Alternate Wetting and Drying Decreases Methylmercury in Flooded Rice (*Oryza sativa*) Systems

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In flooded soils, including those found in rice (*Oryza sativa* L.) fields, microbes convert inorganic Hg to more toxic methylmercury (MeHg). Methylmercury is accumulated in rice grain, potentially affecting health. Methylmercury in rice field surface water can bioaccumulate in wildlife. We evaluated how introducing aerobic periods into an otherwise continuously flooded rice growing season affects MeHg dynamics. Conventional continuously flooded (CF) rice field water management was compared with alternate wetting and drying, where irrigation was stopped twice during the growing season, allowing soil to dry to 35% volumetric moisture content, at which point plots were reflooded (AWD-35). Methylmercury studies began at harvest in Year 3 and throughout Year 4 of a 4-yr replicated field experiment. Bulk soil, water, and plant samples were analyzed for MeHg and total Hg (THg), and iron (Fe) speciation was measured in soil samples. Rice grain yield over 4 yr did not differ between treatments. Soil chemistry responded quickly to AWD-35 dry-downs, showing significant oxidation of Fe(II) accompanied by a significant reduction of MeHg concentration (76% reduction at harvest) compared with CF. Surface water MeHg decreased by 68 and 39% in the growing and fallow seasons, respectively, suggesting that the effects of AWD-35 management can last through to the fallow season. The AWD-35 treatment reduced rice grain MeHg and THg by 60 and 32%, respectively. These results suggest that the more aerobic conditions caused by AWD-35 limited the activity of Hg(II)-methylating microbes and may be an effective way to reduce MeHg concentrations in rice ecosystems.

Abbreviations: %Fe(II), ferrous iron as a percent of total citrate-dithionite extractable Fe; %MeHg, the percentage of total Hg that is methylmercury; AWD, alternate wetting and drying; AWD-35, alternate wetting and drying to 35% volumetric moisture content; CF, continuous flooding; DAP, day after planting; $Fe(II)_{AE}$, acid-extractable ferrous Fe; (Fe(III)a, amorphous ferric iron; Fe_T, total citrate-dithionite extractable Fe; FeRB, Fe-reducing bacteria; MeHg, methylmercury; SRB, sulfate-reducing bacteria; THg, total Mg.

Core Ideas

- **We studied how alternate wetting and drying (AWD) water management effects methylmercury (MeHg) dynamics in rice fields.**

- **Alternate wetting and drying reduced MeHg concentrations in soil, water, and rice grain.**

- **Iron speciation indicated that AWD oxidized the soil and regenerated electron acceptors.**

- **Rice yield did not differ between AWD and the control over 4 yr.**

R ice is grown on 161 million ha worldwide and is the staple crop for 3.5 billion people globally (Muthayya et al., 2014). Rice is a semiaquatic plant and is usually grown under flooded conditions to help control weeds, increase nutrient availability, and ensure water supply during drought (Kendig et al., 2003). Flooded conditions also allow rice fields to serve as important wildlife habitat (Czech and Parsons, 2002); however, rice production is also associated with several negative impacts. First, rice requires higher water inputs than other cereal crops (Pimentel et al., 2004). Increasing food demand from a growing population and decreasing water availability (Mekonnen and Hoekstra, 2016) are placing pressure on rice growers to reduce the amount of water used for rice production. Second, the global warming potential of rice is elevated compared with other crops, caused in large part by high methane emissions (Linquist et al., 2012; Wassmann et al., 2010). Finally, flooded conditions can also change the speciation and increase the bioavailability of As and

Hg in the soil, resulting in increased accumulation of both contaminants in rice grain, thus impacting human health (Das et al., 2016; Grandjean et al., 1997; Hong et al., 2016; Linquist et al., 2014; Qiu et al., 2008; Rothenberg et al., 2016).

Methylmercury is a toxic and highly bioaccumulative form of Hg (World Health Organization, 1990), and it is primarily produced by some groups of anaerobic microbes (Gilmour et al., 2013; Parks et al., 2013; Podar et al., 2015). Wetland soils, including rice fields, are important sites of MeHg production (Gilmour et al., 1998; Krabbenhoft et al., 1995; Marvin-DiPasquale et al., 2014; Podar et al., 2015; Windham-Myers et al., 2014). Methylmercury produced in rice field soil is accumulated by rice plants more readily than inorganic Hg (Strickman and Mitchell, 2017; Zhang et al., 2010). Although human exposure to MeHg is well known to occur through fish consumption, rice has been shown to be the primary source of dietary MeHg for people living in regions of inland China where fish consumption is low and Hg contamination is elevated as a result of mining (Feng et al., 2008; Qiu et al., 2008; Zhang et al., 2010). Methylmercury in rice fields can also impact wildlife within those habitats and can harm downstream ecosystems when transported in drainage water (Ackerman et al., 2008; Chan et al., 2003; Crump and Trudeau, 2009). In California's Sacramento Valley, where 25% of US rice is produced (USDA-National Agricultural Statistics Service, 2017), Hg is a concern because of historical mining activities (Churchill, 2000). Methylmercury concentrations in fish and other wildlife are elevated in the Sacramento–San Joaquin Delta (Ackerman et al., 2008; Ackerman and Eagles-Smith, 2010) and regulatory efforts are underway to limit MeHg loads (Delta Mercury Control Program, 2010).

A wide variety of anaerobic microbes have been shown to produce MeHg (Gilmour et al., 2013; Podar et al., 2015; Parks et al., 2013), including Fe-reducing bacteria (FeRB) (Fleming et al., 2006; Kerin et al., 2006), sulfate-reducing bacteria (SRB) (Compeau and Bartha, 1985; Gilmour et al., 1992), methanogens (Hamelin et al., 2011), and syntrophs (Bae et al., 2014). The relative MeHg contribution of these groups can vary depending on the environment (Hamelin et al., 2011; Warner et al., 2003; Yu et al., 2012). Production of MeHg can be limited by the availability of inorganic Hg(II), labile organic C (electron donors), electron acceptors such as Fe(III) and SO_4^{2-}, redox conditions, pH, and temperature (Ullrich et al., 2001). Methylmercury can be degraded by an oxidative or reductive pathway by a wide array of microbes (Marvin-DiPasquale et al., 2003), and MeHg levels in an ecosystem are dependent on the balance of MeHg production and degradation (Paranjape and Hall, 2017). Abiotic degradation of MeHg can also occur via photolytic demethylation, which is an important control of MeHg in many systems (Fleck et al., 2014; Sellers et al., 1996).

