RESEARCH ARTICLE

Influence of *Spartina alterniflora* on the mobility of heavy metals in salt marsh sediments of the Yangtze River Estuary, China

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Abstract Using bio-disturbed sulphide to trace the mobility and transformation of Cu, Pb, Ni and Zn in the sediments of the *Spartina alterniflora*-dominated salt marsh in the Yangtze River Estuary, measurements were made of the seasonal variations of acid-volatile sulphide (AVS) and of the simultaneously extracted metals (SEM) in the rhizosphere sediments. Microcosm incubation experiments recreating flooding conditions were conducted to evaluate the effect of AVS and other metal binding phases upon the dynamics of Cu, Pb, Ni and Zn in the salt marsh sediments. The results demonstrate that the ratio values of SEM/AVS have a significant seasonal variation in the rhizosphere sediments and that the anoxic conditions in the sediments were likely enhanced by *S. alterniflora* during the summer and autumn compared with the anoxic conditions resulting from the native species *Phragmites australis* and *Scirpus mariqueter*. The incubation experiments suggest that Fe(III) and Mn(IV/III) (hydr)oxides provide important binding sites for heavy metals under oxic conditions, and sulphide provides important binding sites for the Cu and Pb under anoxic conditions. Our observations indicate that the mobility of heavy metals in the salt marsh sediments is strongly influenced by biogeochemical redox processes and that the invasive *S. alterniflora* may increase the seasonal fluctuation in heavy metal bioavailability in the salt marsh ecosystem.

Keywords Heavy metal · Iron (hydr)oxide · Acid-volatile sulphide · Salt marsh · *Spartina alterniflora*

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Introduction

The accumulation and cycling of heavy metals have been a concern for salt marshes in estuarine ecosystems (Williams et al. 1994; Spencer et al. 2003; Reboreda and Caçador 2007; Koretsky et al. 2007, 2008a, b; Duarte et al. 2010) that have been greatly affected by intensive human activities such as domestic, agricultural and industrial waste discharge and mining operations. Because heavy metals may accumulate in and potentially be released by the sediments of salt marshes, the sediments act as potential sources of heavy metal contamination to the surrounding aquatic ecosystem and may prove toxic to aquatic life. More importantly, metals can be taken up by aquatic organisms, thus entering the food chain where the metals could be transferred to the upper trophic levels to become potential sources of toxicity to humans (Pan and Wang 2012).

The various binding phases, such as the organic matter, carbonate, Fe(III) and Mn(IV/III) (hydr)oxides and sulphide phases, are important for the mobility and bioavailability of heavy metals during the biogeochemical redox processes that occur in sediments (Tessier et al. 1979; Yu et al. 2001; Weber et al. 2009a; Borch et al. 2010). Under oxic conditions, Fe(III) and Mn(IV/III) (hydr)oxides are regarded as important sorbent phases for heavy metals in sediments (Zachara et al. 2001; Cooper et al. 2006; van Griethuysen

et al. 2006). However, these oxides are reductively dissolved in sub-oxic conditions, not only releasing dissolved Fe(II) and Mn(II) to pore water but also releasing associated trace metals, which could make trace metals more mobile and bioavailable (Koretsky et al. 2007; Weber et al. 2009a). In anoxic conditions, one of the dominant processes controlling the dynamics of chalcophile metals is bacterial sulphate reduction. This reduction could lead chalcophile metals, such as Cu, Cd, Pb and Hg (Cooper and Morse 1998; Morse and Luther 1999; Burton et al. 2006), to react with sulphides and be sequestrated in insoluble metal-sulphide precipitates. Thus, acid-volatile sulphide (AVS), which is principally generated by sulphate reduction, and the amount of simultaneously extracted metals (SEM), which is defined as the molar sum of the Cd, Cu, Ni, Pb and Zn released during digestion in either 1.0 or 6.0 M HCl at room temperature for 30 min or 1 h (O'day et al. 2000), are commonly used to evaluate the potential bioavailability of trace metals and their subsequent toxicity to benthic biocommunities (Yu et al. 2001; Poot et al. 2007). When the ratio value of SEM/AVS is <1, the sediment may not be toxic for benthic organisms; however, the sediment may be considered potentially toxic when the ratio value is >1.

