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Water management impacts rice methylmercury and the soil microbiome

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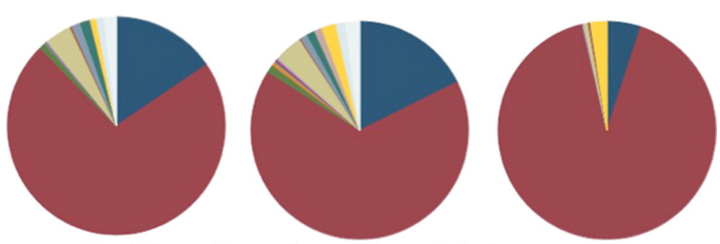
HIGHLIGHTS

- Rice was cultivated using four water-saving methods.
- Methods were flooded, alternating wetting and drying (AWD), or furrow-irrigated (FI).
- Rice methylmercury was low in AWD fields and lowest in FI fields.
- Soil microbiotas differed between flood/AWD fields and FI fields.
- Microbial genera known to contain mercury methylators were lowest in FI fields.

GRAPHICAL ABSTRACT



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ABSTRACT

Rice farmers are pressured to grow rice using less water. The impacts of water-saving rice cultivation methods on rice methylmercury concentrations are uncertain. Rice (*Oryza sativa* L. cv. Nipponbare) was cultivated in fields using four water management treatments, including flooded (no dry-downs), alternating wetting and drying (AWD) (with one or three dry-downs), and furrow-irrigated fields (nine dry-downs) (n = 16 fields). Anoxic bulk soil was collected from rice roots during the rice maturation phase, and rice grain was harvested after fields were dried. Total mercury and methylmercury concentrations were determined in soil and polished rice samples, and the soil microbiome was analyzed using 16S (v4) rRNA gene profiling. Soil total mercury did not differ between fields. However, compared to continuously flooded fields, soil and rice methylmercury concentrations averaged 51% and 38% lower in the AWD fields, respectively, and 95% and 96% lower in the furrow-irrigated fields, respectively. Compared to flooded fields, grain yield was reduced on average by <1% in the AWD fields and 34% in the furrow-irrigated fields. Additionally, using 16S (v4) rRNA gene profiling, the relative abundance of genera (i.e., highest resolution via this method) known to contain mercury methylators averaged 2.9-fold higher in flooded and AWD fields compared to furrow-irrigated fields. These results reinforce the benefits of AWD in

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reducing rice methylmercury concentrations with minimal changes in rice production yields. In the furrow-irrigated fields, a lower relative abundance of genera known to contain mercury methylators suggests an association between lower concentrations of soil and rice methylmercury and specific soil microbiomes.

1. Introduction

Rice ingestion is an important dietary source for methylmercury (MeHg), a potent neurotoxin (Hong et al., 2016; Rothenberg et al., 2014). This is mainly attributed to cultivation of rice under standing water, which turns rice paddies into productive zones for mercury (Hg) methylation (Rothenberg et al., 2014; Windham-Myers et al., 2014a). In submerged rice fields, anaerobic microorganisms convert less toxic inorganic Hg to MeHg (Alpers et al., 2014; Marvin-DiPasquale et al., 2014; Rothenberg and Feng, 2012; Windham-Myers et al., 2014b), which is efficiently accumulated in rice grain (Rothenberg et al., 2011, 2012, 2015; Windham-Myers et al., 2014c; Yin et al., 2013). Microbial Hg methylation is typically concentrated in the oxic/anoxic interface (0–4 cm depth) (Marvin-DiPasquale et al., 2003); however, in rice paddies, pore water MeHg concentrations were highest up to 20 cm depth (Rothenberg and Feng, 2012). This is due in part to rice root exudates, including oxygen (Colmer, 2003) and labile carbon (Marvin-DiPasquale et al., 2014; Windham-Myers et al., 2014b). Root exudates increase the abundance and activity of anaerobic microbes at depth (Kim and Liesack, 2015), and promote dissolution of inorganic Hg(II), i.e. substrate for Hg methylating microorganisms (Rothenberg and Feng, 2012).

In Asia and the U.S., rice farmers are pressured to reduce freshwater use for rice cultivation (Bouman et al., 2007; California Air Resources Board, 2015; Farooq et al., 2009). Water reductions are needed 1) to help resolve water scarcity and increase water availability to other sectors (Bouman and Tuong, 2001; Bouman et al., 2007; Farooq et al., 2009), 2) to meet nutritional demands that accompany global population growth (Morison et al., 2008), 3) to mitigate methane emissions (i.e., greenhouse gases) from flooded paddies (California Air Resources Board, 2015; Linquist et al., 2015), and 4) to reduce groundwater depletion (Massey et al., 2014). Water-saving rice cultivation also reduces rice arsenic concentrations, an added benefit (Linquist et al., 2015). One of the most widely implemented methods is alternating wetting and drying (AWD), which involves multiple, carefully timed periods of nonsubmergence (Bouman et al., 2007). AWD is recommended over more aerobic rice cultivation methods (e.g., furrow-irrigation) because rice yields are not compromised, while rice yields are reduced on average 16–34% using furrow-irrigation (Bouman et al., 2007).