Rice cultivation practices that result in unsaturated soil conditions can save water (Carrijo et al., 2017) and provide other benefits including reductions in methane emissions (Li et al.,2006; Wassmann et al., 2010; Xu et al., 2015) and rice grain arsenic concentrations (Das et al., 2016; Linquist et al.,

2014), However, increased rice grain Cd has also been reported (Norton et al., 2017). These practices can vary in severity, duration, and the timing of unsaturated periods, from unsaturated soil throughout the growing season (aerobic rice) to AWD in which discrete dry-down events are introduced into an otherwise flooded growing season. Compared with aerobic rice, AWD has a lower yield penalty and increases the number of wet-dry cycles, resulting in fluctuating redox conditions (Borin et al., 2016).

Alternate wetting and drying could affect Hg(II)-methylating microbes in multiple ways. Wet–dry cycles may enhance MeHg production by converting Hg to forms that are more easily methylated (Marvin-DiPasquale et al., 2014) and electron acceptors are regenerated when soil dries (Eckley et al., 2015; Marvin-DiPasquale et al., 2014; Singer et al., 2016). These increases in substrate availability can stimulate the activity of Hg(II)-methylating microbes, resulting in spikes of soil MeHg when dried soils were reflooded (Marvin-DiPasquale et al., 2009; Rothenberg and Feng, 2012). In contrast, the few studies of more aerobic rice cultivation practices reported reductions in grain MeHg relative to flooded controls (Peng et al., 2012; Rothenberg et al., 2016; Wang et al., 2014), and aerobic periods limited the activity of anaerobic, Hg(II)-methylating microbes, thus reducing MeHg production (Rothenberg et al., 2016; Wang et al., 2014).

Few studies have tested the effect of AWD on MeHg in rice fields. In a controlled and replicated field experiment, we tested whether MeHg and THg concentrations differed between the bulk soil, surface water, rice straw (stems and leaves), and rice grain for rice fields cultivated using CF and AWD-35. Alternate wetting and drying regenerates electron acceptors, which may promote Hg methylation; however, more aerobic conditions may reduce anaerobic microbial activity. Thus the impacts on MeHg concentrations on paddy soil, surface water, and, most importantly, rice grain are uncertain. Iron speciation in soil was measured to determine if the AWD-35 treatment was regenerating electron acceptors and disrupting anaerobic conditions as expected. Studies were conducted throughout a full growing season and monitoring continued throughout the following fallow season to determine if AWD effects persisted.

METHODS
Site Description

A replicated field experiment was conducted at the Rice Experiment Station (39°27′47″N, 121°43′35″W) near Biggs, CA. This location has a Mediterranean climate with warm, dry summers and mild, wet winters. Average high and low temperatures were 30.8 and 15.6°C respectively during the growing season (May–September) and 13.2 and 4.9°C during the fallow season (October–February) [California Irrigation Management Information System (California Department of Water Resources, 2017)]. The soils are a Esquon-Neerdobe complex (fine, smectitic, thermic Xeric Epiaquerts and Duraquerts) with 45% clay, 26% silt, 29% sand, 0.08% total N, 1.06% organic C, pH 5.0, 34.2 $cmol_c$ kg^{-1} cation exchange capacity, and 0.36 dS m^{-1} electrical conductivity (Pittelkow et al., 2012).

Treatments

A field experiment was established in 2012 and continued through to 2015 to evaluate various AWD treatments relative to a continuously flooded control. The 2012 to 2014 management is described fully in LaHue et al. (2016). For the purposes of the current study described here, we compared two treatments from this larger study: AWD-35 and CF. The plots were 0.2 ha in size and each treatment was replicated three times in a randomized complete block design. To prevent lateral seepage between flooded and drained plots, the plots were separated by levees and ditches. The treatments here were applied to the plots during 2012 to 2015 without rerandomizing. For this study, plant and soil samples were initially collected at harvest in 2014 and the remaining samples were collected throughout the 2015 growing season and the following fallow season.

Management of AWD-35 plots differed from the CF plots in that, beginning after canopy closure, at about 50 d after planting (DAP), AWD-35 plots were subjected to two wet–dry–wet cycles (dry-downs). During each dry-down, irrigation of the plot was halted and the water level in the field was allowed to subside though evapotranspiration, percolation, and seepage (no surface drainage of water was needed to achieve dry-downs). The plots were reflooded when the soil reached approximately 35% volumetric water content (Supplemental Fig. S1). Soil water content was measured using volumetric water content sensors installed in each plot (Model 10HS, Decagon Devices, Inc., Pullman, WA). At canopy closure, fertilizer N uptake was complete, so dry-downs after this time resulted in minimal N losses resulting from nitrification or denitrification and production of N_2O (LaHue et al., 2016). Waiting until canopy closure to begin dry-downs also served to avoid excessive weed pressure (Tuong et al., 2005). Aside from the dry-downs, AWD-35 and CF plots were managed in the same way.

Rice Management

Rice management was consistent with typical conventional rice production in California (University of California Davis Cooperative Extension, 2015). Soil preparation occurred during April and standard recommended fertilizers were applied (50 kg P_2O_5 ha^{-1}, 50 kg K_2O ha^{-1}, and 180 kg N ha^{-1} as urea). All plots received irrigation water from the same mixed source that included surface water from Lake Oroville and well water. The rice variety grown was 'M206', a medium grain, early maturing variety that accounts for most of Californian rice production (http://www.carrb.com/Variety/M-206.htm). Rice was planted on 21 May 2014 and 20 May 2015 by broadcasting pregerminated seed onto the plots and flooding immediately. This simulated the practice used by growers where pregerminated seed is broadcast into flooded fields. Dates of key management events were reported previously for 2012– 2014 (LaHue et al., 2016) and are shown in Table 1 for 2015. Flood water was maintained throughout the growing season in CF plots. During the growing season, water height was managed by adjusting irrigation inflows. All plots were drained 3 wk prior to harvest and no surface drainage occurred prior to this event. A small

Table 1. Dates of management and sample collection events for the 2015 rice growing season and following fallow season.