Tidal flooding and plant growth have significant effects on redox conditions in salt marsh sediments (Williams et al. 1994; Kostka and Luther 1995; Reboreda and Caçador 2007; Koretsky et al. 2008a, b). Although tidal flooding can temporarily limit O_2 diffusion into the sediments, causing anoxic conditions, the tidal flow transport oxygen to sediments, causing oxic conditions in sediments. Plants help to transfer oxygen from the surface to the roots, thus oxidising the rhizosphere sediments (Koretsky et al. 2008a, b). However, plants also release organic carbon into the sediments by way of litter and root exudates, and the introduced organic carbon drives many biotic and abiotic reactions as it decomposes, increasing the reductive conditions in sediments (Hines et al. 1999; Choi et al. 2006; Koretsky et al. 2007; Koretsky et al. 2008a, b; Pereira et al. 2007). The impacts of seasonal variations on the redox conditions of salt marsh sediments are also significant because of soil temperature has a very strong influence on the dynamics of microbial soil reduction (Weber et al. 2010). Thus, there are numerous processes, whether physical and biogeochemical, in isolation or in combination may have affected the redox conditions significantly and ultimately the formation and dissolution of metal phases in sediments (Koretsky et al. 2008a, b; Roychoudhury 2007).

There are large areas of salt marsh in the intertidal zone of the Yangtze River Estuary, China. In recent decades, multiple studies on heavy metals in salt marsh sediments of the Yangtze River Estuary have been undertaken and have shown that there was heavy metal pollution within the intertidal zone (Zhang et al. 2001; Feng et al. 2004;

Zhang et al. 2009). However, the primary focus of most studies has been the total concentrations of the heavy metals found in the sediments (Chen et al. 2000; Feng et al. 2004; Zhang et al. 2009). Few studies have focused on the chemical forms of the heavy metals or the effects of salt marsh plants on heavy metal mobility (Bi et al. 2003; Quan et al. 2007; Zhao et al. 2009). *Spartina alterniflora*, an invasive halophyte, was intentionally introduced to the coastal region of China in 1979 (Quan et al. 2007; Wan et al. 2009) and is now a dominant species in the Yangtze River Estuary. The invasion of *S. alterniflora* has had multiple effects upon the estuarine ecosystems (Li et al. 2009) because *S. alterniflora* can potentially facilitate organic carbon storage (Zhang et al. 2010; Li et al. 2009) and influence the cycles of sulphur (Zhou et al. 2009; Nie et al. 2009) in the salt marsh sediments. These influences suggest that the functional groups of rhizosphere microbial communities in estuarine ecosystems are subject to change. Thus, the *S. alterniflora* invasion could have a potentially significant effect on the biogeochemical redox processes, such as Fe and Mn (hydr)oxide and bacterial sulphate reduction, that control the chemical speciation, bioavailability, toxicity and mobility of many trace metals in the aquatic rhizosphere.

The objective of this study was to examine the spatial and temporal variations of AVS and SEM in rhizosphere sediments. Microcosm incubation experiments were performed in flooding conditions to evaluate the role of AVS and other metal-binding phases that affect the dynamics of Cu, Pb, Ni and Zn in the salt marsh sediments. Another objective was to address the influence of temperature, tides and the invasion of *S. alterniflora* on the mobility and transformation of heavy metals in salt marsh sediments of the Yangtze River Estuary.

