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Rice root exudates affect microbial methylmercury production in paddy soils☆

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ABSTRACT

Microbial methylmercury (MeHg) production in contaminated soil-rice systems and its accumulation in rice pose health risks to consumers, especially those in Asia. However, the mechanism responsible for microbial MeHg production in paddy soils is far from clear. While previous studies examined the effect of soil and microbial factors on soil MeHg levels, in this work we explored the impact of rice cultivation itself on microbial MeHg production, focusing on the root exudate organic matter as a potential source of electron donors for microbial methylators. Effects of the cultivation of two rice cultivars, Heigu246 (H-rice) and Neiwuyou8015 (N-rice), on MeHg production in soils were therefore investigated in pot and batch incubation experiments. Soil MeHg levels measured in H-rice treatment during the heading and harvest stages were 18–49% higher than in the control and 23–108% higher than in N-rice treatment. Consequently, MeHg levels in grain, straw, and root were 38%, 81%, and 40% higher in H-rice than those in N-rice, which was mainly attributed to cultivar-specific MeHg production in soils. Results of the batch experiments suggested that root exudate organic matter could be responsible for MeHg production in soils during rice cultivation, by increasing the abundances of potential microbial methylators. For instance, root exudate organic matter increased copy numbers of Hg methylation genes (*hgcA*) in soils 4.1-fold. Furthermore, the 211% higher concentration of acetate (a key electron donor for microbial methylators) in the root exudate of H-rice could account for the higher MeHg production under H-rice than N-rice cultivation. Our results suggest that root exudate organic matter, especially acetate, as its key component, contributes to the elevated soil MeHg concentrations during rice cultivation. The proposed mechanism provides new insights into the elevated risk of MeHg production in contaminated soil-rice systems, as well as cultivar-specific MeHg bioaccumulation.

1. Introduction

There are global concerns about mercury (Hg), primarily due to the microbial transformation of inorganic Hg to the highly toxic and bioaccumulative species, methylmercury (MeHg) (Nalluri et al., 2014; Blum et al., 2014). The production of MeHg in Hg-contaminated soil-rice systems is a cause of growing concern (Lin et al., 2012; Liu et al., 2014a, 2014b; Xu et al., 2016; Beckers and Rinklebe, 2017), due to the resulting elevated MeHg levels in grains and thus human dietary exposure to MeHg, especially in some heavily polluted areas in Asian countries (e.g., China, Wang et al., 2012). However, the mechanisms responsible for the MeHg production in paddy soils are far from clear. The geochemical factors that influence Hg methylation in soil-rice systems have been studied in a few pioneering studies and include organic matter (Zhu et al., 2015), redox potential (Eh, Wang et al., 2014a), and selenium (Wang et al., 2015a,b). However, in addition to those well-studied geochemical factors, plant itself could also impact Hg biogeochemistry in soils and thus microbial MeHg production, which is less understood and warrants investigation. This may help better understand the reasons why elevated soil MeHg concentrations and MeHg bioaccumulation were normally observed during rice cultivation (Meng et al., 2011; Alpers et al., 2014; Tanner et al.,

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Here, we hypothesized that organic matter contained in the root exudates of rice could potentially be a critical factor leading to enhanced microbial MeHg production during rice cultivation, in view of its concentrations and compositions: (1) The concentration of root-exudate-derived dissolved organic carbon (DOC) in the pore-water of paddy soils is high, thus increasing the organic matter content of paddy soils (Gao et al., 2010; Jia et al., 2013), which may impact Hg methylation in paddy soils (Marvin-DiPasquale et al., 2014; Windham-Myers et al., 2014a; Fleck et al., 2014; Liu et al., 2016); (2) Organic acids such as acetate and oxalate are the dominant components of the root exudates of rice (Koo et al., 2010; Fan et al., 2016). As potential electron donors for microbial methylators, including sulfate-reducing bacteria (SRB) (Compeau and Bartha, 1985; Hao et al., 1996), and iron-reducing bacteria (FeRB) (Kerin et al., 2006; Hori et al., 2010), these compounds could facilitate the microbial transformation of Hg to MeHg in soils, thereby contributing to the elevated MeHg levels associated with rice cultivation (Bachand et al., 2014; Windham-Myers et al., 2014b). Whether root exudate organic matter could enhance microbial activities and thus MeHg production in paddy soils is to be tested, by conducting rice cultivation pot experiment and batch incubation experiments.