Management or sampling event	Samples collected				Date	DAP†
	Soil	Water	Straw	Grain		
Tillage					10 May	-10
Sampling	x				15 May	−5
Fertilizer application					18 May	-2
planting					20 May	0
Initial flood					22 May	2
Sampling	x	x			29 June	40
Canopy closure					3 July	46
Dry-down 1 (AWD-35 only)					8 July	51
Sampling	x				16 July	57
Reflood 1 (AWD-35 only)					16 July	57
Sampling	x	x	x		23 July	64
Dry-down 2 (AWD-35 only)					27 July	68
Sampling	x				3 Aug.	75
Reflood 2 (AWD-35 only)					3 Aug.	75
50% heading					11 Aug.	83
Sampling	x	x	x	x	2 Sept.	105
Final drain					7 Sept.	110
Sampling	x		x	x	28 Sept.	131
Tillage					20 Oct.	153
Sampling	x				21 Oct.	154
Fallow season flood					24 Oct.	157
Sampling		x			30 Oct.	163
Sampling		x			4 Nov.	168
Sampling		x			9 Nov.	173
Sampling		x			20 Nov.	184
Sampling		x			30 Nov.	194
Sampling	x	x			8 Feb.	264
Fallow drain					8 Feb.	264

† DAP, days after planting; AWD-35, alternate wetting and drying to 35% volumetric moisture content.

plot combine (SPC 40, ALMACO, Nevada, IA) was used to measure yield. After harvest, straw was incorporated into the soil and the plots were flooded to facilitate its decomposition during the winter fallow, a common practice in California (Linquist et al., 2006). Drainage from the plots occurred during the first 5 wk of the fallow season and at the end of the fallow season. Plots remained flooded throughout the fallow season.

Sampling Schedule

Initial samples of soil, rice straw, and grain were collected at harvest in 2014. In May 2015, soil samples were collected prior to fertilizer application and planting. Additional sampling occurred at the end of each wet or dry period in AWD-35 plots during the growing season: before the start of a dry-down and prior to reflooding. The final two growing season soil sampling events were before the final drain and at harvest. Water was collected in all plots three times during periods of the growing season when AWD-35 plots were flooded: (i) just before the first dry-down, (ii) between dry-downs, and (iii) just before final draining. Since no surface water export occurred during the growing season, water samples were collected in the middle of the plots. Plant sampling began in July and was conducted concurrently with water samples, as well as at harvest. Fallow season soil sampling occurred after tillage, but before flooding and at the end of the fallow season before

the final drain. Samples of water draining from the plots were collected six times throughout the fallow season, including five samples collected while drainage was occurring early in the season and one when the plots were drained at the end of the season.

Sample collection methods

All samples were collected using trace clean sampling techniques [USEPA Method 1669 (USEPA, 1996)]. Soil samples were collected for MeHg, THg, and Fe speciation analyses. Tillage had thoroughly mixed the plow layer, so soil samples collected after tillage (−5 and 154 DAP) were collected by filling a plastic Ziploc bag (SC Johnson, Racine, WI) with 5 to 10 scoops of soil from the plow layer (0–15 cm) over a 1-m² area. Sampling equipment was cleaned thoroughly between samples to prevent cross-contamination. All other soil samples were collected by inserting 15 cm long by 5 cm diameter plastic cores into the soil. Cores were collected in the spaces between rice plants, with plants being approximately 3 to 5 cm away from the edges of cores. Soil samples primarily consisted of bulk soil; however, some roots were present. It was beyond the scope of this study to examine the rhizosphere explicitly. To decrease variability and ensure more representative samples, two replicate cores were collected in each plot and composited. Overlying water was retained in the headspace to maintain redox conditions. All soil samples were double-bagged and immediately frozen under dry ice for transport to the lab, and were stored at −80°C as soon as practical to arrest or substantially slow any oxidation reactions. The top 5 cm of soil was extruded from replicate cores into plastic bags to maintain anaerobic conditions for homogenization. Subsamples were placed into combusted scintillation vials and stored at −20°C until THg and MeHg analysis. Subsamples for Fe speciation were placed in crimp-sealed serum vials and the headspace was purged with N_2 before storage at −20°C. Overlying water and sediment redox was not explicitly monitored so we could not conclusively reject the possibility that minor changes in redox conditions may have occurred during sample collection, storage, and subsequent processing. However, since all samples were handled using the same methods, any minor changes in redox conditions would be common to all samples and the relative differences observed between treatments were probably not impacted.

Surface water samples were collected for MeHg and THg analysis. New 250-mL polyethylene terephthalate glycol-modified bottles were double-bagged in the laboratory for water sample collection. Bottles were rinsed three times with site water following the clean-hands dirty-hands technique (USEPA, 1996), prior to site water collection in each plot. Care was taken to avoid collecting water where sediment was disturbed. Water samples were field acidified with trace-metal-clean HCl (final concentration 0.5%) and stored under dark conditions prior to analysis. Field blanks and field duplicates were collected for quality assurance. Sample collection occurred between midmorning and mid-day.

Plant samples for MeHg and THg analysis comprised 10 to 15 tillers collected from multiple plants with no visible sediment contamination. When grains were present, samples were divided into straw (stems and leaves) and panicle. Samples were frozen under dry ice for transport to the laboratory and stored at −80°C until further processing. Plant samples were lyophilized, after which, the straw was cut into short pieces and grains were removed from the panicle. Rice grain was analyzed with the husks included to account for all MeHg and THg removed from the field at harvest and to be consistent with previous work in California (Windham-Myers et al., 2014). A coffee grinder cleaned with ethanol between samples was used to grind plant materials to a fine powder before analysis (Drennan-Harris et al., 2013).

Sample Analysis

Water and soil samples were analyzed for THg and MeHg as described by Marvin-DiPasquale et al. (2011). Briefly, THg samples were quantified according to USEPA Method 1631 (USEPA, 2002). Water samples were subjected to heated oxidation in 1% BrCl before quantification. Soil samples were first digested with aqua regia overnight before heated oxidation with 3.7% BrCl and quantification. Plant samples were digested with concentrated HNO_3 at 138 kPa and 126°C for 3 h (Kleckner et al., 2017) before oxidation with 8.3% BrCl and quantification of THg (Marvin-DiPasquale et al., 2011). Methylmercury water samples were distilled, then ethylated (DeWild et al., 2002) and analyzed using a MERX automated MeHg analyzer (Brooks Rand, Seattle, WA). Soil and plant samples were extracted with 25% KOH in methanol at 60°C for 4 h before ethylation and quantification (Marvin-DiPasquale et al., 2011). Quality assurance for each analysis included certified reference material samples, matrix spikes, analytical duplicates, method blanks, and calibration standards (see Supplemental Table S1 to Supplemental Table S3). Finally, the percentage of THg that is MeHg (%MeHg) (100 × MeHg ÷ THg) was calculated for all samples.

