpentinite and mineralized serpentinite	Climara	Haploxerert	Magnesic, minimum horizon development, high smectite content	A 0–5 m B 5–20 cm CR <20 cm
Upland	Knoxville Formation siltstone	Millsholm	Haploxerept	Minimal horizon development with no illuvation	AC 0–10 cm
Upland	Clear Lake Volcanics		Entisol	Thin, lithic soil, mixed; no horizon development	AC 0–10 cm
Riparian (seasonal wetland)	Alluvium	Soboba	Xerofluvent	Sandy, poorly developed	A 0–4 cm C >4 cm
Delta (permanent wetland)	Alluvium	Soboba	Xerofluvent	Sandy, poorly developed, saturated	A 0–18 cm B1 18–25 cm B2 >25 cm

[a] Soil series and order as detailed in Andrews (1972). Areas in the Davis Ck watershed that include serpentinite or volcanic rock with a thin veneer of soil are mapped as rockland. Volcanic soil is similar in composition to the Hambright series, a Haploxeroll that has more extensive soil development.
[b] Horizon depths for riparian and delta soils represent the range found within samples collected in the field.

The supernatants were combined with a PO_4 buffer and $CHCl_3$, shaken and the phases were separated overnight. The $CHCl_3$ layer was decanted and dried under N_2 at 32 °C. Phospholipids were separated from neutral lipids and glycolipids on solid phase extraction columns conditioned with $CHCl_3$, with neutral lipids and glycolipids eluted with $CHCl_3$ and acetone and polar lipids eluted with methanol and air dried under N_2. Polar lipids were subjected to mild alkaline methanolysis to form fatty methyl esters. Extracts were prepared with hexane containing the 19:0 lipid as an internal standard and analyzed by gas chromatography (Hewlett Packard 6890) using a 25 m Ultra 2 (5%-phenl)-methylpolysiloxane column (J&W Scientific). Peaks were identified using FAME standards and MIDI peak identification software (MIDI, Inc., Newark, DE) (Bossio and Scow, 1998).

Nomenclature for fatty acids uses the ratio of the number of carbons to the number of double bonds (e.g., 17:0). The location of a double bond is indicated by the number of carbons the methyl headgroup of the molecule, followed by ω. and the cis (c) or trans (t) orientation of the double bond (e.g., ω6c). Iso-branched (i), anteiso-branched (a) or unspecific branching (br) structures are also indicated. For example i17:1ω7c indicates an iso-branched seventeen-carbon lipid with a cis-oriented double bond seven carbons from the methyl end. The notation 10Me indicates a methyl group on the tenth carbon from the carboxyl end of the molecule (e.g., 10Me16:0). The position of hydroxyl groups are noted (OH) and cy indicates cyclopropane fatty acids.

Of the 137 identified peaks, a group of 23 PLFAs were selected to evaluate microbial community structure based on presence in >75% of samples analyzed at 1 mol% or greater or status as a biomarker for key microbes (Table 2). The total PLFA concentration, expressed as nmol/g, can be used as a relative measure of microbial biomass between sites. Individual PLFAs used to address microbial community structure were converted to mol% to compare microbial composition of different sites independent of overall biomass.

2.4. Statistical methods

Interrelationships between MeHg, individual lipids and different environmental variables that can influence methylation (e.g., pH, Hg, DOC, SO_4^{2-}, NO_3^-) are complex and require multivariate approaches for statistical analysis. The MeHg, Hg, soil leachate and PLFA data were analyzed using principal components analysis (PCA) using Canoco software (Microcomputer Power, Inc., Ithaca, N.Y.). PCA uses indirect gradient analysis to interpret patterns that are extracted from all variation in large, multivariate data sets. This method is analogous to performing multiple linear regressions. (Mendoza et al., 1978; Ter Braak, 1995). All data used for statistical analyses are summarized in Supplementary Table 1.

Table 2
PLFA biomarkers.

PLFA	Biomarker/bioindicator	Reference
14:0, 15:0, 16:0, 17:0, 18:0	Common	
Monoenoic: i15:0, a15:0, i16:0, i17:0, a17:0, cy17:0, cy19:0	Gram+	Wilkinson (1988)
Branched: 16:1ω5c, 16:1ω7c, 18:1ω6c, 18:1ω9c	Gram−	Harwood and Russell (1984)
i17:1 (especially i17:1ω7c)	Desulfovibrio sp. Sulfate-reducing bacteria	Edlung et al. (1985), Scheuerbrandt and Bloch (1962), Taylor and Parkes (1983)
10Me16:0	Desulfobacter sp. Sulfate-reducing bacteria	Taylor and Parkes (1983), Dowling et al. (1986)
10Me18:0 (with 10Me16:0)	Actinomycetes	Kroppenstedt (1985), Kroppenstedt and Kutzner (1972)
18:2ω6c, 18:3ω6c	Fungi	Frostegard and Bååth (1996), Vestal and White (1989)
18:1ω9c	Algae, aerobic bacteria	Findlay et al. (1985), Rajendran et al. (1992)

Table 3
THg, MeHg and PLFA in sediment, tailings and soils in Upper Cache Creek watershed.