More aerobic rice cultivation alters the activity and community structure of microorganisms in paddy soil (Kim and Liesack, 2015; Xiang et al., 2008); however the impact on soil and rice MeHg (increasing or decreasing) is uncertain. In rice fields in California, USA and Guizhou province, China, a single flooding/drying/reflooding cycle was associated with a 3-fold and 4-fold spike in soil MeHg concentrations, respectively (USA: Marvin-DiPasquale et al., 2014; China: Rothenberg and Feng, 2012). In the latter study, pore water sulfate also increased 5.3-fold, suggesting soil MeHg spiked in flooded paddies due to the revival of sulfate-reducing bacteria (Rothenberg and Feng, 2012), one of the most abundant microbial Hg methylators (Gilmour et al., 2013). Based on these limited results, spikes in soil MeHg following reflooding were hypothesized to correlate with elevated rice MeHg concentrations (Rothenberg et al., 2014). However, growing rice aerobically (under both greenhouse and field conditions) was associated with lower rice MeHg and total Hg (THg) concentrations compared to rice cultivated under flooded conditions (Peng et al., 2012; Wang et al., 2014). Differences may reflect the length of time paddy soil is aerobic and the number of reflooding cycles.

The primary objective of this study was to determine the effects of water management on soil and rice THg and MeHg concentrations using a range of water-saving cultivation methods, including continuously flooded fields, AWD fields (with one or three dry-downs), and furrow-irrigated fields. In addition, the impacts of differential water management on soil microbial community structure were examined using 16S rRNA gene profiling, including the relative abundances of bacterial and archaeal genera known to include microorganisms that methylate Hg. We hypothesize that water-saving rice cultivation methods will reduce rice MeHg concentrations, and reduce the abundance of microorganisms that methylate Hg. Results from this study will help inform water managers and rice farmers about water-saving rice cultivation methods that mitigate rice MeHg concentrations.

2. Materials and Methods

2.1. Treatments and experimental design

In 2013, rice germplasm (*Oryza sativa* L. cv. Nipponbare) was obtained from the United State Department of Agriculture Genetic Stocks Oryza Collection (accession # GSOR 100), and grown at the University of Arkansas Rice Research and Extension Center (Stuttgart, Arkansas) using four water treatments × four replicates ($n = 16$ fields). Fields were cultivated using a rice-soybean rotation. Each field was 260 m^2; Nipponbare was planted in 3.3 m^2 blocks at the end of each field and other rice varieties were cultivated in the remaining area. Grain yield (Mg ha^{-1}) was determined for other rice varieties because they were more abundant. All 16 fields were laid out in a randomized complete block design, and separated by packed levees to prevent water movement between plots.

There were four water management treatments ranging from anaerobic to aerobic: 1) Flood, 2) AWD40-Flood, 3) AWD60, and 4) Row40 (=Furrow-irrigated) (Fig. 1). Methods #1–3 were previously described by Linquist et al. (2015). In flooded fields, water was maintained at 10 cm throughout the rice cultivation season. AWD fields were flooded to 10 cm depth and water subsided via evapotranspiration and percolation until soil moisture reached 40% or 60% of saturated volumetric water (measured at 5 cm depth). AWD40-Flood included one dry-down before rice plants reached the reproductive growth stage (green ring stage); after which a 10 cm flood was maintained. AWD60 included three dry-downs throughout the cultivation season. Furrow-irrigated fields were dried until the soil reached 40% of saturation and then re-irrigated by flowing water down the rows; i.e., no standing water. There were a total of nine dry-downs in the furrow-irrigated fields.

2.2. Rice planting and sampling

The soil on all fields was a Dewitt silt loam (fine, smectitic, thermic, Typic Albaqualfs) with a total carbon content of 0.67%, total nitrogen content of 0.075%, and a pH of 5.6 (1:2 dry soil/water). Fertilizers (144, 29, 84 kg urea-nitrogen, phosphorous and potassium ha^{-1}, respectively) were applied prior to the initial flood.

On 23 April 2013, Nipponbare rice was planted in each plot by drill-seeding. On May 23 at the 4–5 leaf stage (i.e., tillering), all fields were flooded to 10 cm for 7–10 days, then treatments were imposed as described above. In the AWD40-Flood fields, flooding resumed on July 5 (73 days after planting). On August 23 (122 days after planting), anoxic bulk soil was collected from rice roots into a plastic bag, and