Study area

The salt marsh of the Dongtan wetland, located on the east coast of Chongming Island, is the largest intertidal zone of the Yangtze River Estuary. The subtropical climate allows a typical seasonal growth cycle for the salt marsh plants in the study area. Our sampling sites are located in the Dongtan wetland (Fig. 1). The high marsh, low marsh and bare flat lie in different elevations of the intertidal zone. The native plant *Phragmites australis* is the dominant species around the high marsh zone, while the pioneer plant *Scirpus mariqueter* dominates the low marsh zone. In the late 1990s, *S. alterniflora* was transplanted to the low marsh zone and quickly expanded towards the high marsh zone (Quan et al. 2007). The monocultures of *P. australis* and *S. mariqueter* have become smaller and smaller since the introduction of *S. alterniflora*, with the monoculture of *S. alterniflora* accounting for 49.4 % of the vegetated area in the Dongtan

Fig. 1 Yangtze River estuary (**a**) and study area (**b**). The sampling cores *A1* and *B1* are located in the monocultures of *P. australis* in the high marsh; cores *A2* and *B2* are in the monocultures of *S. alterniflora*, and core *B3* is in the monocultures of *S. mariqueter* in the low marsh
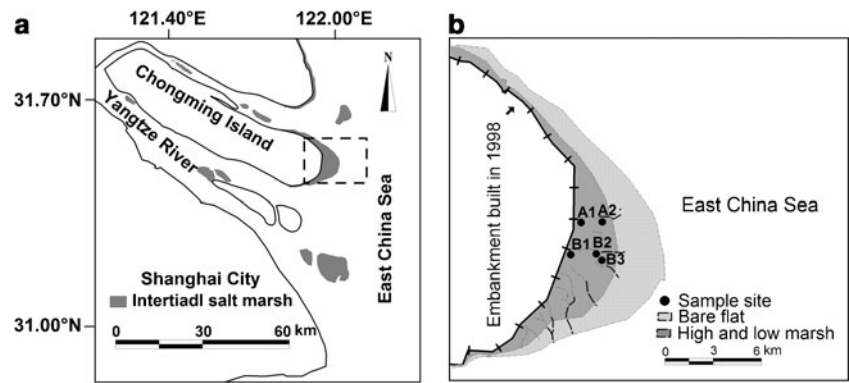
wetland in 2005 (Li et al. 2009). The irregular semi-diurnal tide is the dominant factor controlling sedimentation in the salt marsh (Zhou et al. 2007) and floods the low marsh and bare flat twice per day. The high marsh is flooded during the spring tide, with the strongest flooding occurring in summer and autumn. The tidal range is 2.5 m on average and approximately 3.5 m during spring tides. The peak flow velocity is approximately 2 m/s during spring tides in channels and subtidal slopes; however, the attenuation of hydrodynamics by vegetation is significant. Sediment grain size exhibited a landward/upward decreasing trend, due to the decrease of flow velocity from the subtidal to the salt marsh (Yang et al. 2008). Previous research in the study area showed that the concentrations of heavy metals in sediments were above background levels (Zhang et al. 2001, 2009).

Materials and methods

Sample collection and preparation

Using a plastic bucket, surface water was collected during ebbing of the tidal creek at the high marsh (A1, B1), the low marsh (A2, B2) and the front of the low marsh (B3) (Fig. 1). The sediment profile was logged during the sampling in August 2010. Litter and dark sediment were found at the upper horizon (0–5 cm) of the sediment, and few roots were found at the deeper horizons in the rhizosphere of *P. australis*. Dark or olive-brown sediment and fine roots were apparent at the deeper horizons (approximately >18 cm) in the rhizospheres of *S. alterniflora* and *S. mariqueter*. The root network of *S. alterniflora* was denser than that of *S. mariqueter*. Sediment cores (approximately 35 cm in length each) were sampled with a PVC pipe ($d=8$ cm) at different sites in April, October and December 2009 and August 2010. All of the cores were plugged and sealed with tape in the field. On the same day, the cores were taken to the lab, and sectioning of the sediment from the PVC corers was conducted under ambient atmospheric conditions at the earliest opportunity after extraction. The sediment samples were sectioned at intervals of 0–1, 1–2, 2–4 and 4–6 cm;

below which, the cores were sectioned at 3-cm intervals to a depth of 30 or 33 cm. Each section was immediately placed in a plastic bag, which had as much air removed as possible before sealing and then stored in a bag filled with nitrogen at 4 °C until analysis. After the AVS analysis, the sediments were freeze-dried in a freeze dryer (Freeze Dryer Alpha 1–4 LD, Martin Christ Gefriertrocknungsanlagen GmbH, Germany). Prior to analysis, the dried samples were ground to a powder with an agate mortar and sieved through a 63-μm nylon mesh to remove sand and roots.