Location	Sample type	THg	MeHg	Biomass	Ref.
		μg g^{-1}	ng g^{-1}	nmol PLFA g^{-1}	
Davis Creek Yolo Co., CA	Upland soil	0.2–106	0.05–1.3	7–45	1
Davis Creek Yolo Co., CA	Wetland soil	10–18	0.5–5.1	3–34	1
Davis Creek watershed Yolo Co., CA	Mine tailings	280, 380	0.1, 0.2	5, 34	1
Upper Bear Creek, Colusa Co., CA	Mine tailings	0.04–410	<0.01–6, 57		2
Davis Creek Yolo Co., CA	Stream sediment	0.5, 61	0.2–0.3	3–9	1
Bear Valley, Colusa Co, CA	Stream sediment	0.02–360	<0.01–68		2
Reed Mine, Davis	Sediment + floc 1999	2 ± 2	3 ± 3	214 ± 371	3
Creek, Yolo Co, CA	Sediment + floc 2000	7 ± 10	21 ± 40	223 ± 310	
Sulphur Bank Mine; Clear Lake, Lake Co, CA	Sediment + floc	13 ± 10	17 ± 10	884 ± 344	3
Clear Lake, Lake Co., CA	Lake sediment	2–34	2–7	370–1900	4

[1]This study, showing data from June 2005; [2](Slowey and Rytuba, 2008); values reported for paired Hg and MeHg data; [3] (Batten and Scow, 2003); THg and MeHg values were interpolated from graphics; standard deviations are reported [4](Macalady et al., 2000); standard deviations are reported.

3. Results and discussion

3.1. Variation in Hg and MeHg in Upper Cache Creek

Soil Hg concentrations for samples collected June 2005 were between 0.2 and 106 μg g^{-1} (Table 3), well above 0.06 μg g^{-1} Hg, the mean soil concentration for the conterminous United States (Shacklette and Boerngen, 1984; Smith et al., 2005). Elevated Hg in the volcanic soil (37 μg g^{-1} Hg) may have been related to the hydrothermal event that resulted in the Hg-rich mineralized serpentinite soils (77–106 μg g^{-1} Hg). Other upland soil concentrations were between 0.2 and 0.5 μg g^{-1} Hg, interpreted to be the result of dust transport and volatilization of Hg through historic mining operations. Weathering and transport of mineralized serpentinite and mine tailings (280–380 μg g^{-1} Hg) resulted in stream sediments with elevated Hg concentrations (0.5 and 61 μg g^{-1} Hg). Wetland soils inherited elevated Hg concentrations (10–18 μg g^{-1}Hg) from the stream sediment parent material.

Upland soil MeHg concentrations were between 0.05 and 5.1 ng g^{-1}, with greater concentrations (0.5–5.1 ng g^{-1}) associated with wetland soils (Table 3). Mine tailings in Davis Creek had the greatest Hg concentrations (280 and 380 μg g^{-1}) and the lowest MeHg concentrations (0.1 and 0.2 ng g^{-1}) measured in this study. By contrast, tailings collected in the Upper Bear Creek, a watershed impacted by historic Hg mining approximately 28 km north of Davis Creek, had a broad range of both Hg (0.04–410 μg g^{-1}) and MeHg (<0.01–57 μg g^{-1}) concentrations (Slowey and Rytuba, 2008). Tailings reported by Slowey and Rytuba (2008) included serpentinite, likely at the low range of Hg and MeHg concentrations and calcines, which are enriched in Hg. Davis Creek sediment Hg (0.51 and 61 μg g^{-1}) and MeHg (0.2 and 0.3 ng g^{-1}) concentrations (Table 3) also fell within the lower range of Upper Bear Creek stream sediment Hg (0.02–360 ng g $^{-1}$) and MeHg (<0.01–68 ng g^{-1}) concentrations (Slowey and Rytuba, 2008). Previous work in Davis Creek found a wide range of MeHg concentrations (3 ± 3 and 21 ± 40 ng g^{-1}) that may in part be due to the incorporation of "floc", possibly a biofilm, into the samples (Batten and Scow, 2003). By comparison, sediments from Clear Lake had 17 ± 10 ng g^{-1} (Batten and Scow, 2003) and 2–7 ng g^{-1} MeHg (Macalady et al., 2000).

