

Experimental removal of wetland emergent vegetation leads to decreased methylmercury production in surface sediment

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[1] We performed plant removal (devegetation) experiments across a suite of ecologically diverse wetland settings (tidal salt marshes, river floodplain, rotational rice fields, and freshwater wetlands with permanent or seasonal flooding) to determine the extent to which the presence (or absence) of actively growing plants influences the activity of the Hg(II)-methylating microbial community and the availability of Hg(II) to those microbes. Vegetated control plots were paired with neighboring unvegetated plots in which photosynthetic input was terminated 4–8 months prior to measurements, through clipping aboveground biomass, severing belowground connections, and shading the sediment surface to prevent regrowth. Across all wetlands, unvegetating decreased the activity of the Hg(II)-methylating microbial community (k_{meth}) by 38%, calculated MeHg production potential (MP) rates by 36%, and pore water acetate concentration by 78%. Decreases in MP were associated with decreases in microbial sulfate reduction in salt marsh settings. In freshwater agricultural wetlands, decreases in MP were related to indices of microbial iron reduction. Sediment MeHg concentrations were also significantly lower in unvegetated than in vegetated plots in most wetland settings studied. Unvegetating effects were correlated with live root density (percent volume) and were most profound in vegetated sites with higher initial pore water acetate concentrations. Densely rooted wetlands had the highest rates of microbial Hg(II)-methylation activity but often the lowest concentrations of bioavailable reactive Hg(II). We conclude that the exudation of labile organic carbon (e.g., acetate) by plants leads to enhanced microbial sulfate and iron reduction activity in the rhizosphere, which results in high rates of microbial Hg(II)-methylation and high MeHg concentrations in wetland sediment.

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1. Introduction

[2] Methylmercury (MeHg) is a significant contaminant of aquatic food webs in many regions of the world. MeHg production occurs primarily in sediment or peat substrates and is facilitated largely by sulfate-reducing bacteria [Compeau and Bartha, 1985; Gilmour *et al.*, 1992], although iron-reducing bacteria may also play a role [Warner *et al.*, 2003; Fleming *et al.*, 2006; Kerin *et al.*, 2006]. Wetlands have been demonstrated to be particularly active habitats with respect to MeHg production [Zillioux *et al.*, 1993; St. Louis *et al.*, 1994; Lacerda and Fitzgerald, 2001; Marvin-DiPasquale *et al.*, 2003; Hall *et al.*, 2008; Selvendiran *et al.*, 2008]. For MeHg production to be maximized, however, both microbial activity and Hg(II) availability must be plentiful [Benoit *et al.*, 2003; Drott *et al.*, 2007]. Because these terms tend to be inversely

correlated [Gilmour *et al.*, 1998; Benoit *et al.*, 1999], there are only a few landscape and localized habitats that may meet these conditions.

[3] At the landscape scale, tidally and seasonally flooded wetlands undergo periodic wetting and drying, which may lead to enhanced MeHg production [Ulrich *et al.*, 2001; Hall *et al.*, 2008], by stimulating microbial activity and potentially by making Hg(II) more available to microbes via the reoxidation of reduced sulfur species [Yee *et al.*, 2007]. At the local scale, the thin surface sediment interface and the larger root:soil interface (rhizosphere) share these fluctuating redox conditions and labile carbon supply that are capable of promoting high microbial MeHg production. Periodically inundated systems share these soil conditions as they commonly have densely rooted surface soils, which coincides with the zone where MeHg production is typically the greatest [Gilmour *et al.*, 1998] and where MeHg pools are most likely to become suspended or diffuse into surface waters [Langer *et al.*, 2001].

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[4] Vegetation can influence sediment biogeochemistry in both terrestrial and wetland ecosystems through plant:soil feedbacks [Ehrenfeld *et al.*, 2005]. A primary influence on sediment biogeochemistry is rhizosphere activity and physiology [Marschner, 1986]. Root:soil interactions affect a number of processes and geochemical characteristics in the rhizosphere zone, including (1) microbial community structure and activity [Bagwell *et al.*, 1998; Hines *et al.*, 1989; Borga *et al.*, 1994; Westover *et al.*, 1997], (2) dissolved organic carbon quality [Hines *et al.*, 1994; Garland, 1996; Cheng *et al.*, 2003], (3) the concentration and availability of electron acceptors to microbes [Roden and Wetzel, 1996; Blaabjerg and Finster, 1998; Lee *et al.*, 1999], and (4) nutrient/contaminant speciation [Marins *et al.*, 1997; Windham and Ehrenfeld, 2003; Jacob and Otte, 2003]. In the current report, we test the hypothesis that there is a direct linkage between the processes of microbial MeHg production in wetland surface sediment and the impact of emergent wetland plants on biogeochemical and microbial processes in the rhizosphere zone, especially in terms of Hg(II) availability and microbial Hg(II)-methylation rates. To test this linkage, short-term vegetation removal experiments were performed in a diverse suite of wetlands settings across the salinity gradient in the San Francisco Bay-Delta. This report represents a cross-system analysis of MeHg production across wetland types and its response to experimental devegetation.

2. Methods

2.1. Site Descriptions

[5] Emergent wetland plant devegetation experiments were conducted from December 2005 to October 2007 as part of four separate wetland studies within the San Francisco Bay-Delta (SFB-Delta) region. The specific sampling regions (Figure 1) included (1) historic tidal salt marshes in northern San Francisco Bay (Petaluma), (2) younger, subsiding tidal salt marshes in south San Francisco Bay (Alviso), (3) agricultural and nonagricultural managed freshwater wetlands in the northwest region of the SFB-Delta (Yolo Bypass), and (4) a seasonally inundated freshwater river floodplain in the northeast SFB-Delta (Cosumnes River). Within each study region, different subhabitats were identified based upon hydrology and dominant vegetation type (Table 1). In Petaluma, two types of vegetated subhabitat were sampled, on the basis of distance to slough channels, edge sites (<1 m from a slough) and interior sites (>15 m from any slough). In Alviso, three vegetated subhabitats were sampled (low, mid, and high marsh) along a 1 m elevation gradient that increased with distance from Alviso slough. In the Yolo Bypass Wildlife Area, three types of flooded agricultural wetlands were studied (white rice, wildrice and fallow fields), and three nonagricultural managed wetland areas, one seasonally flooded and two permanently flooded. Of the two agricultural fallow fields, one was devoid of vegetation (barren fallow) and the other had a densely rooted mixed plant community (vegetated fallow). For the purposes of data analysis, these two distinctly different fallow fields were treated separately. In the Cosumnes River Floodplain, a single rush-dominated community was studied in an otherwise diverse mosaic of vegetation types. All sampling regions also included naturally nonvegetated habitats (slough channels or open water ponds) near the vegetated sites, which

were also sampled for comparison to vegetated (control) and experimentally devegetated sites.

2.2. Experimental Field Manipulation

[6] Four to eight months prior to sample collection, 1 m² devegetation plots were established in each area, so that for every vegetated plot there was a neighboring, manipulated devegetated plot with similar initial edaphic conditions. Sampling dates and replicates are listed in Table 1. When vegetation was present, all aboveground biomass (live and dead) was clipped to the ground surface and removed from the plot. A spade was used to cut roots with a 30 cm deep slit along the edge of the plots to inhibit root growth and root-mediated inputs to the devegetated plots. The plots were then covered with professional-grade water-permeable landscape cloth, to shade the sediment and inhibit vegetation regrowth during the study period. Plots were revisited 2–3 times during the growing season to retrench devegetated plots and to measure primary productivity in adjacent vegetated (control) plots. Sample names and edaphic variables are listed in Table 2 for comparison between all sites and between vegetated and naturally nonvegetated habitats. All sites had naturally occurring nonvegetated habitats sampled at the same time as the vegetated plots and the experimentally devegetated plots.

2.3. Field Sampling

[7] At the peak of the growing season (June–December depending on the wetland type), plots were revisited and the landscape cloth lifted to access the underlying sediment surface. To assure wetted soil conditions, tidal saltmarsh sites were only sampled after inundation, during low tide periods during spring tide events. Root density and depth profiles were collected from both vegetated and devegetated plots by taking 30 cm deep cores, which were temporarily preserved on wet ice to slow microbial processes, and were subsequently sectioned into 2 cm depth intervals in the laboratory. Surface sediment (0–2 cm depth) was sampled concomitantly in neighboring devegetated and vegetated plots, using 2 cm deep (6 cm i.d.) precut polycarbonate core rings. Between 5 and 10 surface core “patties” were collected per plot and composited into two glass mason jars (1 pt). These surface sediment composites were homogenized briefly and subsampled in the field for total mercury (THg), reactive inorganic mercury (Hg(II)_R), and MeHg, then frozen immediately on dry ice. Subsequently, the mason jars were topped off with additional sediment (leaving no head space), stored on ice and returned to the laboratory within 24 h. Parameters measured in the field at the time of sediment collection included temperature, oxidation reduction potential (Eh), and pH, all measured with field portable instruments and probes (Oakton© 300 pH/mV meter, ColeParmer© 05990–55 ORP platinum electrode).