Incubation microcosm experiments

To simulate field conditions and evaluate the role of the AVS and other metal-binding phases that affect the dynamics of Cu, Pb, Ni and Zn in the salt marsh sediments, microcosm experiments were set up to investigate the geochemical conditions and processes that occur during changing redox conditions. Approximately 2 kg of wet sediment was collected from the sediment-water interface boundary (0–5 cm) of the salt marsh. For the microcosm experiments, the sediment was air dried, passed through a 63-μm sieve and mixed to homogeneity. A series of independent microcosm incubation experiments were conducted at 27±2 °C. The sediment was submerged with Milli-Q water and incubated for 1–50 days in the dark. In the first batch, approximately 3 g of sieved surface sediment and 20 mL Milli-Q water, which was degassed using N_2 for 3 h, were put into 30-mL plastic centrifuge tubes and then shaken gently to achieve homogenisation. The resulting microcosm was kept in contact with the atmosphere. The second batch was similar to the first except that an external carbon source (0.1 g of powdered *S. alterniflora* aboveground tissue) was added to the sediment and the tubes were sealed to increase anoxic conditions. The blank samples were prepared along with the microcosm samples. Each microcosm sample was performed in triplicate. The incubation samples were harvested at spatial intervals of 1, 3, 5, 10, 15, 20, 30 and 50 days. The results are given as the mean± standard deviation.

Chemical analysis

Prior to centrifugation, the pH and dissolved oxygen in the incubated solution were measured by inserting specific electrode probes into the tubes under N_2 conditions. The tubes were centrifuged at $2,000 \times g$ for 15 min, and the aqueous samples were extracted by glass syringes. Under ambient atmospheric conditions, each aqueous sample was then filtered through a 0.45-μm cellulose acetate membrane and preserved by acidification using nitric acid (pH of <2). Surface water temperature, pH and electrical conductivity were measured in the field.

The procedures for the AVS analysis, including those conducted on the fresh wet sediments and the incubated-sediment paste, were adopted from the method described by Lin et al. (1997) and Lee et al. (2000). Approximately 2 g of sediment was added to the reaction flask and sparged for 2 min with N_2 (100 cm^3 min^{-1}). The sulphide in the sediment was liberated by extraction with 1 M HCl for 40 min at room temperature and then trapped in a solution of 0.2 M Zn $(CH_3COO)_2$ and 0.1 M CH_3COONa with a continuous N_2 flow to form ZnS. Each trap was quantified using the methylene blue method, with an estimated detection limit of 0.02 μmol g^{-1} dry weight. The recovery of S(II) was 97 ± 6 % ($n=3$), which was validated using $Na_2S \cdot 9H_2O$. For the AVS of all of the sediment samples, triplicate determination of approximately 10 % of the samples revealed that the analytical precision was within 20 %. Prior to analysis, the simultaneously extracted soil suspension was centrifuged at $2,000 \times g$ for 15 min and then filtered through a 0.45-μm cellulose acetate membrane.