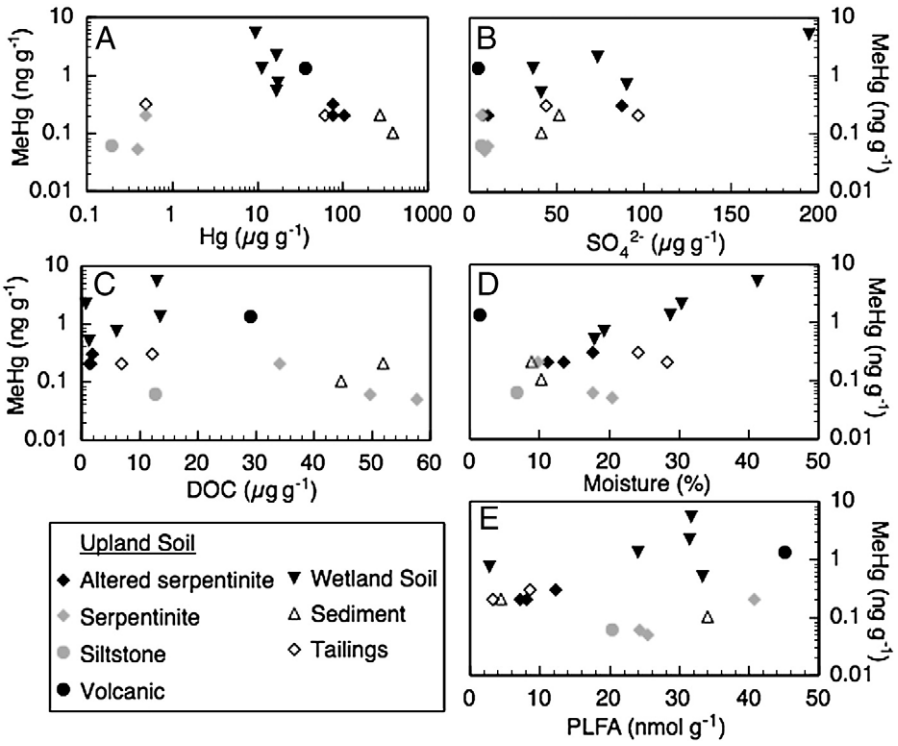

Fig. 2. A) Total H and MeHg concentrations for upland and wetland soils, tailings, and stream sediment collected in June 2005. Data have been plotted in log–log plots. Each data point represents a single sample. Upland soils are identified by parent material, with A, B and C horizons plotted for altered serpentinite and serpentinite soils. Wetland soils include permanent wetland A, B1 and B2 horizons, with A and C horizon for seasonal soils. B) MeHg concentrations as a function of soil and sediment moisture content. C) MeHg concentrations as a function of DOC concentration. D) MeHg concentrations as a function of SO_4^{2-} concentration. E) MeHg concentration as a function of PLFA biomass.

3.2. Biogeochemical constraints on Hg dissolution and methylation

Multiple geochemical and microbial factors can influence the extent to which Hg in soils, tailings and sediment is methylated. Data from the Davis Creek watershed show an upward trend in MeHg with Hg concentration to a threshold level of approximately 17 μg g^{-1} Hg, after which point MeHg concentrations decrease with increasing Hg (Fig. 2A). This trend may be explained by differences in the form of Hg in higher-concentration material or by the suppression of microbial Hg methylation by increasing concentrations of Hg.

Cinnabar and metacinnabar (HgS) are highly insoluble forms of Hg (Schwarzenbach and Widmer, 1963; Sillen, 1964). Assuming cinnabar is the primary form of Hg in altered serpentinite soils, volcanic soil, tailings, and stream sediment, the dissolution of HgS to Hg^{2+} is the limiting reaction in forming MeHg. In aquatic ecosystems, the dissolution of HgS is enhanced in the presence of elevated sulfide concentrations at pH>6 (Hurley et al., 1994; Wang and Driscoll, 1995). The methodology used for soil–water extracts would have resulted in the rapid conversion of sulfide to sulfate in the presence of oxygen. Thus, elevated SO_4^{2-} concentrations to some extent reflect the presence of H_2S in wetland soils, which are likely to be reduced when saturated. Increasing SO_4^{2-} concentrations in wetland soils (37–195 μg g^{-1} SO_4^{2-}) are associated with a general increase in MeHg concentrations (Fig. 2B), with the greatest concentration of MeHg (5.1 μg g^{-1}) occurring in the Delta B1 horizon. This horizon exhibited gley (bluish grey) coloration consistent with reducing conditions that support methylation.

Dissolved organic matter, particularly aromatic organic matter, has been shown to dramatically increase mercury release from cinnabar (Ravichandran et al., 1998; Waples et al., 2005). The organic carbon pool is also significant in that it is used by the overall microbial community to build new cells and includes CH_3, which combines with Hg^{2+} to form MeHg. There is no correlative relationship reflected in the plot of DOC with MeHg concentrations (Fig. 2C). A possible explanation is that a relatively small fraction of DOC is present as aromatic organic carbon. Measurements that could estimate aromatic content (e.g., specific UV absorbance) were not made for these samples. There appears to be an excess of water-extractable organic carbon relative to biological (e.g., microbes and vegetation) uptake, particularly in stream sediments, serpenitine soils and the volcanic soil that may influence how DOC relates to MeHg.

Moisture content correlates reasonably well with MeHg concentrations, particularly in wetland soils (Fig. 2D). Increasing soil moisture is accompanied by an increase in water-saturated micropores in soils, facilitating the reduced environment required by both sulfate- and iron-reducing bacteria.

The MeHg concentration increases in general with PLFA biomass, with the relationship more pronounced for high-Hg soils (mineralized serpentinite, volcanic and wetland soils). The PLFA biomass is significant in that no single microbial process is in isolation from others. For example, nitrogen-fixing bacteria and algae break bonds in atmospheric N_2, generating a labile pool of N for the physical structure of microbes. Fungi and heterotrophic bacteria break down complex organic matter from vegetation, generating labile C for structures and releasing CH_4. Because of these interrelationships, it is useful to address the larger microbial community structure when evaluating the formation of MeHg.

3.3. Variation of soil microbial communities with soil type

The trends in biogeochemical variables (Fig. 2) were used to construct a statistical model using principle components analysis (PCA), which interprets patterns extracted from all variations in soil environmental (DOC, NO_3^-, $SO_4^=$, MeHg, Hg, pH) and microbial community data (Fig. 3). Microbial community structure was defined using PLFA data normalized to mole percent of the total PLFA biomass. The hypotheses tested through this model include: 1) MeHg increases with Hg to a threshold concentration, after which MeHg decreases with Hg and 2) MeHg is primarily the product of SRB. Since there is no