[8] Three additional surface sediment patties were collected at each site for analysis of root biomass and root density in the 0–2 cm depth interval. The resulting sediment patties were rinsed of mineral soil and the live roots were manually harvested with forceps, as visually identified by turgidity and color. A subsample of live roots were subjected to a vital stain (1% tetrazolium red) followed by

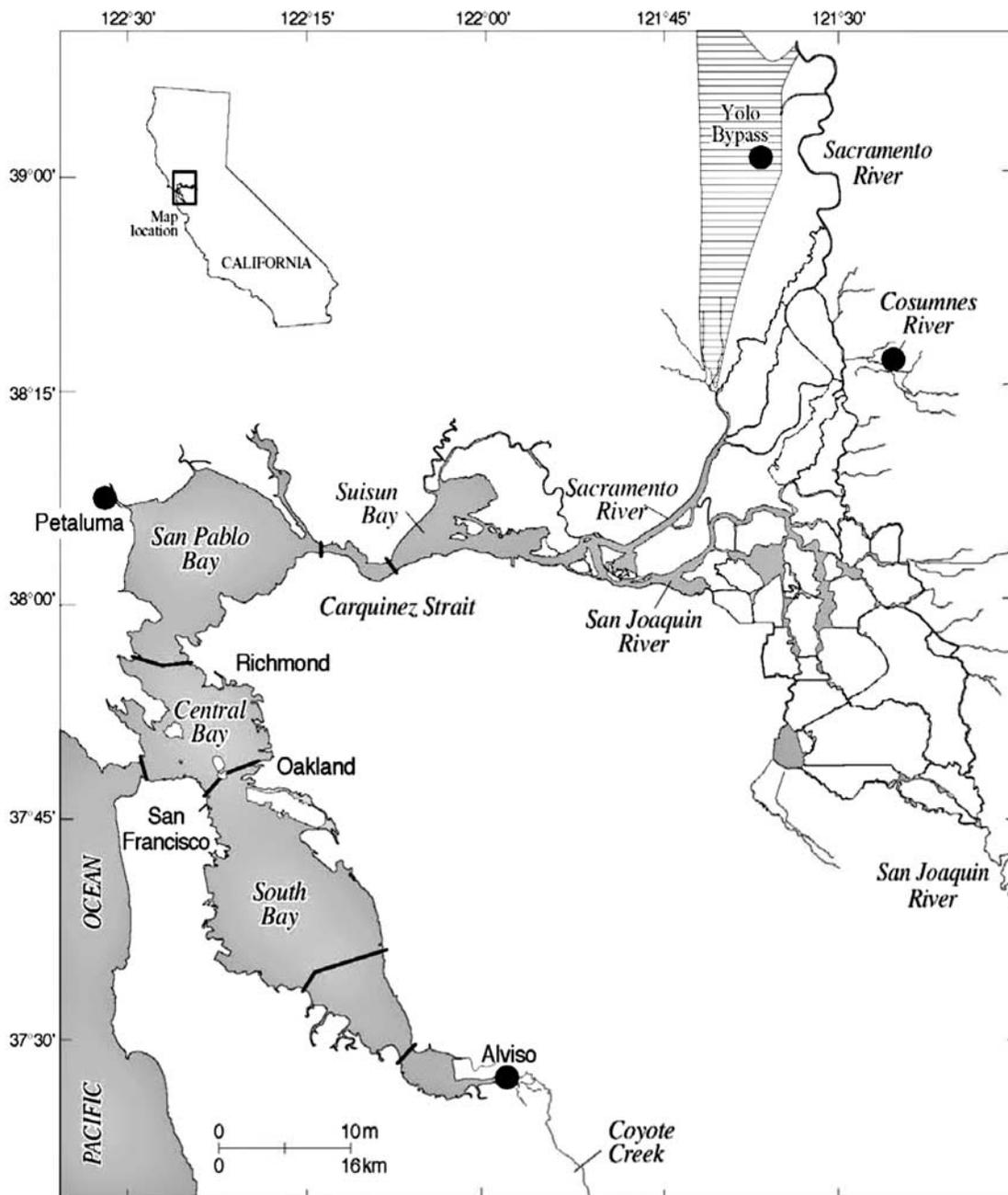


Figure 1. Map of field sites in San Francisco Bay-Delta watershed of California, United States. Map coordinates reported in WGS84. Separate hydrologic units include the Petaluma River, Alviso Slough, Cosumnes River, and the Yolo Bypass flood control channel.

dissection under $40\times$ magnification, which was used to assess errors of commission. Such errors were less than 5% for all samples collected. Live roots for each replicate sediment patty were rinsed thoroughly and then assessed for volume by displacement of deionized water in a 50 or 100 ml graduated cylinder [Böhm, 1979]. These samples were then freeze-dried and weighed to assess root dry biomass. These root density data, collected from discrete 0–2 cm patties, were compared with the 0–2 cm data from the 0–30 cm deep root profiles, and in all cases, the root profile biomass from this 0–2 cm surface interval was

found to be within ± 1 standard deviation of the biomass calculated using the abovementioned surface patties.

2.4. Laboratory Analyses

[9] A summary of all parameters measured is given in Table 3. Field frozen sediment samples for mercury speciation (THg, Hg(II)_R , and MeHg) were analyzed within 3 months of collection. Sediment THg concentrations were analyzed by cold vapor atomic fluorescence analysis (CVAFS) on sediment digestate as described by Olund *et al.* [2004]. Quality assurance for THg included method

Table 1. Description of Subhabitats and Sampling Regimes for the Devegetation Plots During Summer 2006 and 2007 at the Period of Peak Biomass^a

Study Area	Site Types	Dominant Vegetation Type	Maximum Plant Root Depth (cm)	Devegetation Plot Establishment Date	Devegetation Plot Sampling Date	Number of Subhabitat Replicates (N)	Total Number of Samples
Petaluma River	Edge	Gumplant (<i>Grindelia stricta</i>)	>50	Apr. 2006	Aug. 2006	6	6
	Interior	Pickleweed (<i>Sarcocornia pacifica</i>) ^b	14	Apr. 2006	Aug. 2006	6	6
	1st slough	—	—	—	Aug. 2006	6	6
Alviso Marsh	3rd slough	—	—	—	Aug. 2006	6	6
	Low	Cordgrass (<i>Spartina foliosa</i>)	12	Mar. 2007	Jul. and Oct. 2007	2	4
	Med	Bulrush (<i>Bolboschoenus maritimus</i>) ^c	>50	Mar. 2007	Jul. and Oct. 2007	2	4
	High	Pickleweed (<i>Sarcocornia pacifica</i>)	21	Mar. 2007	Jul. and Oct. 2007	2	4
Yolo Bypass	Slough	—	—	—	—	5	5
	Flooded White Rice	Rice (<i>Oryza sativa</i>)	24	May 2007	Aug. 2007	2	2
	Flooded Wildrice	Wildrice (<i>Zizania palustris</i>)	29	May 2007	Aug. 2007	2	2
	Flooded Fallow	Mixed (<i>Echinochloa</i> , <i>Cyperus</i>)	14	Jun. 2007	Aug. 2007	2	2
	Seasonally Flooded Wetland	Jointgrass (<i>Paspalum distichum</i>)	—	May 2007	Nov. and Dec. 2007	2	2
	PW-Tule	Tule (<i>Schoenoplectus acutus</i>) ^d	>50	May 2007	Aug. and Dec. 2007	1	2
	PW-Cattail	Cattail (<i>Typha domingensis</i>)	>50	May 2007	Aug. and Dec. 2007	1	2
Cosummes River	PW-open	—	—	—	Aug. and Dec. 2007	2	3 ^e
	Floodplain-Vegetated	Rush (<i>Juncus balticus</i>)	16	Nov. 2005	Jun. 2006	1	1
	Floodplain-Pond	—	—	—	Jun. 2006	3	3
				Summary		N = 29	N = 37

^aItalics denote subhabitats that were nonvegetated.^bFormerly *Salicornia virginica*.^cFormerly *Scirpus maritima*.^dFormerly *Scirpus acutus*.^eRefers to the replicate permanent wetland, sampled only once in December 2007.

