Radial oxygen loss has different effects on the accumulation of total mercury and methylmercury in rice

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Abstract

Background and Aims Mercury (Hg) pollution in paddy fields and rice has aroused great concern in recent years. This study investigated the dynamic changes of radial oxygen loss (ROL) and Fe plaque formation on roots, and their effects on Hg accumulation in rice plants.

Methods A rhizobag experiment was conducted to study temporal variations and correlations between ROL (amounts and rates), Fe plaque formation, total Hg (THg) and methylmercury (MeHg) in plant tissues and in plaque of four rice cultivars during six growth stages.

Results ROL amounts and Fe plaque formation increased from tillering to reproductive stages, then ROL amounts gradually decreased until maturation stage, whereas Fe plaque remained relatively stable. ROL rates continued to decline during the entire growth period. Both ROL amounts and Fe concentrations in plaque were positively correlated with THg concentrations in the plaque, whereas negatively correlated with THg in straw and brown rice. However, there were no significant relationships between ROL, Fe in plaque and MeHg in plaque and in plant tissues.

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Xun Wang and Bing Li the authors equal contribution to this work.

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Conclusions ROL has great effects on THg but not MeHg accumulation in rice plants. Rice cultivars with higher ROLs tend to have a greater ability to reduce THg accumulation in brown rice.

Keywords Dynamic changes · Iron plaque · Mercury · Methylmercury · *Oryza sativa* L · Radial oxygen loss (ROL)

Introduction

Mercury (Hg) is now recognized as a global contaminant which is extremely toxic (Jiang et al. 2006). Methylmercury (MeHg), the most common form of organic Hg, is of greater concern due to its higher toxicity for humans and its ability to be more readily bioaccumulated and biomagnified along food chains (Ullrich et al. 2001). It is generally considered that the consumption of marine products such as fish and shellfish is a major pathway of human exposure to MeHg (Clarkson 1993). However, recent studies have shown that rice (*Oryza sativa* L.), a staple food in Asia, can be another primary source of MeHg for humans, particularly in Hg mining areas and also in certain inland areas in Southwestern China (Feng et al. 2008; Li et al. 2008; Zhang et al. 2010a, b). Therefore, minimizing grain Hg and MeHg in rice production, especially in Hg-contaminated regions, is of great importance.

To adapt to anaerobic conditions, wetland plants such as rice develop aerenchyma to transfer O₂ from the aerial parts to the roots, resulting in O₂ diffusing toward the root apex and its rhizosphere - a process which is termed radial oxygen loss (ROL) (Colmer 2003). ROL can create an oxidizing environment in the rhizosphere and cause precipitation of toxic metals onto the rhizosphere soil and root surfaces (Otte et al. 1989). Previous studies have indicated that rice cultivars with higher rates of ROL possess greater capacities for limiting the transfer of cadmium (Cd) and arsenic (As) to above-ground tissues (Mei et al. 2009, 2012; Wang et al. 2011). Due to the ROL from plants and biological oxidation by microorganisms, substantial quantities of iron (Fe) are transferred to the plaque, leading to a well-defined zone of ferric oxyhydroxide accumulation (Taylor et al. 1984; Macfie and Crowder 1987). Fe plaque is able to sequester metal(loid)s by adsorption and/or co-precipitation, thus affecting the bioavailability of these elements in the rhizosphere, which can influence metal tolerance and

uptake by wetland plants (Otte et al. 1989; Ye et al. 1997; Blute et al. 2004). Hansel et al. (2001) found that Fe plaque formation could reduce lead (Pb) and zinc (Zn) uptake and translocation in the wetland grass *Phalaris arundinacea*. The formation of Fe plaque on roots significantly inhibited the uptake of As by rice, under both glasshouse (Chen et al. 2005) and paddy field conditions (Garnier et al. 2010). It has also been reported that adding ferrous iron to sulphidic wetland sediments decreased Hg bioavailability and net methylation due to a decrease in sulphide activity and a concomitant decrease in the concentration of dissolved mercury (Mehrotra et al. 2003; Mehrotra and Sedlak 2005). However, the exact effects of ROL and Fe plaque formation on Hg and MeHg uptake by rice plants have not been reported.

