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Influence of rice straw amendment on mercury methylation and nitrification in paddy soils

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ABSTRACT

Currently, rice straw return in place of burning is becoming more intensive in China than observed previously. However, little is known on the effect of returned rice straw on mercury (Hg) methylation and microbial activity in contaminated paddy fields. Here, we conduct a microcosm experiment to evaluate the effect of rice straw amendment on the Hg methylation and potential nitrification in two paddy soils with distinct Hg levels. Our results show that amended rice straw enhanced Hg methylation for relatively high Hg content soil, but not for low Hg soil, spiking the same additional fresh Hg. methylmercury (MeHg) concentration was significantly correlated to the dissolved organic carbon (DOC) content and relative abundance of dominant microbes associated with Hg methylation. Similarly, amended rice straw was found to only enhance the potential nitrification rate in soil with relatively high Hg content. These findings provide evidence that amended rice straw differentially modulates Hg methylation and nitrification in Hg contaminated soils possibly resulting from different characteristics in the soil microbial community. This highlights that caution should be taken when returning rice straw to contaminated paddy fields, as this practice may increase the risk of more MeHg production.
Main finding: Rice straw amendment enhanced both Hg methylation and nitrification potential in the relatively high, but not low, Hg soil.

1. Introduction

The production of neurotoxic methylmercury (MeHg) by anaerobic microbes in paddy fields has raised widespread concern due to its uptake and accumulation by rice (Liu et al., 2014a; Meng et al., 2014; Rothenberg et al., 2014; Zhang et al., 2010). The recent identification of mercury (Hg) methylating *hgcAB* genes provides a foundation for evaluating microbial Hg-methylation potential in the environment (Parks et al., 2013; Gilmour et al., 2013). Previous study has showed highly diverse anaerobic microbes in paddy soils that had potential to convert inorganic Hg into methylmercury (MeHg) (Liu et al., 2014a), which were predominantly distributed in *Deltapreteobacteria*, *Firmicutes*, *Euryarchaeota* and several unclassified groups based on analysis of HgcAB orthologs. In natural ecosystems, however, Hg methylation could not be completely predicted by *hgcAB* carrying microbes, which may be modulated by Hg availability, soil variables such as redox status, SO_4^{2-} and characteristics of dissolved organic carbon (DOC) (French et al., 2014; Graham et al., 2013, 2012b; Liu et al., 2014b).

Rice straw is commonly incorporated into fields to enhance soil nutrient (Mandal et al., 2004; Yadvinder-Singh et al., 2004), and now this agricultural practice is getting more intensive than before in China due to a new policy that prohibits burning straw as this is considered to be one of the most important sources of air pollution. Rice straw is also used to remediate metal-contaminated soils because of its ability of reducing metal reactivity through interaction with its degradation products (Sud et al., 2008; Zhu et al., 2015). Previous studies have suggested that decomposition of rice straw by anaerobic breakdown was the main source of organic carbon in paddy soils (Liesack et al., 2000; Ye et al., 2015), which may affect Hg uptake and methylation by microbes due to its strong binding with Hg^{2+} that reduce Hg bioavailability and reactivity (Barkay et al., 1997; Zhu et al., 2015). However, recent studies argued that dissolved organic matter (DOM) enhanced Hg methylation at relatively low DOM content or under low sulfidic conditions (French et al., 2014; Graham et al., 2012a). This is

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partially consistent with the recent field study indicating a positive correlation between MeHg and soil organic matter content in paddy fields around Hg mining area (Liu et al., 2014a). However, it is difficult to confirm whether DOM directly enhanced Hg methylation by forming available Hg-DOM complexes or indirectly by increasing activity of Hg methylating microbes in the paddy soils (Driscoll et al., 2012; Miskimmin, 1991; Ullrich et al., 2001). Therefore, it is crucial to understand effect of rice straw return on Hg methylation in paddy environments.

In paddy ecosystems, amendment of organic matter may also change microbial community composition, which in turn influences soil functions such as respiration, nutrient cycle and other biogeochemical process (Ryals et al., 2014). Among them, nitrification has been considered to be one of important indicators reflecting soil microbial process (Broos et al., 2005; Liu et al., 2010), and potential nitrification rate (PNR) has been widely used as an endpoint in evaluating soil quality (Liu et al., 2012; Smolders et al., 2001; Subrahmanyam et al., 2014). Experimental PNR involves ammonia oxidation from to ammonium to nitrite, which is the first and rate limiting step of nitrification (Brochier-Armanet et al., 2008; He et al., 2012). This pivotal process was generally presumed to be performed by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). AOB have been suggested to be sensitive to various soil perturbations (Bissett et al., 2013), while AOA seems to be insusceptible to environmental changes due to strongly rigid cell membranes and other cellular macromolecules (Cray et al., 2013). For example, previous studies have indicated the positive effects of organic matter on function and community of ammonia-oxidizers attributing that organic matter provides more available ammonium during degradation (He et al., 2007; Kalvelage et al., 2013). In addition, it has been reported that Hg pollution influenced on AOB activity and community composition based on a short-term microcosm experiment (Liu et al., 2010). Up to date, however, little is known on the responses of ammonia-oxidizers to amended rice straw in Hg contaminated paddy soils.