Table 2. Sediment Properties in Vegetated Subhabitats and Nonvegetated Shallow Water Environments for Each Wetland Studied^a

Site Names	Root Density (% vol)	Peak Biomass (kg m ⁻²)	Sediment Pore Water Cl (mM)	Sediment Organic Content (%LOI)	Sediment THg (ng g ⁻¹)	Sediment MeHg (ng g ⁻¹)	Sediment %MeHg of THg	Sediment k _{meth} (d ⁻¹)	Sediment Hg(II) _R (ng g ⁻¹)	Sediment MP (pg g ⁻¹ d ⁻¹)
PET-Edge	1.2 ± 1.9	2.1 ± 0.4	279 ± 21	11.9 ± 0.5	333 ± 6	2.0 ± 0.5	0.6 ± 0.1	0.002 ± 0.001	6.6 ± 1.0	9 ± 1
PET-Interior	18.1 ± 13.8	2.3 ± 0.6	374 ± 22	21.4 ± 3.0	256 ± 15	3.7 ± 1.5	1.4 ± 0.5	0.122 ± 0.020	0.6 ± 0.1	83 ± 26
PET-slough1	0	0	313 ± 16	12.4 ± 1.3	280 ± 14	1.6 ± 0.4	0.6 ± 0.2	0.030 ± 0.007	0.2 ± 0.2	7 ± 2
PET-slough3	0	0	308 ± 21	7.7 ± 0.5	379 ± 17	0.7 ± 0.1	0.2 ± 0.1	0.005 ± 0.002	0.5 ± 0.4	3 ± 1
ALV-Low	3.5 ± 2.0	2.0 ± 0.2	412 ± 20	7.1 ± 0.4	317 ± 51	0.8 ± 0.3	0.2 ± 0.1	0.036 ± 0.017	5.6 ± 2.6	73 ± 5
ALV-Med	2.8 ± 1.3	3.4 ± 2.6	506 ± 10	11 ± 0.8	320 ± 42	0.8 ± 0.6	0.2 ± 0.1	0.015 ± 0.009	8.9 ± 4.1	51 ± 33
ALV-High	10.1 ± 7.9	1.5 ± 0.5	512 ± 10	13 ± 0.5	328 ± 76	1.7 ± 1.2	0.6 ± 0.4	0.017 ± 0.008	8.6 ± 6.4	49 ± 65
ALV-slough 3	0	0	396 ± 12	6.3 ± 0.6	559 ± 25	0.6 ± 0.3	0.1 ± 0.1	ND	1.2 ± 1.7	ND
YOLO-White Rice	3.6 ± 1.2	2.1 ± 0.3	4.2 ± 0.5	6.2 ± 0.3	411 ± 21	1.0 ± 0.4	0.2 ± 0.1	0.102 ± 0.094	1.2 ± 0.2	105 ± 78
YOLO-Wildrice	6.1 ± 4.7	2.1 ± 0.3	8.4 ± 3.1	6.3 ± 0.5	298 ± 11	0.7 ± 0.1	0.2 ± 0.1	0.104 ± 0.065	0.6 ± 0.1	66 ± 29
YOLO-Fallow-Mixed	2.8 ± 0.9	0.4 ± 0.3	4.2 ± 0.0	6.5 ± 0.1	204 ± 38	1.0 ± 0.3	0.5 ± 0.1	0.136 ± 0.020	1.8 ± 0.1	217 ± 33
YOLO-Fallow-Barren	0 ± 0	0 ± 0	2.8 ± 0.0	5.9 ± 0.2	135 ± 28	0.7 ± 0.3	0.5 ± 0.1	0.015 ± 0.001	0.8 ± 0.1	12 ± 1
YOLO-Seasonal Wetland	4.1 ± 1.0	0.7 ± 0.3	1.4 ± 0.1	9.6 ± 0.2	169 ± 12	1.3 ± 0.1	0.8 ± 0.1	0.075 ± 0.008	0.1 ± 0.1	8 ± 1
YOLO-PW-Tule	8.6 ± 2.1	3.9 ± 1.4	2.1 ± 0.8	7.2 ± 0.3	125 ± 26	1.3 ± 1.1	0.9 ± 0.4	0.300 ± 0.129	0.2 ± 0.1	67 ± 11
YOLO-PW-Cattail	1.7 ± 0.9	3.1 ± 0.8	2.0 ± 0.7	9.6 ± 0.4	138 ± 31	1.1 ± 0.8	0.8 ± 0.4	0.365 ± 0.145	0.3 ± 0.1	100 ± 63
YOLO-PW-openwater	0	0	1.9 ± 0.8	6.1 ± 0.2	134 ± 6	0.5 ± 0.1	0.4 ± 0.1	0.096 ± 0.079	0.2 ± 0.1	15 ± 23
CRF-vegetated	17.8 ± 5.0	1.45	0.4 ± 0.1	10.8 ± 0.5	109 ± 69	9.2 ± 0.4	8.7 ± 0.4	0.027 ± 0.002	1.4 ± 0.4	39 ± 3
CRF-pond	0	0	0.1 ± 0.0	7.5 ± 0.0	181 ± 13	1.7 ± 0.1	0.9 ± 0.1	0.001 ± 0.001	0.3 ± 0.2	1 ± 1

^aData are reported for the time of peak biomass in either 2006 (Petaluma and Cosumnes Rivers) or 2007 (Alviso Marsh and Yolo Bypass). Error is ±1 standard deviation; N = 3–6 replicates, except for Yolo Bypass Fallow fields, where data represent the mean and difference for 2 replicates. All vegetation and sediment data are reported in dry weight. PET, Petaluma River; ALV, Alviso Marsh; YOLO, Yolo Bypass Wildlife Area; CRF, Cosumnes River Floodplain. Italics denote subhabitats that were nonvegetated.

Table 3. Description of Scientific Parameters Measured (Abbreviations)^a

Parameter Code	Units	Description
sed k_{meth}	days ⁻¹	Sediment microbial Hg(II) methylation rate constant
sed Hg(II) _R	ng g ⁻¹ dry weight	Sediment inorganic “reactive” mercury
sed MP	pg g ⁻¹ d ⁻¹ dry weight	Calculated sediment methylmercury production rate (k_{meth} Hg(II) _R)
sed MeHg	ng g ⁻¹ dry weight	Sediment methylmercury concentration, dry weight
sed SR	nmol g ⁻¹ d ⁻¹ dry weight	Sediment microbial sulfate reduction rate
sed TRS	μmol g ⁻¹ dry weight	Sediment total reduced sulfur
sed AVS	μmol g ⁻¹ dry weight	Sediment acid volatile sulfur
sed Fe(II)	mg g ⁻¹ dry weight	Sediment (acid-extractable) ferrous iron
sed aFe(III)	mg g ⁻¹ dry weight	Solid phase amorphous (poorly crystalline) ferric iron concentration
pw DOC	mg L ⁻¹	Porewater dissolved organic carbon
pw acetate	μmol L ⁻¹	Porewater acetate
pw S ²⁻	μmol L ⁻¹	Porewater sulfide
pw Fe(II)	mg L ⁻¹	Porewater ferrous iron
pw Cl	mmol L ⁻¹	Porewater chloride
Redox	mV	Sediment oxidation-reduction potential (E _h)

^aHere sed is sediment and pw is porewater.

blanks <0.1 ng/L, field duplicates within ±25%, laboratory duplicates within ±10%, matrix spikes within 75–125% recovery, and certified reference material (ERM580) within 80–120% recovery. Sediment Hg(II)_R concentration is a proximate measure of the pool of inorganic Hg(II) most readily available for Hg(II)-methylation [Marvin-DiPasquale et al., 2006], and was analyzed by stannous chloride reduction followed by CVAFS [Marvin-DiPasquale and Cox, 2007]. Quality assurance for Hg(II)_R included method blanks (<1 pg), field duplicates (when available) within ±25%, and laboratory duplicates within ±30%. Because the samples were collected as part of four unique field studies, with different collaborative research teams, sediment MeHg was extracted using two different methods: (1) alkaline (potassium hydroxide and methanol) extraction [Bloom, 1989] in the case of Yolo Bypass and Alviso samples and (2) via methylene chloride [DeWild et al., 2004] in the case of Cosumnes River and Petaluma samples. Following either extraction, samples were analyzed for MeHg by ethylation, gas separation, pyrolysis, and CVAFS [U.S. Environmental Protection Agency, 2002]. Quality assurance for all data were determined with method blanks <1 pg, field duplicates within ±30%, laboratory duplicates within ±25%, matrix spikes within 65–135% recovery, and certified reference material (IAEA 405, IAEA580) within 70–130% of the certified value. Percent MeHg (%MeHg) was calculated on the basis of the dry weight concentrations of MeHg divided by THg (×100), as %MeHg is a common index of mercury methylation rates in field samples [Lacerda and Fitzgerald, 2001; Conaway et al., 2003; Lambertsson and Nilsson, 2006].