It is notable that the ROL and Fe plaque of rice roots could change in amount and composition during growth (Nanzyo et al. 2010; Wang et al. 2013). Wang et al. (2013) reported that amount of ROL per plant and Fe plaque increased dramatically from tillering to ear emergence stages and then decreased at the grain-filling stage. Nanzyo et al. (2010) found that the quantity of root Fe plaque reached its peak at the tillering stage, after which it gradually decreased. Furthermore, dissolved organic matter (DOM) secreted by roots might influence the formation of Fe plaque since DOM could provide protons and electrons for reductive dissolution of Fe plaque (Zhang et al. 2012). Aulakh et al. (2001) reported that the level of DOM changed during the entire growth period, reaching its peak in the earing and flowering stage and then gradually decreased. The results from the above previous studies suggest that changes in ROL and Fe plaque during the growth period of rice plants may also have great effects on THg/MeHg uptake and accumulation. However, little information is available on the effects of the dynamic changes of ROL and Fe plaque on the accumulation and distribution of Hg and MeHg in rice plants and other wetland plants. The study therefore aims to investigate the dynamic changes of ROL and Fe plaque formation and temporal variations in Hg and MeHg accumulation and distribution in rice plants at six definable growth stages (tillering, elongation, ear emergence, flowering, grain-filling and maturation) and their relationships, and so reveal the potential roles of ROL and Fe plaque on Hg and MeHg uptake by rice plants.

Materials and methods

Pre-culture of rice seedlings

Two *indica* rice cultivars, Tianyou196 (TY196) and Huayouguangkangzhan (HY) and two *japonica* cultivars, Nanjing 35 (NJ35) and Zixiangnuo (ZX), were selected due to their differences in Hg accumulation properties according to our previous study (Li et al. 2013). The two cultivars, TY196 ($22.8 \pm 0.7 \text{ ng g}^{-1}$ THg) and NJ35 ($20.5 \pm 2.7 \text{ ng g}^{-1}$ THg), have a lower Hg accumulation in brown rice than that in the other two cultivars, HY ($30.1 \pm 3.1 \text{ ng g}^{-1}$ THg) and ZX ($28.5 \pm 2.2 \text{ ng g}^{-1}$ THg). Seeds were surface sterilized with 30 % *v/v* hydrogen peroxide (H_2O_2) for 30 min and washed thoroughly with deionized water. They were then germinated in acid-washed quartz sand and distilled water was used to moisten the seeds and to irrigate the germinated seedlings for 7 days. Seedlings were subsequently transferred to plastic containers (12 L, 80–100 seedlings per container) and grown in 1/4-strength solution for 25 days with the following nutrient composition ($\mu\text{mol L}^{-1}$): NH_4NO_3 500, K_2SO_4 200, CaCl_2 400, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1500, KH_2PO_4 1.3, Fe-EDTA 50, H_3BO_3 10, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 1.0, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 1.0, $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ 5.0, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.5 and $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$ 0.25. The nutrient solution was left without any forced aeration, adjusted to pH 6.0 with NaOH or HCl and changed every 5 days.

Pot experiment under waterlogged conditions

Soil was collected from the plough layer (0–20 cm) of a paddy field, located in Wanshan Hg mining area in Guizhou Province, Southwest China. The soil pH (measured in the water extract following the method of ISO 10390: 2005) was 7.0, and the soil contained 2.34 % total C (Total Organic Carbon Analyzer, Shimadzu, Japan), 0.14 % total N (Automated Discrete Analyzer, SmartChem 200, Alliance, France), $46 \mu\text{g g}^{-1}$ THg and 3.7 ng g^{-1} MeHg (following method of USEPA 1630: 2001). After being air-dried, sieved to < 10 mesh and homogenization, soil was supplemented with basal fertilizers [125 mg N kg^{-1} soil as $(\text{NH}_2)_2\text{CO}$, 80 mg P kg^{-1} and 125 mg K kg^{-1} soil as KH_2PO_4 and K_2SO_4], mixed thoroughly and equilibrated for a month. A cylindrical nylon rhizobag (30 μm nylon mesh, 8 cm diameter and

11 cm length) was designed to separate rhizosphere from non-rhizosphere zones. The rhizobag, which was separated longitudinally into two halves by a thin plastic card in the centre and filled with 500 g dry sand (the same acid-washed quartz sand as that used at the germination stage), was then placed in the centre of a PVC pot (14 cm diameter and 18 cm high), filled with 1.5 kg paddy soil (Cheng et al. 2014). The system was inundated with 2 cm water above the soil surface for 2 weeks before planting.