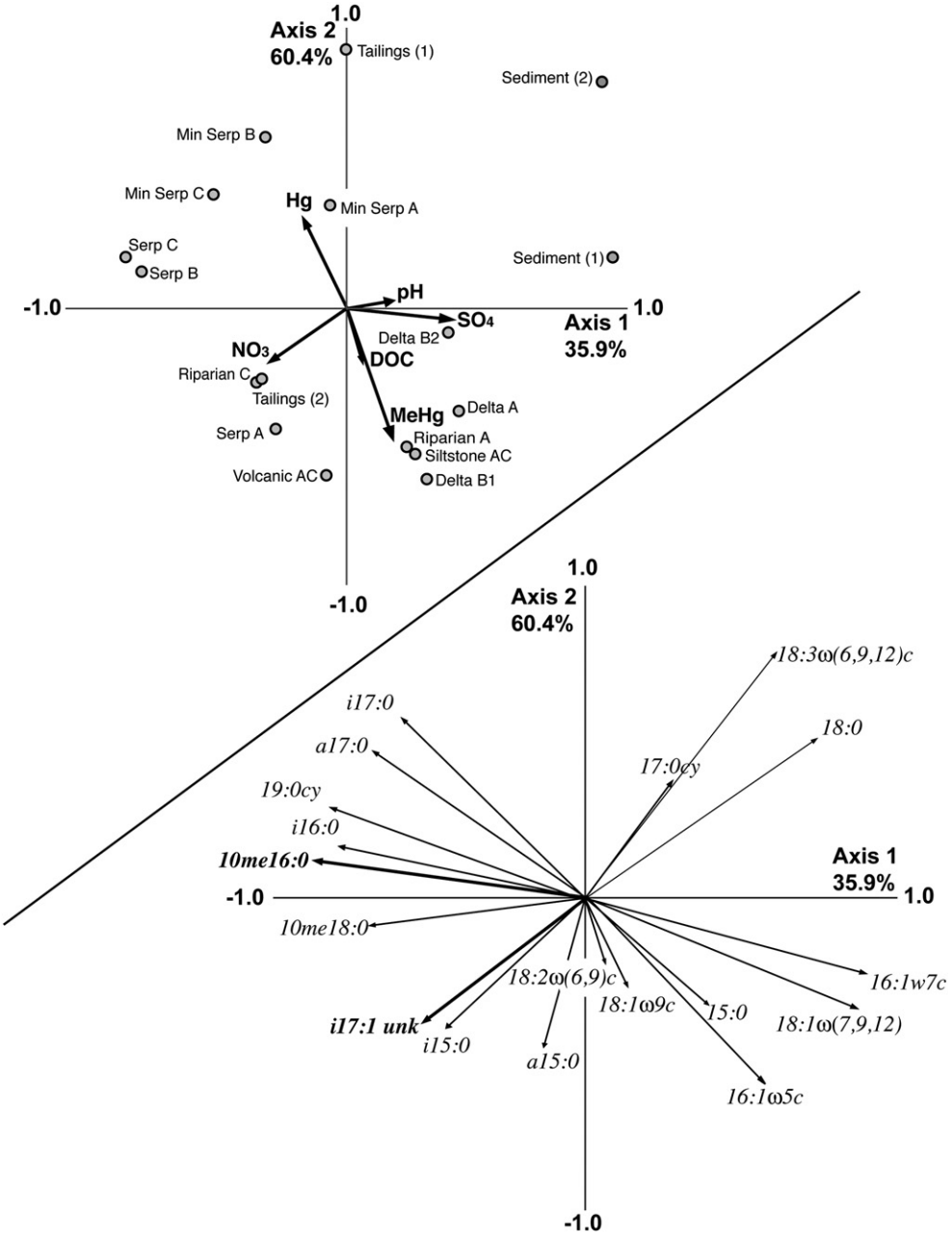

Fig. 3. Principal components analysis (PCA) of PLFA biomarkers and environmental data (Hg, MeHg, SO_4^2, DOC, NO_3, and pH) for soils, tailings and sediment collected June 2005. Axis 1 explains 35.9% of variance and Axis 2 explains 60.4% of the variance between PLFA and environmental data. Plots are on the same set of coordinates. Samples are represented as points with vectors showing the general direction of PLFA and environmental variable increase. Angles between vectors indicate relationships between variables. Acute angles show positive correlations, angles >90° show negative correlations. The relationship between environmental and PLFA vectors and sample points can be defined by projecting a perpendicular line between a given sample point and a vector.

recognized PLFA biomarker for IRB, the relationship between Fe-reducers and MeHg cannot be adequately tested using this approach.

Data were fit to matrices, with vectors pointing in the expected direction of the steepest increase of values for individual variables. Vector length is a measure of fit for the variables with the ordination axes and angles between vectors indicate correlations between individual variables, but not necessarily a correlation between sample points. For example, the acute angles between DOC and MeHg indicate these variables are correlative. However, the shorter length of its vector indicates that DOC is not as good of a fit within the model as MeHg. The correlation between variables is negative when the angle is larger than 90° (e.g., 16:1ω7c and 19:0cy; THg and MeHg). The relationship between a sample and a vector for a given environmental or PLFA variable can be determined by projecting a perpendicular line from the sample point to the vector. These projections can be used to approximate the optima of individual samples with respect to values of that variable.