[10] Sediment samples preserved on wet ice in mason jars were returned to the laboratory and subsampled within 24 hours under anaerobic conditions for microbial Hg(II)-methylation and sulfate reduction rates, using standard radioactive isotope amendment approaches (²⁰³Hg(II) and ³⁵SO₄²⁻, respectively [Marvin-DiPasquale and Agee, 2003]). The resulting Hg(II)-methylation rate constant (k_{meth}) provides a measure of the activity of the Hg(II)-methylating microbial community in a given sample, relative to an addition of “bioavailable” ²⁰³Hg(II) (as HgCl₂). Site/treatment specific methylmercury production potential rates (MP) were subsequently calculated as a first-order process from values of k_{meth} and independently measured concentrations of Hg(II)_R [Marvin-DiPasquale et al., 2003].

[11] Additional sediment measurements included percent water, percent organic content (as loss on ignition; %LOI), grain size distribution expressed as percent fines (%fines; size fraction <63 μm), bulk density and porosity, as detailed by Marvin-DiPasquale and Cox [2007]. Pore water was separated from the remaining bulk sediment by centrifugation at 3000 rpm followed by filtration (0.45 μm). Subsamples for pore water sulfate, chloride, and acetate were immediately frozen in crimp-sealed vials under anaerobic conditions. Sulfate and chloride were analyzed by ion chromatograph [Dionex Corporation, 1992] and acetate was analyzed by high-performance liquid chromatograph using a Shimadzu VP series chromatograph with a UV-visual detector (SPD-10AVP) set at 210 nm [Mueller-Harvey and Parkes, 1987]. Pore water dissolved organic carbon (DOC) subsamples were analyzed within 24 h for UV absorbance at 254 nm and then preserved with phosphoric acid at 0.2% v/v, and subsequently assayed for DOC concentration via an automated high-temperature DOC analyzer [Qian and Mopper, 1996]. Pore water sulfide was preserved with an antioxidant buffer and assayed via a selective ion electrode [Clesceri et al., 1998]. Pore water ferrous iron (PW Fe(II)) was preserved by acidification with hydrochloric acid at 0.2% v/v and assayed via the Ferrozine colorimetric assay [Gibbs, 1979]. Acid volatile sulfur (AVS) and total reduced sulfur (TRS) were analyzed colorimetrically [Cline, 1969] after extraction with an HCl/TiCl or CrCl extraction, respectively, into zinc acetate [Fossing and Jørgensen, 1989; Roden and Tuttle, 1993]. Whole sediment iron speciation included acid extractable Fe(II) and amorphous (poorly crystalline) ferric iron (aFe(III)) [Lovely and Phillips, 1987], as well as crystalline ferric iron (cFe(III)) [Roden and Zachara, 1996]. For Yolo Bypass and Alviso Marsh sediment, frozen sediment samples were also analyzed for benthic chlorophyll-a concentration, using acetone extraction followed by centrifugation and spectrophotometric analyses [Parsons et al., 1984].

[12] Net changes in individual iron species concentration (normalized per day) were calculated in two of the study areas, PET tidal marshes and YOLO agricultural fields, using the concentration difference between two dates during the April–August growing season. Although Fe(III)-reduction rates were not directly measured in short-term incubations, as were rates of microbial sulfate reduction, the conservative

behavior of total iron concentrations in these surface sediment samples through time ($18\text{--}20\text{ mg g}^{-1}$) allowed us to calculate an average daily rate of change in sediment pools of Fe(II), aFe(III) and cFe(III) over the growing season. Availability of aFe(III) was also used as an indicator of conditions favorable for iron reduction [Roden, 2008].

2.5. Statistics

[13] Statistical analyses were performed using SPlus 7.0 [Insightful Corporation, 2001]. Data from the 29 paired plots were categorized by site and/or treatment (vegetated control plot versus devegetated plot). Because of the large range in values for individual parameters among the four study areas, we do not report absolute difference between vegetated and devegetated plots. Instead, we focus on the relative effects of devegetation, as a way to interpret the major vegetation effects across multiple habitat types. For each site specific vegetated-devegetated plot pair, a relative metric for the magnitude and direction of the devegetation effect (%DevegEffect) had on a given parameter (e.g., $X = k_{\text{meth}}$, Hg(II)_R , MeHg, etc...) was calculated as the % difference between devegetated and vegetated control plots, such that

$$\%DevegEffect = \left(\frac{X_{\text{vegetated plot}} - X_{\text{devegetated plot}}}{X_{\text{vegetated plot}}} \right) 100.$$

Normality of each parameter was assessed with Kolmogorov-Smirnov tests, and nonparametric data were log-transformed for normality. Comparisons based on vegetation status alone (control versus devegetated) were made using one-way analysis of variance (ANOVA; $df = 1$) and reported in Table 4. One-way ANOVA was also used to test differences between parameter specific %DevegEffect data grouped by freshwater versus saline habitat and grouped by agricultural versus nonagricultural freshwater wetlands (Table 4). Although the devegetation effect was profound enough for some measured parameters to warrant direct ANOVA comparisons of vegetation status (vegetated versus devegetated), the calculation of the %DevegEffect metric for paired plots provides a clearer sense of the devegetation effect across a continuum of wetland conditions. Pairwise t tests were used to compare paired plots for relative influences of devegetation within habitat categories and within each replicate subhabitat. Significance was determined after adjustment for multiple comparisons using the smallest critical point from posthoc tests (e.g., Bonferroni, Scheffe, Fisher, Tukey). Correlation analyses were used to determine the extent to which the %DevegEffect for given parameters were related to each other (correlation of effects, using only significant %DevegEffect comparisons). Only significant correlations are reported ($p < 0.05$), as assessed by comparison with t_{crit} for a two-tailed distribution and $df = 1$.

3. Results

3.1. Site Characteristics

[14] Across wetland types, the general trend for sediment %MeHg was higher concentrations in the vegetated wetlands and lower concentrations in the neighboring naturally nonvegetated shallow water environments (sloughs, ponds and the open water zones of permanent wetlands, Table 2).

Table 4. ANOVA and Pairwise t Test Results for All Data Grouped as Either Vegetated (Control Site) and Devegetated^a

Factor	ANOVA			Pairwise t Test (P)	Average %Deveg Effect
	N	F	p		
Sed MP	57	5.360	0.024	<0.05	$-36 \pm 17\%$
Sed Hg_R	57	0.0389	0.844	>0.05	ns
Sed k_{meth}	57	8.004	0.006	<0.05	$-38 \pm 17\%$
PW DOC	61	10.017	0.002	<0.05	$-34 \pm 10\%$
PW acetate	61	29.827	<0.001	<0.05	$-78 \pm 9\%$

^aHere sed is sediment and pw is porewater and df is 1 for all contrasts listed. Error terms represent ± 1 standard deviation, and nonsignificant differences are indicated as ns.

When all factors were analyzed by subhabitat category, root density showed the best Pearson product moment correlation with sediment MeHg concentrations ($R = 0.75$) and with %MeHg ($R = 0.65$). Organic content (%LOI) was also correlated with root density ($R = 0.61$), but showed weaker relationships with sediment MeHg concentration ($R = 0.43$) and %MeHg ($R = 0.37$). The two most densely rooted habitats, Petaluma marsh interior and Cosumnes River floodplain with mean root densities of $\sim 18\%$, were also the only two sites where mean sediment %MeHg that exceeded 1%. Aboveground peak biomass was not correlated to root density, but did have significant positive relationships with surface sediment %LOI ($R = 0.46$) and THg ($R = 0.65$). The variability in the absolute value of devegetation response in sediment MeHg concentration across habitats is most closely associated with initial root density, suggesting an effect of rhizosphere surface area on biogeochemistry ($R = 0.65$).