Rice seedlings were carefully transplanted into the rhizobags, with two seedlings in each rhizobag, one each side of the central separator. There were 4 replicates per treatment. Soil in each pot was kept submerged using deionized water and the seedlings were grown in a glasshouse with the following conditions: temperatures day/night were 25/20 °C, day/night relative humidity was 60/80 % and 16 h of light with a $>350 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photon flux density.

Harvest and sampling

After transplanting, plants were harvested at their tillering stage (the stage when side shoots are produced, day 26), elongation stage (when rice plants grow at their fastest rate, and stems are elongating, day 42), ear emergence stage (when developing ears are visible, day 59), flowering stage (when most ears are flowering, day 72), grain-filling stage (when grains are being filled, day 85) and maturation stage (when grains are ripe, day 101), respectively. The two plants from a pot were separated and washed thoroughly with deionized water. One plant was used for determining the ROL and the other for DCB extraction of Fe plaque on root surfaces. After Fe plaque was extracted, the plants were separated into roots (all stages), straw (a mixture of stems and leaves in all stages), panicle (flowering stage), grains (grain-filling stage), husk and brown rice (maturation stage). All the harvested plant samples were freeze-dried and the freeze-dried weight was measured. The samples were then ground to a fine powder and stored at 4 °C for further analysis.

ROL measurement

ROL amounts were measured using a titanium(III) citrate buffer method (Kludze et al. 1994). The

actual ROL amount was calculated using the following formula:

$$\text{ROL amount} = c(y-z)$$

$$\text{Rate of ROL} = c(y-z)/g$$

where ROL is measured as $\mu\text{mol O}_2 \text{ plant}^{-1} \text{ h}^{-1}$, **c** is the initial volume of Ti^{3+} added to each tube (in L), **y** is the concentration of Ti^{3+} in the solution of the control (without plants) (in $\mu\text{mol Ti}^{3+} \text{ L}^{-1}$), **z** is the concentration of Ti^{3+} in solution after 6 h treatment with plants (in $\mu\text{mol Ti}^{3+}$ in solution $\text{plant}^{-1} \text{ L}^{-1}$), and **g** is the root dry weight (in kg).

DCB extraction of Fe plaque

Iron plaque on fresh root surfaces of rice plants was extracted using the dithionite-citrate-bicarbonate (DCB) method (Otte et al. 1989). The whole root system from each plant was harvested for the DCB extraction and the dry weight of root was measured after extraction. The roots were washed twice with deionized water and then incubated for 60 min in 40 ml cold solution of dithionite-citrate-bicarbonate (0.03 M sodium citrate and 0.125 M sodium bicarbonate with the addition of 0.6 g sodium dithionite). After incubation, roots were rinsed three times with deionized water and the rinsings added to the DCB extract. The resulting solution was made up to 100 ml with deionized water for analysis. Since it is hard to obtain the exact mass of plaque, the comparison of the Fe plaque formation on root surfaces between treatments was based on the ‘per gram of root dry weight’. Such expression has been widely used in similar studies in wetland plants (Taylor and Crowder 1983; Otte et al. 1989; Ye et al. 1997).

Sample analysis

Concentrations of THg and Fe in DCB-extracted solutions were determined by Atomic Absorption Spectroscopy (AAS, Z-5000, Hitachi, Japan) (for THg) and Inductively-Coupled Plasma Optical Emission Spectrometry (ICP-OES, Optima 2000 DV, Perkin Elmer, USA) (for Fe), respectively. The plant samples were ground to a fine powder and mixed completely. Then 0.2 g was randomly collected from each sample and microwave-digested in concentrated HNO_3 (16 mol L^{-1}) and THg in the digests measured by atomic

fluorescence spectrometry (AFS, Beijing Titan Instrument Co., Ltd). For MeHg analysis, plant samples (0.2 g, the same as above) were prepared by KOH-methanol/solvent extraction and determined with a MERX Automatic Methylmercury System (Brooks Rand Laboratories, Seattle, WA), following method 1630 (USEPA 2001). Blanks, tea standard material (GBW-08303) (China Standard Materials Research Center, Beijing, P.R. China), lobster standard material (TORT-2) (National Research Council of Canada) were used for quality control. Recoveries from the reference materials and matrix spikes ranged from 95 to 102 % for THg analysis, 97 to 104 % for Fe analysis, and from 86 to 104 % for MeHg analysis.