Sediment Fe speciation was analyzed as detailed by Marvin-DiPasquale et al. (2008). Sediment was extracted with weak acid, then acid-extractable ferrous iron ($Fe(II)_{AE}$) was quantified by spectrophotometric determination (absorbance at 562 nm) with Ferrozine (Sigma-Aldrich, St. Louis, MO) (Lovley and Phillips, 1986). After determination of $Fe(II)_{AE}$, samples were reduced with hydroxylamine HCl and total acid-extractable Fe (Fe_{TAE}) was quantified. Amorphous ferric iron ($Fe(III)_a$), which is available for reduction by FeRB, is calculated by difference [$Fe(III)_a = Fe_{TAE} − Fe(II)_{AE}$] (Lovley and Phillips 1987a). Finally, total extractable Fe (Fe_T) in soil was measured by dithionite-citrate extraction and quantification of Fe(II) in the extractant with Ferrozine (Roden and Zachara, 1996). Total extractable Fe is the sum of the three Fe fractions [$Fe_T = Fe(III)_c + Fe(III)_a + Fe(II)_{AE}$], where $Fe(III)_c$ is crystalline ferric Fe. As an indicator of soil redox status, ferrous iron as a percent of total citrate-dithionite extractable iron [%Fe(II)] was calculated [$100 × Fe(II)_{AE} ÷ Fe_T$]. Quality assurance for each analysis included matrix spikes, analytical duplicates, method blanks, and calibration standards (see Supplemental Table S4 to Supplemental Table S7).

Statistical Analysis

Spatial and temporal autocorrelation was a concern in this analysis because plots were sampled repeatedly throughout the study. To address this issue, these data were analyzed via linear mixed effects regression analysis, the standard method of addressing autocorrelation arising from repeated measures (Pinheiro and Bates, 2000; Zuur et al., 2009). This method accounts for repeated measures and allows for the robust, simultaneous assessment of the effects of treatment and sampling date on response variables.

R statistics software (version 3.3.3, R Core Team, 2017) was used for data analysis and plotting. Separate statistical models were fitted for each matrix (soil, water, straw, and grain) and analyte (MeHg, THg, etc.). All models were initially fitted with lme4 (Bates et al., 2015) with the structure y = treatment + sampling date + (treatment × sampling date) + (1| block) + (1| plot), where y is the response variable, treatment is a categorical variable with the levels AWD-35 or CF, sampling date is a categorical variable with levels for each sample collection event, treatment × sampling date is an interaction term, and (1| block) and (1| plot) are random intercepts for block and plot, respectively. Only one sampling event occurred in 2014 (at harvest), so the effect of year was accounted for by the fixed effect for sampling event. Backward stepwise regression via the step function in the lmerTest package (Kuznetsova et al., 2016) was used to select models by sequentially removing nonsignificant random effects followed by fixed effects. A significance level of $p < 0.05$ was used for all tests. The significance of random effects was tested first by using likelihood ratio tests of models (fitted via restricted maximum likelihood) with and without the random effect. After the random effects structure was selected, models were fitted via maximum likelihood. The significance of fixed effects, beginning with interactions, was tested with p-values calculated via F-tests with Satterthwaite's approximation for degrees of freedom. A lack of significant random effects indicated a lack of autocorrelation. In cases where random effects for both block and plot were deemed to be not significant, two-way ANOVAs with the same fixed effects structure [y = treatment + sampling date + (treatment × sampling date)] were used to test the significance of fixed effects.

Standard diagnostic plots were used to check assumptions of normalcy and homogeneity of variance, and natural log transformations were used successfully to correct for any violations of assumptions. The influence of unusual observations (5 THg values, <2 SD from the mean) was tested by conducting the analysis both with and without them. Outliers were not included in the plots and are discussed further in the supplemental information. For models with a significant interaction between treatment and sampling date, significant differences in least-squares means between treatments were tested for each sampling event using the lsmeans package (Lenth, 2016), with p-values adjusted for multiple comparisons following Tukey's method. Values reported in the text are mean ± SD.

Two additional model structures were tested with the same model selection protocol described above. Water data were modeled as y = treatment + season + (treatment × season) + (1| block) + (1| plot), where season is either growing or fallow, because previous studies noted elevated water MeHg and THg during the fallow season (Tanner et al., 2017). A subset of rice grain and straw concentration and yield data collected at harvest, were modeled as y = treatment + year + (treatment × year) + (1| block) + (1| plot) where year is either 2014 or 2015.

RESULTS AND DISCUSSION
Soil Hg and MeHg Dynamics

Time series of soil Hg and Fe parameters in soil are shown in Fig. 1. Soil THg was 23 ± 4 ng g^{-1}, and no differences were detected between treatments or sampling events (Fig. 1a). Soil MeHg concentrations and %MeHg [%MeHg is a metric often used as an indicator of net Hg(II) methylation efficiency (Drott et al., 2008)] were generally elevated in CF plots (0.12 ± 0.04 ng g^{-1}, 0.49 ± 0.02%) compared with AWD-35 (0.07 ± 0.04 ng g^{-1}, 0.26 ± 0.02%). There was a significant interaction between treatment and sampling event ($p < 0.05$, Fig. 1b), showing that MeHg and %MeHg decreased in response to dry-downs. Soil MeHg and %MeHg were similar between CF and AWD-35 plots before 51 DAP, when the first dry-down occurred (Fig. 1b). After AWD implementation, soil MeHg and %MeHg decreased in AWD-35 plots relative to CF, with significant differences (Tukey-corrected $p < 0.05$) between treatments observed in the later part of the growing season, shortly before the final drain, during harvest, and after fall tillage (Fig. 1b). Decreases in soil MeHg and %MeHg the during aerobic periods are consistent with previous studies (Peng et al., 2012; Rothenberg et al., 2016; Wang et al., 2014). Interestingly, a spike in MeHg concentrations was observed during the first dry-down (57 DAP) in both CF and AWD-35 (Fig. 1b). Other authors have reported a pulse of MeHg after soils are flooded (Marvin-DiPasquale et al., 2009; Rothenberg and Feng, 2012; Zhao et al., 2016) but peaks in concentration occurred sooner than observed here. Samples collected at 40 DAP and 64 DAP showed no hint of elevated concentrations, so studies with higher sampling frequency are needed to understand the elevated concentrations at 57 DAP. A more detailed understanding of temporal changes in soil MeHg throughout the rice growing season and over the course of wet–dry–wet cycles would help optimize the number, timing, and duration of AWD dry-downs for MeHg control.