The negative correlation between MeHg and Hg reflects the threshold concentrations of Hg for MeHg formation identified in Fig. 2A. Samples plotting in the Axis 2 positive direction (mineralized serpentine soil, tailings (1), sediment (2)) had microbial communities that were strongly influenced by Hg concentrations. Mineralized serpentinite soils and the B- and C-horizons of serpentinite soils show a strong relationship with vectors for cy19:0. This biomarker has been associated with anaerobic Gram-negative bacteria (Vestal and White, 1989) as well as Gram-negative bacteria under nutritional (Wilkinson, 1988; Kieft et al., 1995) or moisture stress (Wilkinson, 1988) and associated with slow microbial growth in serpentinite soils (deGrood

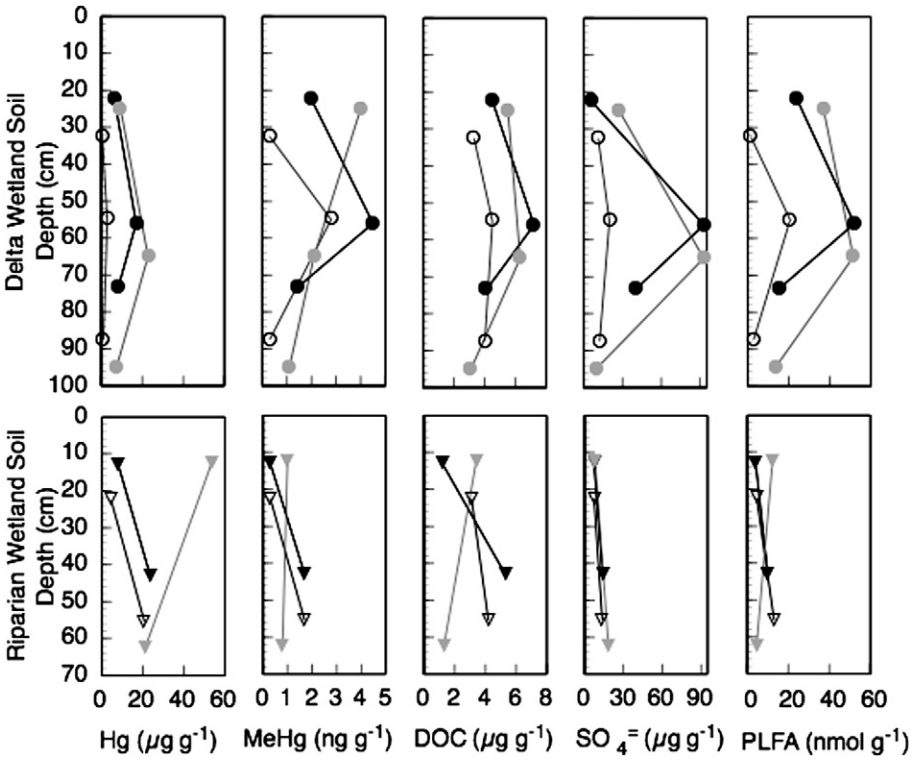

Fig. 4. Depth profiles of wetland soils collected in March, 2005, showing Hg, MeHg, DOC, $SO_4^=$, and PLFA concentrations. Triplicate samples were plotted as individual profiles to demonstrate variations within each group of soils, with data points placed at the midpoint of each horizon depth interval. Delta soils in permanent wetlands and riparian soils in seasonal wetlands are shown.

et al., 2005). Serpentinite B and C horizons and to a lesser extent, mineralized serpentine soils also associated with 10Me16:0, a biomarker for *Desulfobacter* sp. (Taylor and Parkes, 1983; Dowling et al., 1986). Soils forming on serpentinite had elevated Cr and Ni concentrations (1900–2500 mg Cr kg^{-1}; 1600–2500 mg Ni kg^{-1}; (Morrison et al., 2008), and the presence of these toxic trace metals, in addition to the elevated Hg concentrations associated with mineralization, may favor stress-adapted bacteria and *Desulfobacter*.

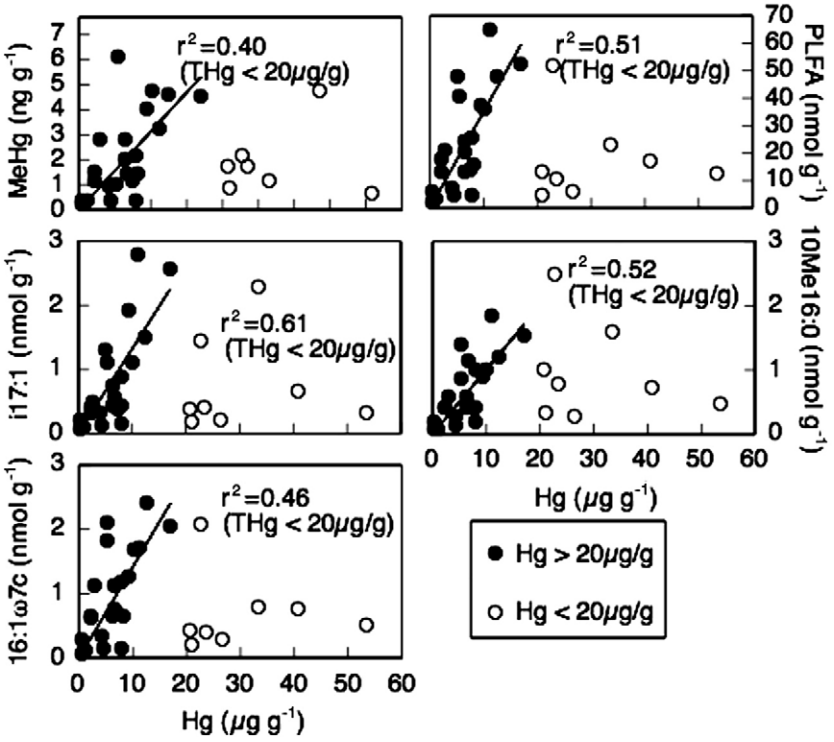

Fig. 5. Data from all horizons of riparian and delta wetlands from December 2004 and March 2005 showing the effect of Hg concentrations on MeHg concentration, PLFA biomass and concentrations of selected PLFA biomarkers.