Statistical analysis

Data were analyzed using the statistical software package SPSS 17.0 and summarized by means $\pm$ standard errors (SE). Means were compared by least significant difference (LSD) at the 5 % level. Coefficients of determination (R^2) and significance probabilities (P) were computed for linear regression fits.

Results

Growth, ROL and Fe plaque formation

The biomass of roots and straw tissues of the four rice cultivars at six growth stages are listed in Table 1. They both increased significantly during the entire growth period and at the same growth stage, significant differences ($P < 0.05$) were detected among the cultivars. Both amounts and rates of radial oxygen loss (ROL) were significantly different ($P < 0.01$) between the growth stages, ranging on average from 1.2 to 16 $\mu\text{mol O}_2 \text{ plant}^{-1} \text{ h}^{-1}$ and from 0.42 to 14 $\text{mmol O}_2 \text{ kg}^{-1} \text{ root d.w. h}^{-1}$, respectively. The dynamic changes of ROL amounts followed this trend: increasing from tillering stage to reproductive stage, then decreasing gradually to the maturation stage (Fig. 1a). However, ROL rates gradually declined down as rice plants grew from tillering stage to maturation stage (Fig. 1b). Additionally, significant differences ($P < 0.05$) in both amounts and rates of ROL were detected among the rice cultivars at the same growth stage, following the trend: NJ35 > TY196 > HY > ZX (Table S1).

Table 1 Biomass of roots and straw tissues of four rice cultivars (TY196, HY, NJ35, ZX) grown in Hg-contaminated paddy soil at six growth stages (tillering, elongation, ear emergence, flowering, grain-filling and maturation stages) (mean $\pm$ SE, $n=4$)

Stage	Root biomass (g)			Straw biomass (g)				
	TY196	HY	NJ35	ZX	TY196	HY	NJ35	ZX
Tillering	0.21±0.03 (D, a)	0.18±0.01 (E, b)	0.22±0.00 (D, a)	0.13±0.02 (D, c)	1.5±0.29 (D, a)	1.2±0.12 (E, a)	0.77±0.12 (D, b)	0.5±0.14 (D, c)
Elongation	0.31±0.03 (C, a)	0.31±0.02 (D, a)	0.31±0.02 (C, a)	0.15±0.03 (D, b)	1.8±0.18 (D, a)	1.9±0.27 (D, a)	1.1±0.13 (D, b)	0.6±1.3 (D, b)
Ear emergence	0.70±0.01 (B, a)	0.65±0.07 (C, a)	0.66±0.03 (B, a)	0.48±0.07 (C, b)	2.3±0.21 (C, b)	2.2±0.31 (D, b)	3.5±0.16 (C, a)	2.6±0.36 (C, b)
Flowering	0.77±0.06 (B, a)	0.68±0.15 (C, b)	0.70±0.09 (B, b)	0.66±0.11 (B, b)	4.6±0.67 (B, b)	4.1±1.0 (C, c)	5.6±1.1 (B, a)	5.3±1.1 (B, a)
Grain-filling	0.93±0.10 (AB, a)	0.90±0.13 (B, a)	0.97±0.05 (A, a)	0.78±0.16 (A, b)	5.8±1.4 (B, b)	5.2±0.90 (B, c)	7.5±1.0 (A, a)	6.2±1.5 (A, b)
Maturation	1.3±0.20 (A, a)	1.2±0.13 (A, a)	0.95±0.12 (A, b)	0.80±0.08 (A, c)	8.0±2.2 (A, a)	7.0±0.87 (A, b)	7.4±1.4 (A, b)	6.1±0.76 (A, c)