[15] Regression analyses across the full range of data suggest a functional importance of root density across-plot conditions in surface sediment. Using %MeHg as an independent surrogate for Hg(II)-methylation efficiency, our data show that calculated MP based on the microbial rate constant (k_{meth}) and sediment concentrations of Hg(II)_R, explain a significant portion of the variability in %MeHg in the salt marshes (Petaluma, $r^2 = 0.45$, $p = 0.028$; Alviso $r^2 = 0.60$, $p < 0.001$) but not in the freshwater nonagricultural or agricultural Yolo and Cosumnes wetlands. Of all ancillary measurements, root density was among the best predictors of pore water acetate concentrations, k_{meth} , and %MeHg within individual field types (Figures 2a–2c), but this was not the case across all field types ($p > 0.05$).

3.2. Devegetation Effects Among Wetlands

[16] Both ANOVA and pair-wise comparison of vegetated control and devegetated plots showed that in all wetlands MP rates and k_{meth} values were decreased by an average of $36 \pm 17\%$ and $38 \pm 17\%$, respectively, as a result of devegetation (Table 4), while sediment Hg(II)_R concentrations did not exhibit a significant %DevegEffect ($p < 0.05$). Pore water DOC and acetate concentrations were also significantly lower in devegetated plots (across all plot pairs), with mean %DevegEffect values of -34 ± 10 and $-78 \pm 9\%$, respectively.

[17] The efficacy of live root reduction in the devegetation plots was greater than 80% in all but two out of 37 paired samples. There were also no differences between control and devegetated plots in initial conditions and no significant changes in sediment state variables (bulk density, porosity, %organic, %water, %fines, pH, temperature) due

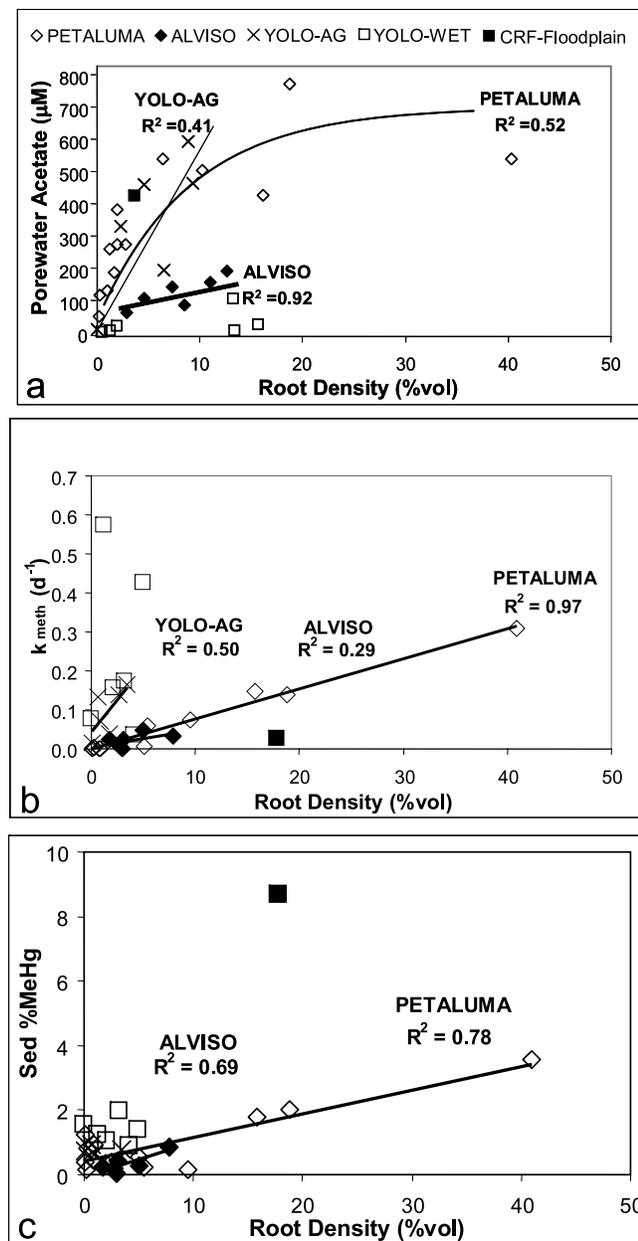


Figure 2. Linear regressions from summer 2006 and 2007 data sets illustrating the relationship between (a) acetate concentrations and root density (%volume), (b) k_{meth} and root density (%volume), and (c) %MeHg concentration in sediment and root density (%volume). Data are coded by ecosystem: PETALUMA (salt marsh), ALVISO (salt marsh), YOLO-AG (agricultural wetlands), YOLO-WET (managed wetlands), and CRF-Floodplain (river floodplain).

Table 5. ANOVA Results Testing the Differences Between Average %DevegEffect for Data Grouped by Either Freshwater or Saline Study Areas^a

Factor	ANOVA			%DevegEffect Freshwater	%DevegEffect Saline
	N	F	p		
%DevegEffect Sediment MP	26	0.586	0.450	-36 ± 20%	-25 ± 17%
%DevegEffect Sediment PWDOC	26	4.365	0.047	-20 ± 11%	-42 ± 12%
%DevegEffect Sediment PWFe(II)	26	5.016	0.034	-17 ± 8%	-37 ± 14%

^aHere df is 1 for all contrasts listed. Error terms represent ±1 standard deviation.

to the devegetation treatment, with the exception of a 5°C increase in temperature in the Cosumnes floodplain.

[18] The extent to which devegetation decreased MP was not significantly different between sites grouped as either saline or freshwater wetlands (Table 5). In contrast, for these same two groupings, pore water DOC and Fe(II) concentrations were decreased to a significantly greater extent in saline wetlands, as a result of devegetation. Within freshwater wetlands, none of the %DevegEffect factors varied significantly between wetland sites grouped as agricultural and those grouped as nonagricultural.

3.3. Petaluma Salt Marsh

[19] Vegetated Petaluma interior marsh (pickleweed-dominated) sites had %MeHg values that were more than twofold greater than vegetated marsh edge (gumplant-dominated) sites (Table 2), and sediment %MeHg was significantly correlated with calculated MP rates ($R = 0.69$). Devegetation decreased MP rates by 48% in marsh interior plots (Table 5). This decrease was largely driven by the associated decrease in the activity of Hg(II) methylating bacteria (%DevegEffect for $k_{\text{meth}} = -75\%$), and not by a decrease in Hg(II) availability, as the %DevegEffect for Hg(II)_R was nonsignificant. Devegetation in the marsh interior also led to a decrease in microbial sulfate reduction rates (-73%) and associated sediment reduced sulfur pools (pore water sulfide, sediment TRS, sediment AVS), which was reflected in a concomitant increase in sediment redox (65 ± 40 mV). Devegetation also significantly decreased pore water DOC and acetate in interior plots (48 and 95%, respectively), as well as in marsh edge plots (59 and 97% respectively), but only interior plots exhibited significant decreases in MP, k_{meth} and microbial sulfate reduction (SR). However, both marsh subhabitats exhibited a significant decrease in pore-water sulfide and porewater Fe(II) concentrations as a result of devegetation (Table 6). Because of high variability in iron pools between plots, calculated daily rates of change for individual iron species were not measurable in most plots, and were not significantly influenced by devegetation in either interior ($p = 0.24$) or edge ($p = 0.39$) habitats.