Desulfovibrio sp. biomarker i17:1 (Scheuerbrandt and Bloch, 1962; Taylor and Parkes, 1983; Edlung et al., 1985) plotted in the negative quartile for Axes 1 and 2. The serpentinite A horizon, riparian C horizon and tailings (2) correspond to this area of the ordination plot. When comparing the position of sample points to either SRB biomarker vector, the model suggests that several soils and tailings (serpentinite A, B and C horizons; mineralized serpentinite C horizon, Riparian C horizon, Tailings (2)) have a combination of the two SRB groups that potentially contribute to methylation. Other soils and sediment (delta A horizon, delta B2 horizon, sediment (1), sediment (2)) don't project onto the vector for either SRB PLFA biomarker. These sample points project onto the 16:1ω7c vector, a common PLFA that is also a major constituent of the cellular membrane for a *Geobacter metallireducens* isolate (Lovley et al., 1993). Although this association does not demonstrate methylation by *Geobacter* sp. in these samples, the overall model does suggest that methylation by SRB is not the only process forming MeHg in this sample suite.

3.4. Temporal and spatial variations in wetland soils

Seasonal and permanent wetland soils were examined for variations in biogeochemistry and microbial community structures using samples collected December 2004 and March 2005. Permanent wetland soils in the delta had three distinct horizons (A, B1, B2) with gley soil (indicating anoxic redox conditions) at the B1 horizon accompanied by a faint hydrogen sulfide odor. Seasonal wetland soils in the riparian area were poorly developed, but were influenced by a dynamic piezometric surface. Profiles of Hg, MeHg, DOC, $SO_4^{=}$, and PLFA biomass show distinctions between these two wetland soils (Fig. 4). The reduced B1 horizon in delta soils show an increase in Hg, DOC, and $SO_4^{=}$ concentrations, as well as PLFA biomass. Two of the three delta profiles also show an increase in MeHg concentration in the B1 horizon. The soil structure in delta soils allows a stabilized environments that may enhance Hg accumulation as well as dissolution due to enhanced availability of $SO_4^{=}$ and DOC at this horizon. The poorly developed riparian soil shows a more narrow range of MeHg, DOC and $SO_4^{=}$ concentrations, with lower PLFA biomass. There is no consistent pattern of concentration increases or decreases with depth for the riparian soil.

Although there are differences in chemistry between delta and riparian soils, some generalizations can be made by plotting all wetland soil data together. The Hg threshold noted in soils, sediments and tailings (Fig. 2) was seen in wetland soils collected in December and March (Fig. 5). There is a positive correlation between Hg and MeHg ($r^2 = 0.40$) and PLFA biomass ($r^2 = 0.51$) at Hg concentrations below 20 μg g^{-1}. After this concentration, the relationship becomes less distinct. Specific lipid biomarkers also follow this pattern, with positive correlations between individual biomarkers i17:1 ($r^2 = 0.61$), 10Me16:0 ($r^2 = 0.52$), and 16:1w7c ($r^2 = 0.46$) at Hg concentrations below 20 μg g^{-1}. The apparent threshold concentration identified for wetland soils as well as upland soils, sediment and tailings indicate that Hg concentrations between 17 and 20 μg g^{-1} inhibit microbial growth and biotic MeHg methylation. While this effect in itself is not surprising, it is notable that microbial biomass persists at lower levels and microbial Hg methylation continues even at Hg concentrations exceeding 20 μg g^{-1}.

Seasonal shifts in wetland biogeochemistry can be seen in MeHg between December 2004 and March 2005. Following the dry summer to fall, December rainfall initiated increased flow in Davis Creek, flooding seasonal riparian wetlands and increasing the soil moisture content in permanent wetlands. Soil moisture is strongly correlated with MeHg concentrations ($r^2 = 0.71$ in December; $r^2 = 0.64$ in March; Fig. 6). Soil nutrient concentrations were greater in December (5–24 μg g^{-1} DOC; 1–116 μg g^{-1} $SO_4^{=}$ with one anomalous value of 818 μg g^{-1}; 1–34 μg/g NO_3^{-1}) early in the wet season. As vegetation growth increases into March, plant uptake of nutrients decreases nutrient concentrations available for microbial activity (1–7 μg g^{-1}