No differences in Fe speciation were evident before AWD-35 dry-downs were initiated (Fig. 1, Supplemental Fig S2). Generally, %Fe(II) increased when plots were flooded and decreased on drying (Fig. 1d). For all sampling events during the flooded portion of the growing season, %Fe(II) was 62–67% in CF plots, whereas dry-downs in AWD-35 plots resulted in oxidation of significant amounts of Fe(II). In response to the first dry-down, %Fe(II) in bulk soil decreased from 60 to 6.2% and remained below 31% for the remainder of the growing season. The pool of ferric iron that is readily available to FeRB for use as an electron acceptor, measured here as Fe(III)$_a$ (Lovley and Phillips, 1986), was undetectable in all CF samples collected under flooded conditions. In AWD-35 plots, Fe(III)$_a$ was undetectable before the first dry-down (40 DAP) but increased to 3 mg g^{-1} in response to drying. It should be noted that only bulk soil was
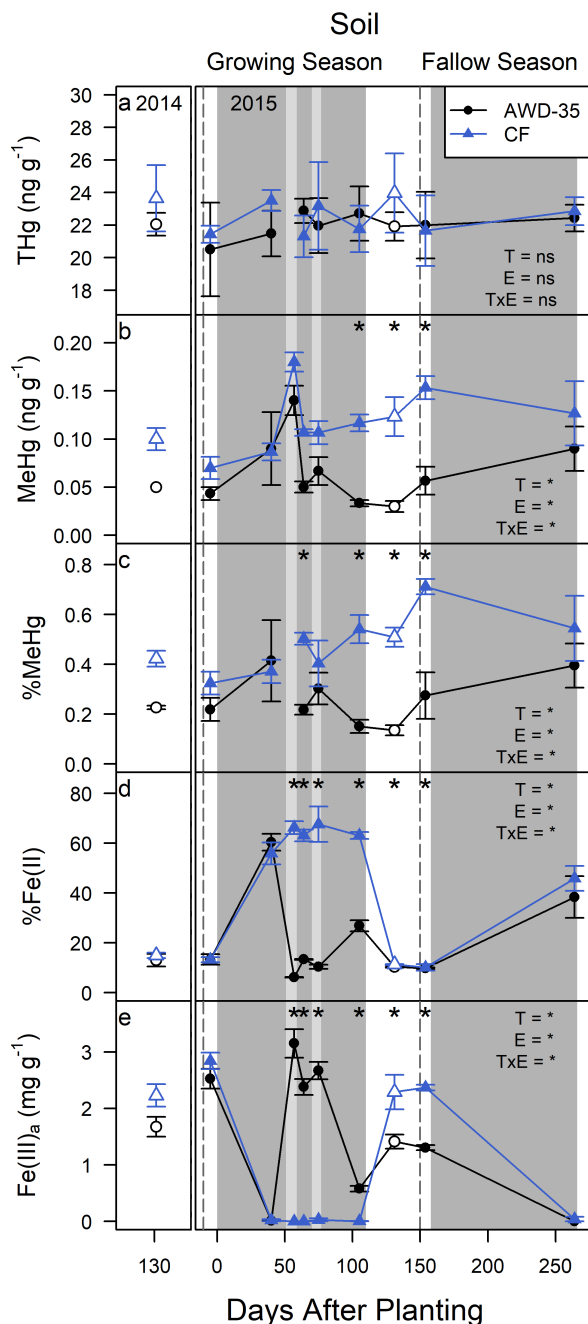
Fig. 1. Mean ± SE (*n* = 3) of (a) total Hg (THg), (b) methylmercury (MeHg), (c) the percentage of MeHg (%MeHg), (d) ferrous iron as a percentage of total citrate-dithionite-extractable Fe (%Fe(II), and (e) amorphous ferric iron (Fe(III)$_a$) in soil on a dry weight basis for alternate wetting and drying (AWD-35) and continuously flooded (CF) treatments. Samples collected at harvest in 2014 are shown in the left panel and 2015 growing and fallow season data are on the right, with the *x*-axis showing days after rice was planted (DAP). Hollow points indicate samples were collected at harvest. Shading indicates whether plots were flooded: dark gray, both AWD-35 and CF were flooded; light gray, drying events where AWD-35 plots were dry and CF plots were flooded; no shading, both AWD-35 and CF were dry. Samples were generally collected immediately before changes in hydrologic conditions. Tillage occurred at -10 DAP and 150 DAP (vertical dashed lines). The significance of fixed effects is noted on the right side of each plot: T, treatment; E, sampling event; T × E, interaction between treatment and sampling event; *, *p* < 0.05; ns, not significant. When a significant interaction was present, asterisks (*) are shown above sampling events for which the difference between treatments was significant (*p* < 0.05).

collected in this study and it is likely that Fe(III)$_a$ was present in the rhizosphere in CF plots and during flooding in AWD-35 (Kirk and Bajita, 1995). Although S species were not measured in this experiment, thermodynamics predict that oxidation of H$_2$S to SO$_4^{2-}$ will occur at a lower redox potential than the oxidation of Fe(II) to Fe(III). Thus, the oxidation of Fe(II) in AWD-35 plots was probably accompanied by the oxidation of reduced S species (if present), producing SO$_4^{2-}$. Changes in Fe speciation in response to flooding and drying show that the AWD-35 treatment resulted in oxidation of the soil and regeneration of electron acceptors.