Besides its potential effects on soil MeHg levels, root exudate organic matter may contribute to cultivar-specific MeHg accumulation in rice (as high as 37-fold, Peng et al., 2012; Li et al., 2013; Wang et al., 2014b; Wang et al., 2015a,b). The cultivar-specific MeHg accumulation in rice was mainly attributed to cultivar-specific MeHg uptake by the rice roots and the subsequent translocation of MeHg within rice tissues. By contrast, little consideration has been given to the potential effects of the rice cultivation itself on microbial MeHg production in soils. The root exudates of different rice cultivars differ in their concentrations and compositions (Jones, 1998; Kerdchoechuen, 2005; Wang et al., 2015a,b). The concentration of root-exudate-derived DOC in the pore-water of paddy soils ranges from 17 to 235 mg/L (Lu et al., 2002; Jia et al., 2013). Furthermore, variations in the composition of root-exudate-derived DOC include that of acetic acid (a key electron donor for microbial methylators), the concentration of which ranges from 5 to 20 mg/L (Bolan et al., 1994; Fan et al., 2016). We thus hypothesized that these differences in the organic matter concentration and composition of root exudates differentially affect microbial activities and thus microbial MeHg production in soils during the cultivation of different rice cultivars. Cultivar-dependent microbial MeHg production in soils (explored in this study), together with cultivar-specific MeHg uptake by roots (examined in previous studies, Peng et al., 2012; Li et al., 2013), may thus explain the variations in MeHg accumulation in different rice cultivars. Testing these hypotheses would improve the mechanistic understanding of microbial MeHg production and bioaccumulation in contaminated soil-rice systems, including cultivar-specific MeHg accumulation. Furthermore, it would allow the risk of MeHg contamination of rice to be minimized, by selecting low Hg-accumulating rice cultivars.

We therefore examined the contributions of rice cultivation and the cultivation of different rice cultivars to the production of MeHg in paddy soil, by comparing MeHg levels in paddy soils with or without rice cultivation as well as the differences between different rice cultivars in pot experiment. The potential differences in soil MeHg levels were then elucidated by conducting batch incubation experiments to examine the effect of root exudate organic matter: soil MeHg levels were examined and compared in microcosm in which Hg-contaminated soils were incubated with root exudate or its key components, acetate and oxalate. The results of this study contribute to explaining the reasons for the different soil MeHg levels during rice cultivation or in the cultivation of different rice cultivars.

2. Materials and methods

2.1. Soils, rice, chemicals, and containers

The soil used for pot and batch experiments was collected from a Hg-contaminated rice paddy (0–15 cm depth) in the vicinity of a Hg mine located in Xunyang, Shanxi Province, China, one of the largest Hg-mining districts in China (Qiu et al., 2012). The soil (referred to as XY soil) was air-dried, ground, and sieved through a 2-mm mesh before use. Total mercury (THg) and MeHg levels in the soil were 33.1 ± 0.4 mg/kg and 3.8 ± 0.4 μg/kg, respectively. Other soil characteristics are listed in Table S1. Two rice cultivars, Heigu246 (*indica*, H-rice) and Neiwuyou8015 (*indica*, N-rice), were selected from common rice cultivars and used in the pot experiment. Our preliminary experiments demonstrated that, when cultivated in the same soil, the two cultivars differed in their grain MeHg levels (56 ± 5.4 μg/kg for H-rice and 39 ± 2.3 μg/kg for N-rice, $p < 0.05$). They were therefore appropriate to explore the mechanisms responsible for cultivar-specific differences in microbial MeHg production and bioaccumulation.

Chemicals and containers used in our study were described in Supplementary data, text.

2.2. Pot experiment

In the pot experiment, H-rice and N-rice were cultivated in Hg-contaminated XY soil to quantify potential changes in soil MeHg levels due to the rice cultivation and the cultivation of different rice cultivars. Three treatments of 12 replicate pots each were established: control (XY soil without rice cultivation), H-rice (H-rice cultivated in XY soil), and N-rice (N-rice cultivated in XY soil). Two kg of soil was added to each pot together with basic fertilizers (128 mg N, as $CO(NH_2)_2$/kg soil, 82 mg P/kg, and 103 mg K, as KH_2PO_4/kg soil). On July 5, 2015, the soil was saturated with deionized water. On July 8, 2015, 14-day-old seedlings of H-rice and N-rice cultivated in low-Hg soil were transplanted into pots (3 seedlings per pot) and placed in a greenhouse (Nanjing City, Jiangsu province, China) at an ambient temperature of 15–38 °C. During rice cultivation, the soils were flooded with deionized water (~3 cm above the soil surface).

The soil MeHg concentration, pH, and Eh and the DOC level in the pore-water were measured at different times during rice cultivation. At each soil and pore-water sampling time, 3 out of the 12 replicate pots of a treatment were randomly selected and sampled. The number of replicates, i.e., 3 seedlings/pot and 3 replicate pots/treatment, was chosen to achieve low relative standard deviations (RSDs) in soil and tissue MeHg levels, according to our previous experiences in conducting pot experiments (e.g., Wang et al., 2015a,b; Zhu et al., 2016; Shu et al., 2016; Tang et al., 2017). Sampling was conducted only once per pot, to avoid potential changes in soil characteristics and plant growth due to the sampling procedure. On days 0 (seedling transplantation), 33 (tillering stage), 78 (heading stage), and 138 (harvest stage), rhizosphere soils and pore-water samples (details specified below) were placed in polypropylene centrifuge tubes, immediately vacuum-packed, and stored at −20 °C in a laboratory freezer before further processing within 24 h. At that time, the tubes were defrosted, unpacked within an anaerobic glove box filled with nitrogen gas, and capped. The capped tubes were centrifuged at 4000 rpm for 20 min and returned to the glove box, where the supernatants were removed and the MeHg concentration of the soils was measured as described in Section 2.5. Soil pH and Eh were

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