3.4. Alviso Salt Marsh

[20] In Alviso salt marsh vegetated control sites, %MeHg was low (0.2 to 0.6%) across the marsh elevation gradient (Table 2). Sediment %MeHg was correlated with calculated MP rates ($R = 0.76$) across all Alviso subhabitats studied. The low marsh (cordgrass-dominated) subhabitat exhibited the most pronounced response to devegetation, with a 81% decrease in MP, a 91% decrease in k_{meth} , and a 20% decrease in MeHg concentration (Table 6). All three marsh elevations exhibited a decrease in pore water acetate concentration (34–95%) as a result of devegetation, while pore water

Table 6. Percent Devegetation Effect Data for Sediment and Pore Water Parameters at Peak Biomass During 2006 and 2007^a

Sampling Region	Subhabitat	Salinity	Management	sed k_{meth}	sed Hg(II) _R	sed MP	sed MeHg	sed %MeHg	sed SR	sed acetate	sed S^{2-}	sed Fe(II)	sed Fe(II)	sed aFe(III)	sed DOC	sed TRS	sed AVS	sed Cl	Root Density
PET	Edge	Saline	—	ns	ns	ns	ns	ns	ns	-97	-47	-69	ns	ns	-59	ns	ns	ns	-92
	Interior	Saline	—	-75	ns	-48	-48	-41	-73	-95	-50	-39	-19	ns	-48	-36	-21	ns	-80
	Low	Saline	—	-91 ^b	ns	-81 ^b	-20	-11	ND	ND	ns	ns	ns	ns	-46	-51	ND	ns	-68
ALV	Med	Saline	—	ns ^b	ns	ns ^b	ns	ns	ND	-34	ns	+57	ns	ns	ND	ns	ns	ns	-99
	High	Saline	—	ns ^b	ns	ns ^b	-21	ns	ND	-42	-58	ns	ns	ns	-52	ns	ns	ns	-99
	White Rice	Fresh	Ag	-48	ns	-64	-38	-16	ns	-63	ns	ns	+17	-24	-47	ns	ns	-28	-99
YOLO	Wildrice	Fresh	Ag	-67	ns	-67	ns	ns	ns	-93	ns	ns	+16	ns	ns	ns	ns	-37	-99
	Fallow-mixed ^e	Fresh	Ag	-56	-82	-92	-55	-42	-81	-63	+68	+87	ns	-93	ns	+26	ns	-13	-95
	Fallow-barren ^d	Fresh	Ag	-67	+81	ns	-49	-19	-49	-93	-72	-50	ns	+21	ns	-58	ns	ns	ns
	Seasonal Wetland ^d	Fresh	Non-Ag	-17	ns	ns	-35	-21	ns	-79	ns	+30	ns	ns	ns	ns	ns	ns	-87
	Tule Wetland ^c	Fresh	Non-Ag	-87	+83	ns	-41	-23	-80	-98	ns	-38	ns	ns	ns	-71	-80	ns	-93
CRF	Cattail Wetland ^c	Fresh	Non-Ag	ns	+24	ns	-14	-41	ns	-99	-45	-30	ns	ns	ns	-10	-26	ns	-99
	Floodplain ^e	Fresh	Non-Ag	-60	-30	-73	-13	ns	-83	-90	-56	-34	ns	-63	-80	ns	-37	ns	-98

^aHere sed is sediment and pw is porewater. Percent devegetation effect data as calculated by %DevegEffect = $(X_{devegetated\ plot} - X_{vegetated\ plot}) / (X_{devegetated\ plot} / X_{vegetated\ plot}) \times 100$. Values in bold indicate statistically significant differences between vegetated and devegetated sites for a given subhabitat parameter (X), as assessed using pairwise t tests ($p \leq 0.05$). ND, no data collected; ns, nonsignificant. PET, Petaluma River; ALV, Alviso Marsh; YOLO, Yolo Bypass Wildlife Area; CRF, Cosumnes River Floodplain. Abbreviated terms defined in Table 3.

^bStatistical tests are based on data from homogenized composited samples from 2 replicate field sites in October 2007.

^cStatistical tests are based on laboratory subsampled replicates ($n = 2$) of homogenized composited samples from a single field site.

^dStatistical tests are based on field-sampled replicates ($n = 2$) of homogenized composited samples from a single field site.

DOC was significantly decreased in the low and high marsh elevations only. While rates of microbial SR were not measured in the Alviso plots, sediment TRS decreased 51% in the low marsh subhabitat as a response to devegetation.

3.5. Yolo Bypass and Cosumnes River Freshwater Wetlands

[21] Despite differences in hydrology and vegetation among the freshwater wetland types studied, the activity of Hg(II)-methylating bacteria (as k_{meth}) consistently decreased (17–87%) as a result of devegetation, in all subhabitats except in the cattail dominated wetland (Table 6). Similarly, sediment MeHg concentration significantly decreased (13–55%) in all subhabitats except for wild rice fields. The effect of devegetation on sediment Hg(II)_R concentration was more varied with a decrease in the vegetated fallow field and the Cosumnes River floodplain, and an increase in the barren fallow field and in both the tulle- and cattail-dominated wetlands, and non-significant changes in both rice field settings and in the Yolo seasonal wetland. The combined effect of k_{meth} and Hg(II)_R concentrations on calculated MP rates thus yielded decreased rates of MP in devegetated plots of both rice field subhabitats and the vegetated fallow field in Yolo, and in the Cosumnes R. floodplain. As was observed in the saltmarsh environment, pore water acetate consistently decreased (63–99%) with devegetation across all freshwater subhabitats (Table 6). Agricultural fields showed the strongest devegetation responses with respect to solid phase iron species, including an increase in sediment Fe(II) and a decrease in sediment aFe(III) concentrations, whereas concentrations for the more abundant cFe(III) fraction were varied and not significantly different between treatments. Despite sulfate loading to both white and wild rice fields through fertilizer application ($>50\text{--}75\text{ kg SO}_4^{2-}\text{ acre}^{-1}$), no significant affect from devegetation was observed in the white or wild rice fields for microbial SR rates or for reduced sulfur species concentrations. Devegetation-driven decreases in microbial SR rates were observed, however, in both fallow field settings and in the two most densely rooted freshwater wetlands (tule and floodplain; Table 6).

[22] Comparisons of iron speciation over the agricultural growing season showed significant daily decreases in crystalline Fe(III) and increases in reduced Fe(II) between consecutive sampling dates (flood-up to August), indicating iron reducing activity (Figure 3). Devegetated plots in the rice fields had higher calculated Fe(II) production ($p = 0.031$), while in contrast, aFe(III) production was measurably greater ($p = 0.039$) in vegetated plots (Figure 3). In contrast to the similar responses among the replicate white rice and wild rice fields, changes in iron speciation over time were quite different between the two fallow field replicates as a response to devegetation (or “the tarp effect”). In the barren fallow field, devegetation led to an increase in sediment aFe(III), concomitant with a decrease in TRS, and a significant increase in sediment redox conditions (+207 mV). In the vegetated fallow field, devegetation led to a decrease in sediment aFe(III) and an increase in TRS, with no significant change in sediment redox. Similarly, calculated rates of change for iron species showed that devegetation stimulated FeII buildup in the vegetated fallow field, but

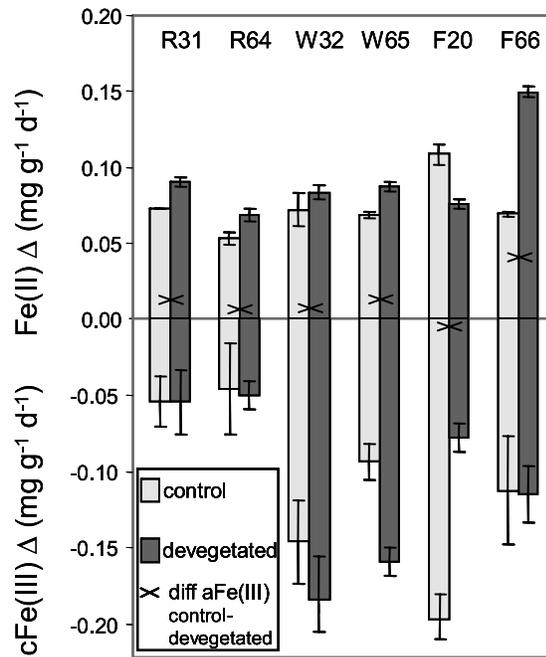


Figure 3. Calculated rates of change (Δ) in sediment cFe(III) and Fe(II) pools for both vegetated control and devegetated plots in Yolo Bypass agricultural fields (only). Effect of devegetation on amorphous Fe(III) pools is shown as a single value (crosses) representing the absolute difference between control and devegetated plots. Error bars reflect compounded standard differences for lab replicates ($n = 2$). White rice is R31 and R64. Wildrice is W32 and W65. Fallow-barren is F20. Fallow-mixed is F66.

a net reduction of Fe(II) over time in the barren fallow field (Figure 3).