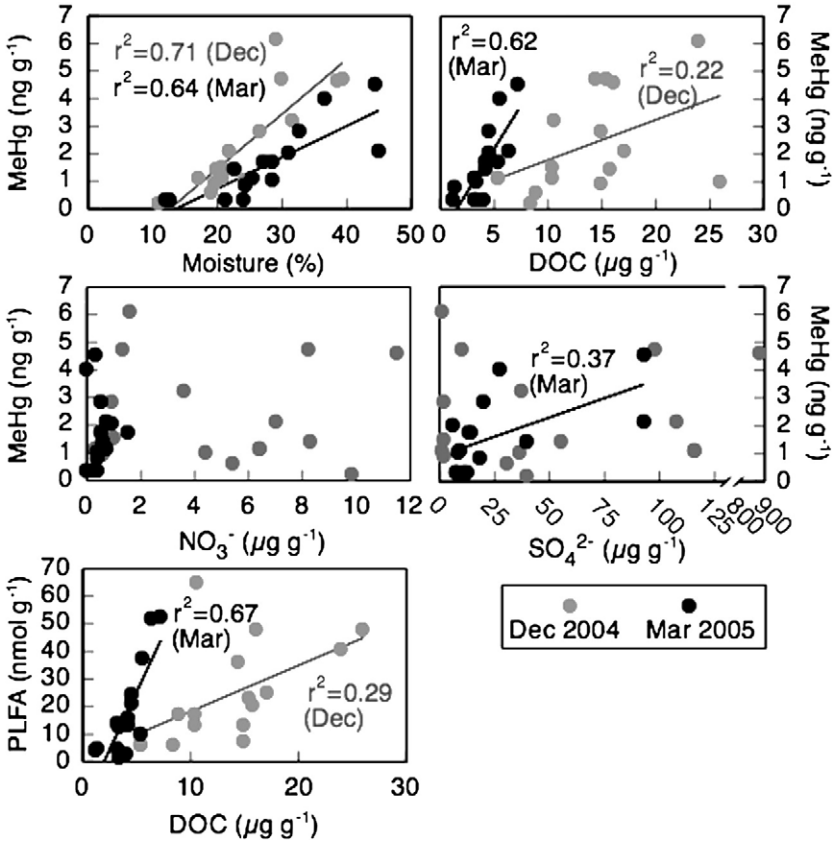

Fig. 6. Data from all horizons of riparian and delta wetlands showing seasonal differences in the relationship between MeHg and nutrient concentrations.

DOC; 6–93 μg g^{-1} $SO_4^{=}$; 0–1.5 μg/g NO_3^{-1}). Vegetation decay during the dry late spring to summer months releases nutrients back into the soil pore waters. Reduced nutrient pools result in an increased degree of correlation of MeHg to DOC concentrations in March ($r^2 = 0.62$) relative to December samples ($r^2 = 0.22$), suggesting that the available organic carbon pool becomes limiting for microbial Hg methylation. The relatively low correlation between MeHg and $SO_4^{=}$ concentrations ($r^2 = 0.37$) suggests that even in March, there is an excess of $SO_4^{=}$ in these wetland ecosystems relative to the amount of S required to sustain methylation by SRB. These shifts in nutrient concentration drive variations in soil microbial communities.

Seasonal shifts in biogeochemistry and microbial community structure for wetland soils were modeled using PCA (Fig. 7). Axis 1 explains 29.9% of data variability with 59.7% environmental and microbial data variability explained by Axis 2. There is a weakly correlative relationship between Hg and MeHg in this model, reflecting wetland soils with actively growing vegetation communities. All nutrients were positively correlated with MeHg, Delta A and B1 horizon soils from December plotted in areas that are perpendicular to the vector for *Desulfovibrio* sp. (i17:1). There is a shift in delta soil microbial communities indicated by an arrow in Fig. 7. This shift was produced by decreased nutrient concentrations (Fig. 6), with a decrease in vectors for 18:1ω(7,9,12)c, a biomarker for aerobic bacteria and algae, and vectors for common PLFAs 16:1ω5c and 16:1ω7c. This shift also increases the degree of sample correspondence to the vector for the *Desulfobacter* sp. biomarker 10me16:0. Riparian wetland soils do not show any notable seasonal shifts or changes with soil horizon in this model.