All else being equal, the increased availability of electron acceptors in AWD-35 plots would be expected to increase the activity of FeRB and SRB, and thus increase Hg(II)-methylation. The opposite occurred in this experiment: %MeHg decreased in response to the AWD-35 treatment. Anaerobic microbes require appropriate redox conditions for growth (Liesack et al., 2000), so one explanation for the decrease in MeHg is that dry-downs oxidized the soil, resulting in less favorable redox conditions for Hg(II) methylators. The rapid oxidation of Fe during dry-downs (Fig. 1d) indicates that the soil was oxidized, so anaerobic microbial activity probably ceased temporarily (except in anaerobic microsites), along with MeHg production. Fe(III) reduction, SO$_4^{2-}$ reduction, and methanogenesis typically occur sequentially along redox gradients (which can exist at multiple scales temporally or spatially) because electron acceptors with higher reduction potentials yield more energy, allowing FeRB to outcompete SRB and methanogens when Fe(III) is present (Liesack et al., 2000; Lovley and Phillips, 1987b). Upon reflooding, Fe(III)$_a$ was available to support microbial Fe(III)-reduction in AWD-35 plots (Fig. 1e). Consistent with this, LaHue et al. (2016) reported that methane emissions occurred throughout the growing season in CF plots, but ceased after the first dry-down and remained negligible for the remainder of the growing season in AWD-35 plots, and Wang et al. (2014) reported that copy numbers of the *srAB* gene, a measure of SO$_4^{2-}$-reduction, were lower in soils in aerobic rice water management treatments. These results suggest that the activity of methanogens and SRB is inhibited by aerobic treatments such as AWD-35. Changes in redox conditions caused by AWD-35 may have shifted the microbial community to one that produces less MeHg: Warner et al. (2003) found that Hg(II)-methylation under Fe(III)-reducing conditions occurred at a lower rate than under SO$_4^{2-}$-reducing conditions, but MeHg degradation rates were similar, leading to lower net MeHg production under Fe(III) reduction. Increased rates of MeHg degradation in AWD-35 plots could also have caused lower MeHg concentrations relative to CF; however, we were unable to differentiate between decreases in MeHg production or increases in MeHg degradation. Finally, it is possible that AWD-35 altered the availability of inorganic Hg for methylation. Concentrations of inorganic reactive Hg, an operationally defined fraction believed to be available for methylation, decreased in rice fields during flooded periods and increased when the soil was dried (Marvin-DiPasquale et al., 2014). Thus changes in Hg bioavailability due to AWD-35 would

be expected to promote Hg(II) methylation. Since MeHg decreased, changes in Hg bioavailability were probably outweighed by the impacts of more aerobic redox conditions.

Water Hg and MeHg concentrations

MeHg concentrations in AWD-35 plots were 68 and 39% lower than CF in the growing and fallow seasons, respectively (Fig. 2). Differences in water MeHg within the field plots probably stem from differences in soil MeHg concentrations. In contrast to MeHg, THg concentrations in water did not differ between treatments. Both MeHg and THg concentrations in water were significantly higher during fallow season sampling events than in the growing season (Fig. 2). This is consistent with previous studies of MeHg and THg in California rice fields (Tanner et al., 2017). Although the reasons for this pattern are uncertain, elevated concentrations during the fallow season may be caused by the release of MeHg and THg temporarily stored in the soil or decomposing rice straw (Bachand et al., 2014; Windham-Myers et al., 2014). Another possibility is that decomposing straw may promote Hg(II) methylation by stimulating microbial activity (Marvin-DiPasquale et al., 2014); however, others have found that changes in straw management had complex (Zhu et al., 2015), insignificant (Tanner et al., 2017), or contradictory (Eagles-Smith et al., 2014) effects on MeHg concentrations.

The AWD-35 water MeHg concentrations continued to be lower than CF throughout the following fallow season (Fig. 2b, 163–264 DAP), indicating that AWD-35 may have a lasting effect on water MeHg concentrations beyond the season in which they were applied. MeHg in rice field water and its effects on

wildlife are a concern in California, where rice fields provide an important habitat during the fallow season (Czech and Parsons, 2002). Though we did not measure exported MeHg loads, differences in MeHg concentration in water suggest that the net export of MeHg from the AWD-35 fields would also be reduced relative to the CF fields. Drainage water from Sacramento Valley rice fields ultimately flows into the Sacramento–San Joaquin Delta (Tanner et al., 2017), where elevated MeHg concentrations have had negative impacts on wildlife fitness (Ackerman et al., 2008; Hoffman et al., 2011). Thus the widescale adoption of AWD has the potential to affect MeHg concentration at the watershed scale. This highlights the potential of AWD for MeHg management and suggests that more research is warranted on the long-term impacts of AWD on MeHg in rice field water and its export.

Plant Uptake of Hg and MeHg

Partitioning of MeHg and THg in above ground biomass is consistent with previous studies (e.g., Meng et al., 2010), with most MeHg content found in the grain and the majority of THg found in the straw (Table 2). Between 64 and 105 DAP, straw (stems and leaves) MeHg concentrations decreased (Fig. 3), then did not change significantly from 105 DAP to harvest (131 DAP). Three mechanisms might explain this. First, Meng et al. (2011) observed that MeHg first accumulated in leaves and stems during vegetative growth, then was translocated to rice grain during reproductive growth. Second, growth of rice plants could result in dilution of MeHg in tissues (Meng et al., 2011). Finally, loss of MeHg may have occurred via *in planta* demethylation (Strickman and Mitchell, 2017; Xu et al., 2016). Straw MeHg was signifi-
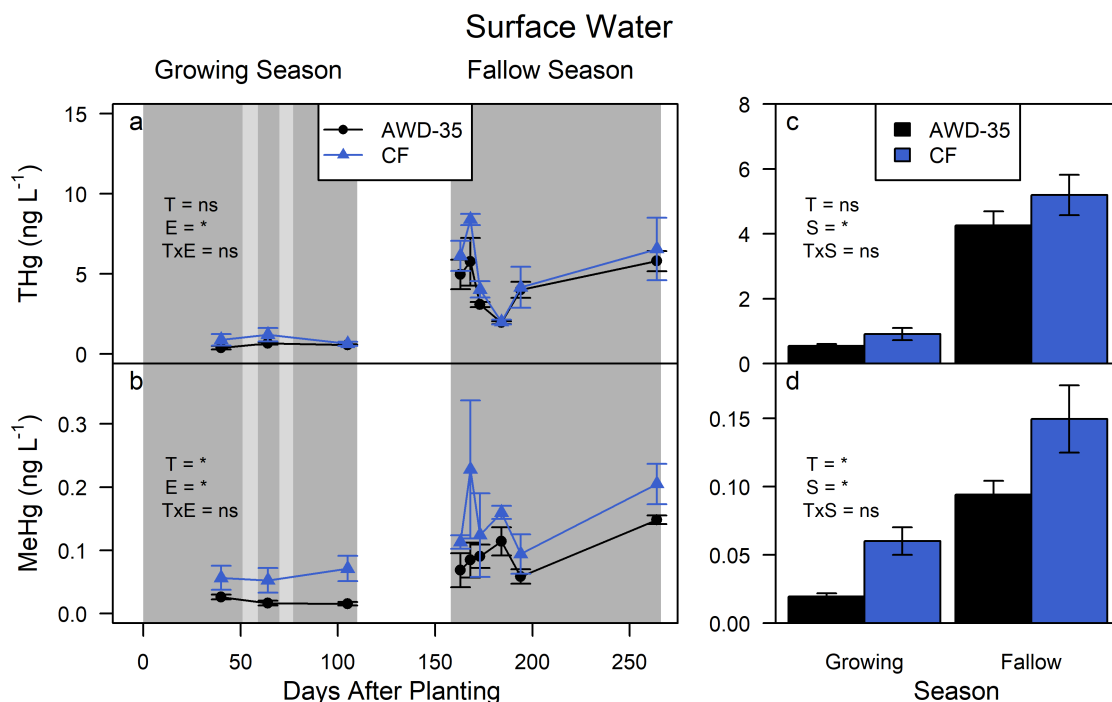