3.6. Relationship Between Microbial Devegetation Effects: Implications for Sulfur and Iron Cycling

[23] Pearson correlation analysis was used to assess the correspondence of devegetation effects among the parameters assessed, and to identify significant biogeochemical interactions. When compared across all wetland settings, the %DevegEffect for aFe(III) positively correlated with both the %DevegEffect for Hg(II)_R ($R = 0.66$) and the %DevegEffect for MP ($R = 0.73$, Figure 4). Thus, in wetlands where sediment aFe(III) concentration were significantly decreased because of devegetation, MP showed the most substantial decreases (Figure 4). Because a decrease in aFe(III) is indicative of net Fe(III) reduction, or a lack of Fe(II)-reoxidation back to aFe(III), this relationship suggests that Fe(II)-reoxidation may be important in driving higher rates of MP in the vegetated (control) sites, by resupplying aFe(III) as an electron acceptor for a subset of the Fe(III)-reducing microbial community that may be involved in Hg(II)-methylation (e.g., geobacter [Rodén, 2008]). Because the most significant devegetation effects associated with mercury cycling (ie. k_{meth} , MP, %MeHg, Hg(II)_R and MeHg concentration) were predominantly associated with significant changes in iron speciation in the freshwater subhabitats studied, our data show important linkages between iron

biogeochemistry and MeHg production dynamics in agricultural and floodplain freshwater wetlands.

4. Discussion

[24] All indices of MeHg production, (including calculated MP rates, %MeHg, k_{meth} , and MeHg concentration) in surface sediment were generally greater in the presence of actively growing emergent vegetation, compared to devegetated plots, across all wetland regions studied. Removal of active photosynthetic processes and root growth led to an average of 36% lower MP rates (range = 0–92%), and 38% lower k_{meth} values (range = 0–91%) (Table 6). These results confirm that actively growing vegetation commonly influences MeHg production in wetland surface sediment. Because k_{meth} values generally decreased as a result of the devegetation treatment, while Hg(II)_R concentrations had limited and variable responses to devegetation (Table 6), it appears that the primary influence of actively growing vegetation on Hg(II)-methylation is most commonly the stimulation of microbial activity. In all cases, devegetation led to significant decreases in pore water acetate (average = –78%, range = –34 to –99%), consistent with decreases in microbial activity as a function of decreased availability of suitable electron donors for heterotrophic terminal processes.

4.1. Microbial Methylation Activity as the Primary Factor in Methylmercury Production

[25] Lambertsson and Nilsson [2006] and others [e.g., Korthals and Winfrey, 1987] argue that organic matter availability is the primary control on Hg(II)-methylation, citing comparative studies across multiple wetland types in which %MeHg was used as a measure of methylation efficiency. Further, Drott *et al.* [2008] argued that the activity of the microbial Hg(II)-methylating community (i.e., k_{meth}) is the primary factor controlling sediment %MeHg concentrations. With a fourteenfold wider range of k_{meth} values than those used by Drott *et al.* [2008], our experiment, which was effectively a removal of fresh organic matter inputs to wetland surface sediments, supports the hypothesis that (1) sediment MeHg concentrations are a function of in situ microbial activity (k_{meth}) and (2) carbon inputs are the primary controlling factor on MeHg production and sediment MeHg accumulation. These experimental results further make the linkage between emergent plant root density, pore water acetate concentrations, k_{meth} , and %MeHg, and experimentally shows that the loss of photosynthetic organic inputs to wetland surface sediment leads to a decrease in all estimates of methylmercury production rates (k_{meth} , MP, and for most sites, sediment %MeHg.)

[26] Sediment MeHg concentrations were significantly lowered in 91% of devegetated plots, suggesting that decreased MP directly leads to decreased sediment MeHg concentrations. The %DevegEffect on sediment MeHg, however, was not correlated with the %DevegEffect on MP across ecosystems, suggesting that our wetland sites have different MeHg retention, loss, and/or benthic degradation rates. Drott *et al.*'s [2008] comparison of methylation and demethylation rates across diverse wetlands show wide ranges of methylation potential (0.0002–0.024 $k_{\text{meth}} \text{ d}^{-1}$), and a narrow, consistent range of demethylation potential (0.01–0.1 $k_{\text{demeth}} \text{ d}^{-1}$). Because of this differential variability

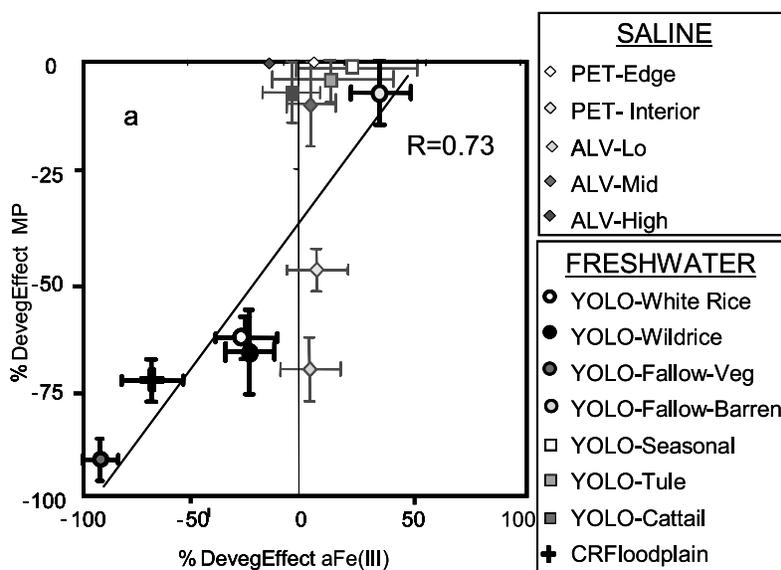


Figure 4. Correlative relationship for mean subhabitat responses between %DevegEffects for methylmercury production (MP) and amorphous ferric iron (aFe(III)). All data are reported, but data for which both X and Y variables were significantly different from zero are enhanced with bold black lines. Error bars represent the data range for $n = 2$ observations, except for Petaluma marshes, where standard deviations are used ($n = 6$).

ity in rates, sediment MeHg concentrations typically reflect rates of production rather than degradation [Drott *et al.*, 2008].

4.2. Role of Vegetation in Carbon Supply

[27] Carbon supply associated with the presence of actively growing plants appeared to be the dominant factor controlling microbial Hg(II)-methylation activity across all study regions, as pore water acetate concentration dropped precipitously in all devegetated sites (mean %DevEffect = -78%), and more generally, pore water DOC decreased by an average of 34% following devegetation. Although acetate concentration is not a measure of the rate of acetate turnover [Hines *et al.*, 1994], microbial Hg methylation was correlated with acetate concentration across all sites (except the permanently flooded wetlands), suggesting that acetate is a good proximate measure of electron donor supply for heterotrophic Hg(II)-methylating microbes. By virtue of their physiological adaptations to anaerobic soils, wetland plants are capable of fermentative respiration under low oxygen conditions [Marschner, 1986]. Under such conditions, glycolysis followed by acetaldehyde production and dehydrogenation, produces both ethanol and eventually acetate, a primary electron donor for microbial respiration, particularly sulfate reduction [Hines *et al.*, 1994] and iron reduction [Lovely and Phillips, 1986]. High pore water acetate concentration was a good predictor of sites with high MP rates, and these sites were commonly represented by E_h values between -100 and $+100$. Low MP rates and low pore water acetate concentrations were found in both highly oxidized Alviso Marsh sites ($E_h \geq 300\text{mV}$) and the highly reducing permanent wetlands of the Yolo Bypass ($E_h \leq -100\text{mV}$). Whereas this might be expected in oxidized soils which do not promote fermentative respiration, the lack of acetate in surface sediments of the more deeply flooded, permanent wetlands in the Yolo Bypass

may signify a different spatial distribution of acetate pools within the sediment:water profile [Jones, 1998].

4.3. Plant Effects on Inorganic Hg(II) Availability

[28] In contrast to expectations, Hg(II)_R concentrations were only rarely higher in control vegetated control sites, compared their paired devegetated sites (i.e., Yolo vegetated fallow field and Cosumnes R. Floodplain, Table 6). We had expected that vegetated plots would be significantly more oxidized than devegetated plots, thereby keeping a larger proportion of the inorganic mercury in the more available Hg(II)_R form, akin to processes affecting speciation of other metals in the rhizosphere zone [Jacob and Otte, 2003]. Further supporting the idea of sediment MeHg production being limited by available carbon rather than available Hg(II), the presence of live plants did not correspond to more oxidized conditions in surface sediment, but rather, devegetated plots often exhibited no significant difference or an increase in sediment redox, and/or lower concentrations of reduced species (e.g., AVS, TRS, sulfide) compared to their paired vegetated plots (Table 6).

4.4. Root Structure and Rhizosphere Microbial Communities

[29] Dense rooting in the upper surface sediment is not just a function of vegetation type, but also reflects a physiological response to hydrology [Howes *et al.*, 1981; McKee and Patrick, 1988]. Thus, hydrology may set the conditions that allow periodically flooded wetlands to produce large amounts of MeHg, not only through direct effects on sediment Hg(II) availability but also through indirect effects on plant productivity, root organic exudates and thus microbial activity.