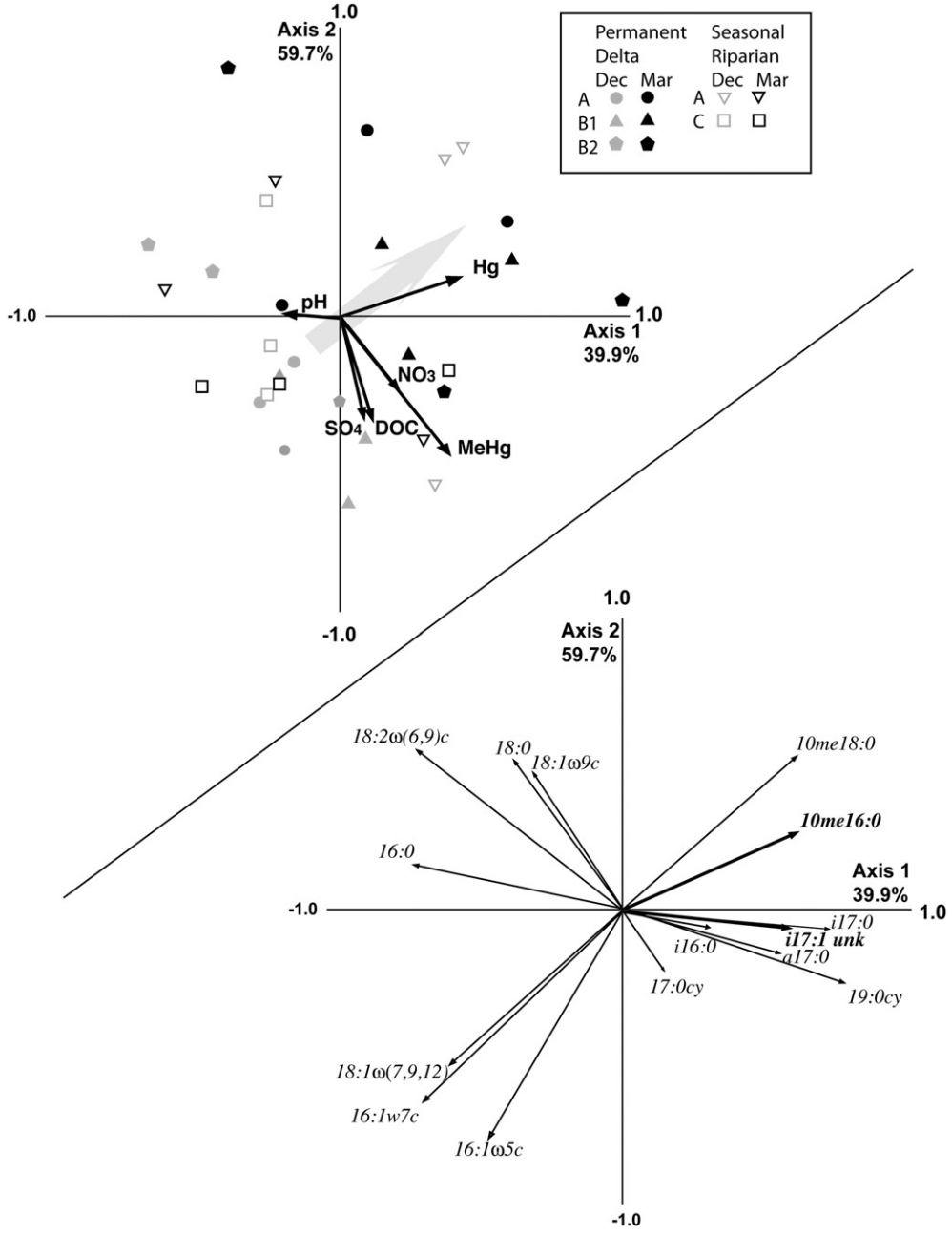

Fig. 7. Principal components analysis (PCA) of PLFA biomarkers and environmental data (Hg, MeHg, pH, nutrients) for permanent (delta) and seasonal (riparian) soils collected in December 2004 and March 2005. Axis 1 explains 39.9% of variance and Axis 2 explains 59.7% of the variance between PLFA and environmental conditions. Plots are on the same set of coordinates. Samples are represented as points with vectors showing the general direction of variable increase. Angles between vectors indicate relationships between variables. Acute angles show positive correlations, angles >90° show negative correlations. The relationship between environmental and PLFA vectors and sample points can be defined by projecting a perpendicular line between a given sample point and a vector. The grey block arrow indicates a general direction of shift for permanent wetlands from December to March.

3.5. Soil, organic matter and mercury

Mercury in the Knoxville mining district occurs primarily as HgS minerals (Kim et al., 2004) with limited solubility, inhibiting the formation of MeHg from the aqueous species Hg^{2+}. However, organic matter, in particular, aromatic organic compounds, have been shown to enhance the dissolution of HgS, possibly through the surface complexation of HgS and oxidation of surface sulfur species by the organic matter (Ravichandran et al., 1998). Sequential extractions performed in a separate study on soils described in the present work found that Hg was present as HgS in the delta and riparian wetland soils and in tailings with Hg bound to oxide minerals (e.g. chromite) in a serpentine soil (Süß, 2006). Laboratory experiments with these soils produced an increase in Hg^{2+} with increasing pH due to solubilization of soil organic matter (SOM) (Süß, 2006). Derived primarily from vegetation, SOM could potentially facilitate the conversion of HgS to Hg(II). For this reason, alluvial soils as well as their sediment parent material should be examined when evaluating the potential for methylation in watersheds.

3.6. Methylation: from headwaters to the San Francisco Bay

Elevated methyl-Hg concentrations are associated with San Francisco Bay estuaries, from 1–12 ng g^{-1} methyl-Hg (Thomas et al., 2002). Cache Creek, a tributary in the Sacramento River watershed, has extensive historic Hg mines in its headwaters, had a MeHg load of 6.7 g MeHg day^{-1} measured over a 31 day period in 2000 (Domagalski et al., 2004). Elevated MeHg concentrations in Cache Creek in turn resulted in the bioaccumulation and trophic transfer of Hg and MeHg in aquatic organisms, including crayfish (Hothem et al., 2007) and fish (Slotton et al., 2004), leading to the release of multiple fish advisories (Gassel et al., 2005). By comparison, most upland soils in the Davis Creek watershed had relatively low MeHg concentrations (<0.3 ng g^{-1} MeHg). The volcanic soil was a notable exception, with 1.3 ng g^{-1} MeHg. Wetland soils had elevated MeHg concentrations (0.5–5.1 ng g^{-1} MeHg) due to the enhancement of methylation through a large pool of DOC and other nutrients created by seasonal cycles of vegetation growth and decay. Thus, the wetlands along Davis Creek Reservoir enhance localized Hg methylation while reducing the downstream movement of Hg-rich sediments resulting from historic mining.

4. Conclusions

The formation of MeHg in the Davis Creek watershed was a function of nutrient supply and soil moisture. Sulfate was not a limiting factor in Hg methylation as concentrations are elevated in all soil pore waters. Total Hg concentrations span three orders of magnitude in this historic Hg mining district, with the greater concentrations appearing to suppress the biological formation of MeHg. The greatest MeHg concentrations were in wetland soils (0.5–5.1 ng g^{-1} MeHg), at least twice the magnitude of MeHg in the stream sediment parent material (0.19–0.25) for these soils. Microbial consortia identified using PLFA biomarkers included *Desulfovibrio* sp. (i17:1), *Desulfobacter* sp. (10me16:0).

The determinants of microbial community structure (nutrients and soil moisture) were related to the formation of MeHg. Wetland soils had much greater concentrations of MeHg than stream sediments, the parent material for these soils. The stability of soil and accumulation of DOC, $SO_4^{=}$, and NO_3^{-} from decaying vegetation supported a larger microbial biomass and more diverse microbial community. Wetland microbial communities varied more significantly with soil or sediment composition than with time.

While the results of this study indicate that MeHg production is magnified in wetland soils, the value of the Davis Reservoir wetlands for Hg-rich sediment entrainment and on-site Hg removal should be considered. A study of constructed wetlands indicated that the benefits of a wetland, including the entrainment of suspended and particulate Hg removal, should be considered together with the risk of MeHg production (Gustin et al., 2006).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemgeo.2009.03.031.

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