Fig. 2. Total Hg (THg) (a, c) and methylmercury (MeHg) (b, d) concentration in rice field water plotted vs. days after planting (DAP) (a, b) and per plot by season (c, d). Points and bars show mean ± SE (*n* = 3 per point). Shading indicates whether plots were flooded: dark gray, both alternate wetting and drying (AWD-35) and continuously flooding (CF) treatments were flooded; light gray, drying events where AWD-35 plots were dry and CF plots were flooded; no shading, both AWD-35 and CF dry. The significance of fixed effects is noted on the left side of each plot: T, treatment; E, sampling event; T × E, interaction between treatment and sampling event; S, season; T × S, interaction between treatment and season; *, *p* < 0.05; ns, not significant.

Table 2. Yield, total Hg (THg), and methylmercury (MeHg) concentrations and content† in rice plant samples collected at harvest.

	2014		2015	
	AWD-35	CF	AWD-35	CF
Grain				
Yield, Mg ha⁻¹	9.24 ± 0.38	8.12 ± 0.98	13.48 ± 0.26	13.83 ± 0.15
MeHg, ng g⁻¹	0.20 ± 0.10	0.37 ± 0.11	0.08 ± 0.03	0.34 ± 0.17
THg, ng g⁻¹	1.12 ± 0.04	1.44 ± 0.09	0.77 ± 0.15	1.33 ± 0.31
%MeHg‡	18 ± 8	26 ± 9	11 ± 4	24 ± 9
MeHg, ng m⁻²	186 ± 95	302 ± 103	112 ± 42	471 ± 240
THg, ng m⁻²	1039 ± 79	1174 ± 183	1040 ± 217	1847 ± 446
Straw				
Straw, Mg ha⁻¹	6.56 ± 0.77	6.53 ± 0.72	13.37 ± 0.12	14.72 ± 0.50
MeHg, ng g⁻¹‡	0.08 ± 0.01	0.07 ± 0.02	0.07 ± 0.01	0.12 ± 0.09
THg, ng g⁻¹	6.4 ± 1.1	8.2 ± 1.4	5.5 ± 4.2	14.1 ± 7.9
%MeHg §	1.26 ± 0.15	0.91 ± 0.29	3.04 ± 3.71	0.83 ± 0.26
MeHg, ng m⁻²	52 ± 9	48 ± 17	89 ± 15	174 ± 120
THg, ng m⁻²	4268 ± 1165	5435 ± 1492	7292 ± 5507	20,443 ± 10,698

† THg or MeHg content (ng m⁻²) = biomass × concentration

‡ MeHg (ng g⁻¹) ÷ THg (ng g⁻¹) × 100

§ %MeHg, percentage of methylmercury; AWD-35, alternate wetting and drying to 35% volumetric moisture content; CF, continuous flooding.

cantly lower in AWD-35 than in CF at 64 DAP, but not at later sampling dates (Fig. 3). In contrast to MeHg, straw THg increased between 64 and 105 DAP and there was no difference in THg concentration between treatments. The different responses of MeHg and THg may be caused by differences in how MeHg and inorganic Hg are accumulated and translocated in the plant. The atmosphere has been shown to be the primary source of inorganic Hg to aboveground parts of the plant (Meng et al., 2012; Yin et al., 2013), whereas MeHg is accumulated from the soil (Strickman

and Mitchell, 2017) and MeHg is more mobile in the plant than inorganic Hg (Meng et al., 2010).

Rice grain grown under AWD-35 management had 60% lower MeHg concentrations and 32% lower THg concentrations at harvest. Reductions in grain MeHg and THg were evident in both 2014 and 2015 (Table 2). Additionally, grain from AWD-35 plots had a lower %MeHg than that from CF plots (Table 2). The only previous study of the effect of AWD on MeHg reported similar reductions in rice grain MeHg, %MeHg, and THg (Rothenberg et al., 2016). Studies which evaluated aerobic rice also reported reductions in grain MeHg (Peng et al., 2012; Wang et al., 2014). Because MeHg in rice grain has been shown to originate in the soil (Strickman and Mitchell, 2017), differences in grain MeHg are probably caused by differences in soil MeHg concentrations between treatments. Differences in rice grain THg cannot be caused by differences in THg exposure from the soil or the atmosphere, since these did not differ between treatments. Approximately 50% of the reduction in rice grain THg between treatments is simply a reflection of differences in MeHg, which is included in THg. The remaining difference in THg concentration could represent Hg that was demethylated within the plant after being initially accumulated as MeHg (Strickman and Mitchell, 2017; Xu et al., 2016).

Concentrations of MeHg and THg in rice grains observed in this study (range: MeHg, 0.06–0.49 ng g⁻¹; THg, 0.61–1.57 ng g⁻¹) were below the range of values found by a recent comprehensive review (Rothenberg et al., 2014). Because the current study and the one previous AWD study (Rothenberg et al., 2016) had similarly low soil THg concentrations (23 ± 4 ng g⁻¹ and ~ 22 ng g⁻¹, respectively), studies are needed to test the effectiveness of AWD at reducing rice grain MeHg concentrations in more contaminated sites. Although aerobic rice production reduced grain MeHg and THg in rice grown in soil with 1000-fold higher THg concentrations (Peng et al., 2012; Wang et al., 2014), AWD has greater potential for adoption by farmers because it has less impact on yield (Carrijo et al., 2017).