Different capital letters within the same column indicate significant difference between the stages at the level of $P<0.05$. Different small letters within the same line and the same tissue indicate significant difference between the cultivars at the level of $P<0.05$

Fe concentrations in plaque on root surfaces increased by 2.6- to 3.9-fold from tillering to ear emergence stages, and then were maintained relatively stable to maturation stage (Fig. 1c). There were also significant differences ($P<0.05$) in Fe concentrations in plaque among the cultivars at the same growth stage, and a similar trend in ROL was also observed (TableS2). A significant positive correlation between ROL amounts and Fe concentrations in Fe plaque of rice plants at six growth stages was also detected ($R^2=0.71$, $P<0.01$) (Fig. 1d).

THg and MeHg concentrations in rice plants and in Fe plaque

Dynamic changes in THg and MeHg concentrations in straw, roots and Fe plaque of the four cultivars are presented in Fig. 2. Both THg and MeHg concentrations in straw tended to decline during the entire growth period (Fig. 2a, d). From tillering to maturation stages, THg and MeHg concentrations decreased by 0.94- to 1.4- and 3.2- to 6.7-fold, respectively. Furthermore, both THg and MeHg concentrations in straw exhibited significant differences ($P<0.05$) among the cultivars, with the highest concentrations in ZX and the lowest in NJ35 (Tables S3, S4). THg concentrations in roots (Fig. 2b) increased by 1.2- to 4.0-fold from tillering to maturation stages, whereas MeHg concentrations in roots tissues (Fig. 2e) decreased by 1.5- to 2.2-fold during the entire growth period. THg concentrations in Fe plaque on roots (except for ZX) gradually increased from tillering to flowering (or grain-filling) stages, and then decreased up to the maturation stage, whereas they increased over the entire growth period for ZX (Fig. 2c). The dynamic change of MeHg concentrations in Fe plaque did not show any obvious trend.

Distributions of THg and MeHg in different plant components of the four rice cultivars during the entire growth cycle are presented in Fig. 3. In general, root tissues played the most dominant role in accumulating THg and increased gradually from tillering to maturation stages, whereas the aerial part accumulated the largest proportion of MeHg. After the ear emergence stage, the %MeHg in rice reproductive organs (panicle, grain and brown rice) increased sharply, reaching a maximum level at the maturation stage. Both THg and MeHg in Fe plaque were relatively lower than in the other parts of rice plants. However, the proportions of

Fig. 1 Dynamic changes of ROL amounts (a), ROL rates (b) and Fe concentrations in Fe plaque (c), and relationship between ROL amounts and Fe concentrations in Fe plaque (d) for four rice

cultivars (TY196, HY, NJ35, ZX) at six growth stages (tillering, elongation, ear emergence, flowering, grain-filling and maturation stages). Data are means $\pm$ SE ($n=4$)

THg in Fe plaque of NJ35 and TY196 were higher than those of HY and ZX.

Relationships analysis

Some interesting relationships were observed between ROL amounts, degrees of Fe plaque formation and THg concentrations in straw and in Fe plaque on root surface of the rice plants (Fig. 4). Significant negative correlations were found between ROL ($R^2=0.50, P<0.01$), Fe concentration in plaque ($R^2=0.60, P<0.01$) and THg concentrations in straw, whereas there were significant positive correlations between ROL ($R^2=0.53, P<0.01$), Fe concentration in plaque ($R^2=0.61, P<0.01$) and THg concentrations in plaque at the six growth stages of the rice plants.

Correlations between ROL and THg concentrations in brown rice, and between Fe concentrations in plaque and THg concentrations in brown rice are presented in Fig. 5. Both ROL ($R^2=0.81, P<0.01$) and Fe concentrations in plaque ($R^2=0.59, P<0.01$) were negatively correlated with THg concentrations in brown rice of four cultivars at the maturation stage. However, there were no clear relationships between ROL, Fe concentrations in plaque and MeHg concentrations in different plant components of the four rice cultivars (data not shown).