[30] Root density (%volume) was correlated with sediment MeHg ($R = 0.78$) and with %MeHg in sediment ($R = 0.61$) across all wetlands studied, except the Yolo perma-

nently flooded wetland. Within salt marshes and agricultural wetlands, root density was the strongest predictor of k_{meth} and sediment %MeHg, a proximate measurement for Hg(II) methylation efficiency (Figures 2b and 2c). Root density, an index of root surface area [Böhm, 1979], is a key factor in structuring rhizosphere communities. Horticultural and microbial studies have shown that root surface area is correlated with microbial biomass [Marschner, 1986], labile carbon supplies such as acetate [Hines et al., 1994], and other root exudates [Cheng et al., 2003]. Although we measured microbial biomass for only one set of experimental paired plots (Petaluma marsh, $n = 24$), root density was positively correlated with microbial biomass, and devegetation led to reduction in microbial biomass (L. Windham et al., unpublished data, 2008).

4.5. Role of Iron Versus Sulfate Reducing Bacteria In Hg(II)-Methylation

[31] In salt marshes, where devegetation decreased Hg(II)-methylation, microbial sulfate reduction rates (where measured) also decreased (Table 6). However, in freshwater rice fields where devegetation decreased Hg(II)-methylation, indices of Fe(III)-reduction were also suppressed, with no accompanying suppression of sulfate reduction rates. The observed association of lower MP rates and greater Fe(II) buildup initially suggests three possibilities: (1) iron-reducing bacteria limit net MeHg production [Warner et al., 2003], (2) the buildup of Fe(II) inhibits MeHg through limiting Hg-sulfide formation [Mehrotra and Sedlak, 2005], or (3) a combination of these scenarios. Clearly, the interaction of reduced and oxidized iron species with both sulfur and mercury are complicated and require a deeper exploration into the meaning of these experimental differences.

[32] At the start of the experiment, within 2 weeks after flood-up of the dry field soils (t_{initial}), cFe(III) dominated the total iron pool in the Yolo agricultural sites. Over the following 44–68 day growing periods, sediment Fe(II) concentrations increased at a net average rate of $50\text{--}100 \mu\text{g g}_{\text{sed}}^{-1} \text{d}^{-1}$ and the cFe(III) concentration decreased at a net average rate of $100\text{--}200 \mu\text{g g}_{\text{sed}}^{-1} \text{d}^{-1}$ (Figure 3). Concentrations of aFe(III), however, only decreased by $2\text{--}8 \mu\text{g}_{\text{sed}}^{-1} \text{d}^{-1}$ and in 3 sites, actually increased slightly, by $2\text{--}19 \mu\text{g g}_{\text{sed}}^{-1} \text{d}^{-1}$ (W65 and the fallow fields). This growing pool of aFe(III), the most bioavailable form of Fe(III) [Lovely and Phillips, 1987; Roden and Wetzel, 1996], suggests that Fe(II) is likely being reoxidized to aFe(III) [Sobolev and Roden, 2001]. Because this pool is being constantly resupplied while cFe(III) is constantly declining, iron reduction rates are likely greatest where aFe(III) pools are increasing [Kostka and Luther, 1995; Roden, 2008]. In agricultural wetlands, sites of measurable aFe(III) production (e.g., vegetated fallow field) were also where sediment MeHg production rates and concentrations were highest; in contrast, sites of low-to-no net aFe(III) production (e.g., devegetated plots, barren fallow field) were where sediment MeHg production rates and concentrations were lowest.

[33] Devegetation had a significant effect on iron speciation, especially when comparing the vegetated fields to the one barren fallow field. In all five vegetated fields, devegetation led to higher net rates of Fe(II) production, lower net rates of aFe(III) production, and higher net rates of cFe(III) consumption. In contrast, in the barren field,

devegetation led to lower net rates of Fe(II) production, higher net rates of aFe(III) production, and lower net rates of cFe(III) consumption. These patterns are consistent with the devegetation patterns seen for other redox-sensitive indices (reduced sulfur species in the vegetated fallow field, redox and Hg(II)_R pools in the barren fallow site, Table 6). After taking the multiple indices into account, we conclude that the reason devegetation leads to such different effects in vegetated versus barren fields has to do with the interplay of labile carbon supply and the reoxidation of reduced species in the rhizosphere zone.

[34] In the barren field, where primary production inputs were low and predominantly algal (calculated daily organic input of $<150 \text{ mg C m}^{-2}$), the main effect of the experimental treatment (landscape cloth) was limitation of carbon supply, which limited microbial activity (as seen for both sulfate reduction rates and k_{meth}). Less microbial activity in the experimental treatment led to less reducing conditions, and a greater reoxidation potential of the soil. Although Hg(II)_R concentrations responded positively (81% increase), the net effect on microbial methylation activity (k_{meth}) likely trumped any changes in Hg(II) bioavailability, and therefore, lower sediment MeHg concentrations were found in the experimental plots from the barren fallow field. In the vegetated fields, where organic inputs were predominantly plant derived, there were two significant effects of the devegetation treatment: (1) inhibition of labile carbon supply and (2) rhizosphere oxidation. First, a lack of acetate supply led to less microbial activity under the landscape cloth (as seen for both SR and k_{meth}). Second, rhizosphere reoxidation in the vegetated control sites resupplied aFe(III) pools whereas under the landscape cloth, pools of Fe(II) built up and aFe(III) remained constant or decreased (Figure 3). These patterns are also consistent with the known, high transpiration rates in these vegetated soils (P. Bachand, personal communication, 2008), which can promote surface soils oxidation [Dacey and Howes, 1984; Howes et al., 1981], and the observed $26 \pm 12\%$ increase in pore water chloride concentrations in vegetated as opposed to devegetated plots (Table 6).

[35] The calculated daily changes in the aFe(III) pool in Yolo agricultural wetlands, although not large, were also correlated with sediment %MeHg in August ($R = 0.70$), whereby sites with increasing aFe(III) concentrations from flood-up to August were also sites with increasing sediment MeHg concentrations. Because these changes in iron speciation are not consistent with the dominance of sulfate reduction, or in the absence of Fe(II) oxidation [Roden, 2008], it appears that active iron cycling (reduction and reoxidation) in the rhizosphere zone of vegetated sites accounts for higher calculated rates of MeHg production.

[36] Indices of Fe(II) production were observed despite large sulfate additions in agricultural rice and wildrice fields. Although there was a devegetation effect on SR rates in both of the fallow fields (Table 6), SR rates in the control plots varied thirtyfold ($6\text{--}174 \text{ nmol S}^{2-} \text{ g}_{\text{dw}}^{-1} \text{ d}^{-1}$) and yet, sediment %MeHg was similar in both sites ($0.5 \pm 0.1\%$). Finally, the decrease in MeHg production due to devegetation was most pronounced in the same locations that the decrease in aFe(III) concentration was also most pronounced (Figure 4). Since the vegetated control sites with the highest net seasonal change in aFe(III) were also

the sites with the highest k_{meth} rates, we propose that iron reducers are the dominant microbial community driving MeHg production in agricultural fields of the Yolo Bypass. If so, this data supports the hypothesis that in some freshwater settings, benthic Fe(III)-reduction can be the dominant microbial process mediating MeHg production. We are currently investigating microbial community structure using molecular approaches to further support this hypothesis.

5. Conclusion

[37] This cross-ecosystem study comparing wetlands with a wide range of sediment and plant characteristics shows that where root densities and associated acetate supplies are high, MeHg production is enhanced. Comparative and experimental tests of vegetation presence and root density identify the role of emergent wetland plants in promoting microbial MeHg production. The availability of inorganic Hg(II) was not consistently altered in the short-term revegetation experiments, suggesting that Hg(II) availability was not a primary factor controlling MeHg production in vegetated sites. This study also provides further experimental evidence for the role of Fe(III)-reducing bacteria in regulating MeHg production and accumulation in freshwater wetland sediment. This revegetation approach was effective across a wide range of ecosystem types, with consistent, thorough reductions in root biomass coupled with only a few measurable experimental artifacts. We conclude that actively growing freshwater and saltmarsh wetland plants promote Hg(II) methylation in the rhizosphere primarily through the exudation of labile carbon products, which stimulate sulfate- and iron-reducing bacterial activity. These field data represent a unique experimental contribution to our understanding of the direct and indirect roles of vegetation on MeHg production.

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