Over 4 yr, rice grain yield did not differ significantly between CF and AWD-35 (Fig. 4). Significant differences occurred between years, with mean yields ranging from 8.6 ± 0.9 Mg ha⁻¹ in 2014 to 13.7 ± 0.3 Mg ha⁻¹ in 2015. Many studies of AWD have reported reductions in yield (e.g., Xu et al., 2015). In a meta-analysis, Carrijo et al. (2017) found that although AWD reduced yields by 5.4% overall, the effect was moderated by the severity and timing of drying events, and soil characteristics such as pH <7 and soil organic C >1% protected against yield reductions. Although the AWD-35 treatment tested here would be considered severe according to Carrijo et al. (2017), soil pH (5.0) and organic C (1.06%) would be expected to protect somewhat against yield reductions. Waiting until canopy closure to begin dry-downs prevented N losses from nitrification and denitrification and limited weed growth, which may have further helped maintain yield.
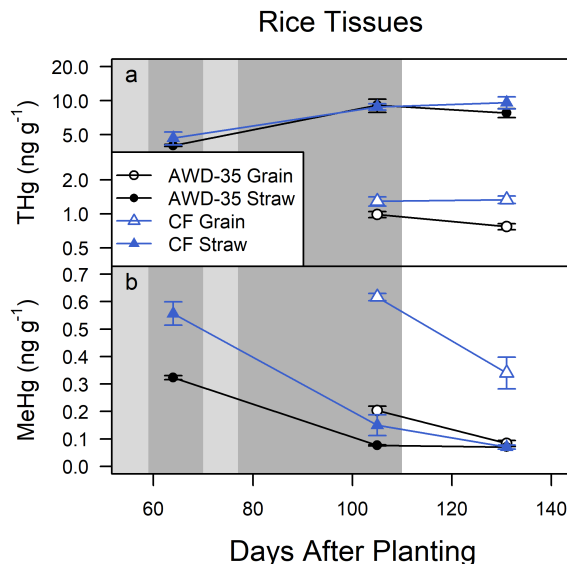
Rice Tissues

Fig. 3. Concentrations of mercury species in plant tissues at multiple sampling dates. (a) methylmercury and (b) total mercury (THg). Note the log-scale *y*-axis for THg. Bars show the mean ± SE (*n* = 3). Rice harvest occurred at 131 d after planting. Shading indicates whether plots were flooded: dark gray, both alternate wetting and drying (AWD-35) and continuous flooding (CF) treatments were flooded; light gray, drying events where AWD-35 plots were dry and CF plots were flooded; no shading, both AWD-35 and CF dry.
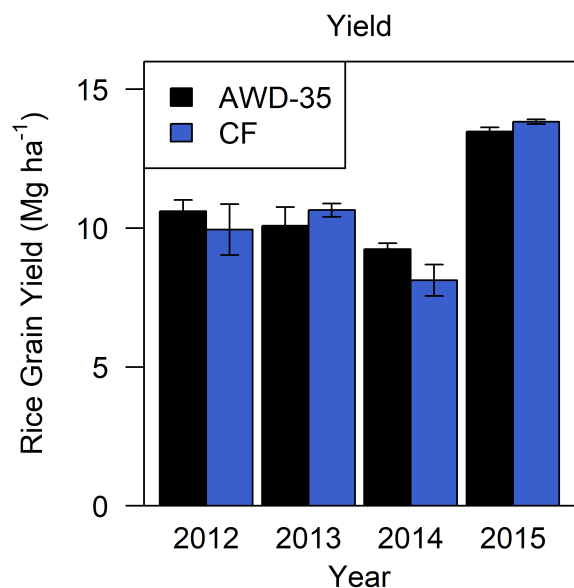
Yield

Fig. 4. Mean ± SE (*n* = 3) rice grain yield in alternate wetting and drying (AWD-35) and continuously flooded (CF) plots. 2012–2014 data were published previously by LaHue et al. (2016).

Conclusion

In the present study, we found that AWD-35 resulted in significant reductions in MeHg concentration in soil, water, and rice grain. AWD-35 resulted in concurrent changes that would be expected to affect MeHg in opposite ways: increasing soil redox would be expected to inhibit methylation, whereas increased availability of electron acceptors and Hg(II) would be expected to promote methylation. Since a decrease in soil MeHg was observed in this study, the effect of redox changes probably outweighed the changes in substrate availability. Only bulk soil was measured in this study, so the results observed here may not apply to soil in the rhizosphere. However, reduced MeHg concentrations in bulk soil were accompanied by reduced concentrations in surface water and plants, suggesting that bulk soil was representative of the MeHg status of the system. Future studies incorporating measurements of the microbial community and its activity (methylation–demethylation rate assays, RNA transcripts for genes related to methylation, demethylation, SO_4^{2-} reduction, etc.) and relevant soil chemistry parameters (redox potential, pH, and S speciation) would serve to clarify the mechanism by which AWD decreases MeHg concentrations. This study is confined to a single site and implementation of AWD, and more work is needed to determine how broadly such results can be expected. Specifically, AWD should be tested in rice-growing areas with higher soil THg and where MeHg accumulation in rice grain is a pressing human health concern. Finally, future research should investigate how changing the severity, timing, or number of dry-downs affects MeHg dynamics and grain yields.

SUPPLEMENTAL INFORMATION

Supplemental Fig. S1. Volumetric soil water content in AWD-35 and CF during dry-downs. Supplemental Fig. S2. Time series of concentrations of a) acid extractable ferrous iron (Fe(II)AE), b) crystalline ferric iron (Fe(III)C), and c) total citrate-dithionite extractable iron (FeT) in soil on a dry weight basis, for alternate wetting and drying (AWD-35) and continuously flooded (CF) treatments. Supplemental Table S1. Certified reference material analyses. Supplemental Table S2. Matrix spikes for MeHg and THg analyses. Supplemental TableS3. Lab duplicates for MeHg and THg analyses. Supplemental Tables S4. Deviation of replicates in acid extracts. Supplemental Table S5. Deviation of replicates for citrate-dithionate extracts. Supplemental Table S6. Recovery of matrix spikes for acid extractions. Supplemental Table S7. Recovery of matrix spikes for citrate-dithionite extractions.

CONFLICT OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest.

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