Discussion

The THg concentrations in straw decreased steadily during the entire growth period, whereas in roots it

Fig. 2 Dynamic changes of THg (a, b, c) and MeHg (d, e, f) concentrations in straw, roots, and Fe plaque for four rice cultivars (TY196, HY, NJ35, ZX) at six growth stages (tillering, elongation,

ear emergence, flowering, grain-filling and maturation stages). Data are means $\pm$ SE ($n=4$)

showed an opposite trend (Fig. 2a, b). To the best of our knowledge, the temporal variations in THg levels in rice

plants have never been reported. Although the THg levels in straw showed a diminishing trend, it is

Fig. 3 Distribution of THg and MeHg in different components of four rice cultivars (TY196, HY, NJ35, ZX) at six growth stages (tillering, elongation, ear emergence, flowering, grain-filling and maturation stages)

premature to conclude that the ‘pre-tillering’ period may be the critical time for Hg ingress into the plant since the amount of THg accumulated in straw kept increasing during the entire growth period. The diminishing trend of THg concentration in straw may be due to the role of ‘dilution’, since the biomass of straw increased rapidly during the entire growth period. Both the concentration and proportion (Fig. 3) of THg in roots showed an increasing trend, suggesting that the entry of THg into the rice plant could be a persistent and continuous process, also indicating that roots play a dominant role

in accumulating THg. Similar findings have been reported in previous studies on rice (Meng et al. 2010; Wang et al. 2014). It has also been reported that rice seed (brown rice) has the highest ability to accumulate MeHg compared to other tissues (Meng et al. 2010; Zhang et al. 2010a). Meng et al. (2011) also observed that both the concentration of MeHg and the mass of MeHg in stalks and leaves decreased sharply when the seed started to form, reaching a minimum level at rice harvest. The main reason for the decreasing trend of MeHg levels in roots and straw is that most of MeHg would be translocated into reproductive organs (panicle, grain and brown rice) when rice seed started to form. This is why both the concentration and proportion of MeHg in roots and straw decreased sharply after the ear-emergence stage. Our results from this study also

Fig. 4 Relationships between ROL amounts and THg concentrations in straw (a) and Fe plaque (c), and between Fe concentrations in Fe plaque and THg concentrations in straw (b) and Fe plaque

(d) for four rice cultivars (TY196, HY, NJ35, ZX) at six growth stages (tillering, elongation, ear emergence, flowering, grain-filling and maturation stages)

Fig. 5 Relationships between mean values of ROL amounts (a) and Fe concentrations in Fe plaque (b) at six growth stages and THg concentrations in brown rice for four rice cultivars (TY196, HY, NJ35, ZX)

suggested that reducing the translocation of MeHg into rice seeds during the ripening period could be an efficient way to decrease the accumulation of MeHg in brown rice.

ROL is an important process for wetland plants such as rice adapting them to waterlogged conditions (Armstrong and Armstrong 2005). In this study, ROL amounts increased as rice plants grew, reaching peak values during the reproductive period, and gradually decreasing up to the maturation stage. Similar results have been reported in a previous study (Wang et al. 2013). Furthermore, there were significant differences in ROLs among the rice cultivars ($\text{NJ35} > \text{TY196} > \text{HY} > \text{ZX}$) at the same stage. It has been reported by other workers that ROL differs markedly not only between wetland species (Li et al. 2011; Yang et al. 2014) but also between cultivars of rice (Mei et al. 2009; Wang

et al. 2011). Wetland species (or cultivars) with higher rates of ROL tend to form more Fe plaque on root surfaces and in the rhizosphere (Li et al. 2011; Yang et al. 2014). However, in the present study, only ROL amounts but not ROL rates, were significantly positively correlated with Fe concentrations in plaque on root surfaces (Fig. 1d). This result indicates that ROL amounts rather than ROL rates play a dominant role in affecting the formation of Fe plaque on root surfaces during the entire growth period of rice plants. It should also be noted that the Fe formation on root surfaces can be influenced by many abiotic factors such as the availability of soil Fe, temperature, pH, CO_2 , soil permeability, etc. (Chen et al. 1980; St-Cyr and Crowder 1988), as well as biotic factors, including ROL and rhizosphere bacteria (St-Cyr and Crowder 1989; Chen et al. 2008). Mendelsohn et al. (1995) reported that ROL is the most

important biotic factor controlling Fe plaque formation. Our study also suggests that the rice cultivars with higher ROL amounts released more O_2 from roots during the reproductive phase of growth, and tend to deposit larger amounts of Fe on the root surfaces. It is also very likely that the increase in ROL with growth is due to the production of fine lateral roots. As the production of fine laterals during the entire growth period was not measured in this experiment, its effect was not clear. The significance of fine lateral root growth should be considered in further studies.