Here, we conduct a microcosm experiment to evaluate how rice straw amendment will influence Hg methylation and nitrification processes in paddy soils with different Hg background levels. We analyzed bacterial community via Illumina high throughput sequencing, which could be also used to identify Hg methylation associating microbes according to reported Hg methylating microbes containing *hgcAB* homologs (Gilmour et al., 2013). We also correlated Hg methylation to DOC content and the Hg methylators in the paddy soils. Soil nitrification potential and community of ammonia-oxidizers were measured to evaluate effects of rice straw amendment on soil microbial function. We tested the following hypotheses: (1) amended rice straw will have different effects on Hg methylation and nitrification in soil with different Hg background levels; and (2) amended rice straw will affect microbial communities accounting for Hg methylation and nitrification in the different soils.

2. Methods and materials

2.1. Experimental design and soil analysis

Soils with relatively low and high Hg background levels were collected at different paddy sites around Wanshan Hg mining areas in China, with an average Hg content of 0.52 and 5.53 mg kg^{-1}, respectively. The two paddy soils have similar chemical properties in pH (7.31–7.40) and organic matter content (5.20% and 5.14%, respectively). Soil samples were passed through a 4 mm sieve and then stored at 4 °C for microcosm experiment. In order to test the effect of rice straw amendment on potential for Hg methylation by indigenous microbial community, freshly spiked inorganic Hg (concentration of 2 mg Hg per kg soil) was introduced into the two soils. We established four treatments as follows: 1) soil with relatively low Hg background level (L); 2) the low Hg soil with 1% straw amendment (LS); 3) soil with relatively high Hg background level (H) and 4) high Hg soil with 1% straw amendment (HS). Briefly, 200 g of soil was introduced in a conical PVC pot (8 cm diameter and 10 cm height), and then the rice straw (0.2 cm in length, with less than 0.1 μg kg^{-1} Hg) was added into the pots and mixed homogenously with soil. The inorganic Hg was added through 150 ml deionized water mixed $HgCl_2$ solution, leading to a paddy condition by flooding the soils with 2.5 cm depth of water. Each treatment was run with four replicates, giving a total of 16 experimental units. All the experimental pots were incubated at room temperature in dark condition for 120 days, and deionized water was added periodically to keep the soils saturated throughout the experiment. At the termination of this experiment, soil samples were collected and passed through a 2.0 mm sieve. One sub-sample was stored at −20 °C for analysis of THg and MeHg and microbial DNA extraction, and another sub-sample was air-dried for general chemical analyses.

2.2. Soil chemical analysis

Ammonium, nitrate and DOC in the soils were extracted with 0.5 M K_2SO4 in a ratio of 1:5 by shaking at 200 rpm for 1 h and filtered using 0.45-μm Millipore filter. The ammonium and nitrate contents in the filtered extract was analyzed within 24 h using a Continuous Flow Analyser (SAN++, Skalar, Holand). DOC content in the extracts was analyzed by TOC analyzer (TOC-L Analyzer, Shimadzu, Japan). Potential nitrification rate (PNR) was assessed using the chlorate inhibition soil-slurry method. In brief, 5 g of fresh soil was placed in a 50 ml falcon tube containing 20 ml of 1 mM $(NH_4)_2SO_4$. Potassium chlorate with a final concentration of 50 mg L^{-1} was added to inhibit nitrite oxidation. After 24 h incubation of the suspension in a dark incubator at 25 °C, NO_2^--N in the soil was extracted using 5 ml of 2 M KCl, then the NO_2^--N concentration in extraction was determined spectro-photometrically at 540 nm with N-(1-naphthyl) ethylenediamine dihydrochloride as an indicator. Potential nitrification rate was calculated as the linear increase in nitrite concentrations during incubation.

2.3. Mercury speciation analysis

For Hg analysis, 0.500 g of each soil sample was first digested with concentrated HNO_3+HCl (10 ml, 1:1 v/v) in a teflon tube at 100 °C for 2 h (Zheng et al., 2008). Total Hg concentration was then determined using an atomic fluorescence spectrometer (AFS, AFS-8220 Jitian Analytical Instrument Co., Beijing, China). A standard reference soil GBW-07405 (GSS-5), obtained from Center for National Standard Reference Materials of China, was included in the analytical process for quality assurance/quality control. MeHg was extracted using $CuSO_4$-methanol/solvent from the soil followed by ethylation and trapping on a Tenax column via N_2-purging based on modifications to the US EPA 1630 method. MeHg in the extract was measured with an automated MeHg analytical system (TEKRAN 2700 GC-CVAFS). Quality controls included measuring analytical blanks and a certified reference material (ERM-CC580) between sample runs. The analytical blanks were <2 pg L^{-1} for MeHg and the recovery of the ERM ranged from 85.7 to 104.2% MeHg.

2.4. Soil DNA extraction and real-time PCR (qPCR)

The total microbial genomic DNA was extracted from 0.25 g of soil using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's