The significant negative correlations between ROL amounts and THg concentrations in straw (Fig. 4a) and brown rice (Fig. 5a) suggest that ROL had indirect effects on THg accumulation in above-ground tissues (straw, brown rice), and rice cultivars with higher ROL amounts tended to have a greater ability to inhibit Hg transfer and accumulation in above-ground parts than those with lower ROL amounts. ROL from roots may have important impacts in regulating Hg bioavailability in the rhizosphere and subsequently Hg accumulation in rice. It has been reported that the mobility and availability of many toxic metals to plants in wetland soils are often regulated by redox potential (Eh) and the associated rhizosphere pH (Gambrell et al. 1991). ROL from rice roots significantly changes Eh and pH in the rhizosphere (Mei et al. 2012). An increase in Eh in the rhizosphere by aerobic treatment could significantly reduce the THg in the soil solution (Peng et al. 2012) and Hg bioavailability (Wang et al. 2014) in rhizosphere soil, and ultimately decrease THg accumulation in brown rice.

The correlations in Fig. 4c, d suggest that ROL might also regulate Hg bioavailability by Fe plaque formation. However, the proportion of THg in Fe plaque was relatively lower than in tissues of rice plants, and the largest amount of THg was accumulated in root tissues (Fig. 3). This may imply that Fe plaque might not play a key role in reducing Hg transfer from roots to above-ground tissues and Hg accumulation in brown rice. The functions of Fe plaque on metal uptake may depend greatly on the amounts of Fe/Mn deposition and metal bioavailability both on root surfaces and in the rhizosphere. Fe plaque could enhance Zn uptake by roots but might show a ‘barrier’ effect when large amounts of Fe are deposited on root surfaces (Otte et al. 1989; Jacob and Otte 2003). The effects of Fe plaque on Hg adsorption only focus on Fe and Hg/MeHg on root surface in the present study. Yang et al. (2014) reported that the

amounts of Fe, Mn and Zn in the Fe plaque in the rhizosphere were larger than those on the root surfaces. It is possible that the adsorption of Hg/MeHg in Fe plaque in the rhizosphere may be greater than that on the root surfaces but this needs further investigation. More in-depth studies on the role of ROL and Fe plaque on Hg and MeHg in both the rhizosphere and on root surfaces are required.

Both ROL amounts and the degree of Fe plaque formation on root surfaces showed no significant correlation with MeHg in plant tissues (brown rice, straw, roots) and in Fe plaque, which suggests that both ROL and Fe plaque had no clear effects on MeHg accumulation. Our data indicate that the effects of ROL and Fe plaque on THg and MeHg in soils and on uptake by rice plant were different and that Fe had a higher affinity for THg than for MeHg. In a similar As study, Chen et al. (2005) found that Fe plaque enhanced As(III) but decreased As(V) uptake by rice. Liu et al. (2006) reported that arsenate was the predominant species of As in Fe plaque, whereas the organic species of As could hardly be detected in Fe plaque.

Conclusions

This study investigated the effects of ROL and Fe plaque on Hg and MeHg uptake and accumulation in rice plants. Our results demonstrated that ROL (amount and rate), Fe plaque formation, THg and MeHg in tissues of rice plants showed great variations over the six growth stages. Rice cultivars with higher ROL amounts tended to have higher degrees of Fe formation on root surfaces and lower THg in straw and brown rice, which suggests that ROL has strong effects on THg uptake and accumulation. However, ROL and Fe plaque have no obvious effects on MeHg accumulation. The present study increases our understanding of the dynamic changes of ROL, Fe plaque formation and their roles in accumulation of THg and MeHg over the life cycle of the rice plant. The effects of ROL on microbial composition and abundance, Fe plaque formation on root surfaces and in the rhizosphere, and their relationships with THg/MeHg accumulation under field conditions all require further investigation.

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