The concentrations of heavy metals, which included the water soluble Fe and Mn and the simultaneously extracted Al, Fe, Mn, Cu, Pb, Ni and Zn, were determined using inductively coupled plasma-optical emission spectrometry (ICP-OES; Varian 710-ES ICP-OES, Agilent, USA). The water soluble Cu, Pb, Ni and Zn from the incubation experiments were measured by magnetic sector inductively coupled plasma mass spectrometry (ICP-MS; Element 2 ICP-MS, Thermo Scientific, Germany). The relative standard deviation of these measurements was always better than 15 %. The concentrations of total Al, Fe, Mn, Cu, Pb, Ni and Zn in the sediments and selected reference materials (GSD-9, China Stream Sediment Reference Material) were analysed by X-ray fluorescence spectrometry (X-ray Fluorescence Spectrometer 1800, Shimadzu, Japan). The metal recoveries were between 90 and 110 % compared with the certified values, and the relative standard deviation of these measurements was always less than 15 %. The total organic carbon (TOC) was determined by titration with $FeSO_4$ after digestion with a $K_2Cr_2O_7$–H_2SO_4 solution at 175 °C in a silicone–oil bath. The sulphate in the surface water and the incubated solution was measured by the turbidimetric method (Kolmert et al. 2000). The water content was determined by drying the samples at 105 °C for 12 h. The grain size distribution was assessed by a laser diffraction particle-size analyser (LS 13320, Beckman Coulter, USA) after the removal of organic matter and carbonates. The statistical analyses were performed with the SPSS 11.5 for Windows® software package.

Results and discussion

General characteristics of sites

An overview of the general site characteristics is provided in Table 1. In the surface water of the salt marsh tidal creek, the pH ranged from 7.2 to 8.2. The temperature increased from approximately 9.0 to 29.0 °C between December and August. The sulphate concentrations ranged from 80.0 to 869 mg L^{-1} and were higher during the winter. The grain-size analysis revealed that the predominant size of sediments is 4–63 μm (silt). The fraction of medium silt (<16 μm) was generally 50 % and correlated positively with the total Cu, Pb, Ni, Zn and TOC ($P<0.05$), which is consistent with previous investigations (Zhang et al. 2001, 2009). The TOC concentrations ranged from 0.4 to 1.4 %, with a mean value of 0.8 ± 0.2 % ($n=147$), with slight differences found for the all sites. The decreasing order of the depth-integrated mean concentrations of the TOC in the rhizosphere sediments was *S. alterniflora*>*P. australis*>*S. mariqueter*. The depth-integrated mean concentrations of the total metal were Cu=35.1 ± 7.6 mg kg^{-1}, Pb=37.6 ± 8.2 mg kg^{-1}, Ni=34.4 ± 3.9 mg kg^{-1} and Zn=110.0 ± 17.1 mg kg^{-1} ($n=147$). The depth-integrated mean concentrations of the simultaneously extracted metal were SEM-Cu=15.8 ± 5.4 mg kg^{-1}, SEM-Pb=15.1 ± 5.1 mg kg^{-1}, SEM-Ni=6.6 ± 2.2 mg kg^{-1} and SEM-Zn=27.2 ± 6.0 mg kg^{-1} ($n=147$) (Tables S1 and S2 in the Electronic supplementary material). The general physical–chemical properties of sediments for batch experiments are provided in Table 2.

Spatial and temporal distributions of AVS and SEM

As a result of oxidation, there was a loss of AVS in the sediments when we sectioned the samples and stored them before analysis. Although we obtained a fresh sample from the inside of the sediment section when the AVS was analysed and finished all of the sample analyses within two weeks, the concentrations of the AVS in the sediments are under-estimated; thus, the AVS results must be interpreted semi-quantitatively.

The AVS concentrations in the sediments exhibited major spatial and seasonal variation among the different sites (Fig. 2). There were two distribution patterns found for the

Table 1 The characteristics of the surface water and the rhizosphere sediments in the salt marsh of Dongtan wetland

Time		Surface water				Sediments				
		T (°C)	pH	Electric conductivity[a] (μS cm^{-1})	Sulphate (mg L^{-1})	n	Water content (%)	Grain size (<16 μm) (%)	TOC (%)	SEM (μmol g^{-1})
Phragmites australis										
A1	12/2009	9.0	7.9	16.1	453.8	10	32.1±6.1	59.2±8.6	1.0±0.2	0.9±0.1
A1	08/2010	28.5	8.0	15.1	80.0	11	34.7±2.9	64.7±5.9	0.8±0.2	1.0±0.1
B1	12/2009	10.0	7.2	21.3	428.9	12	27.2±3.1	38.2±4.4	0.7±0.1	0.8±0.1
B1	08/2010	28.2	8.1	8.2	120.4	12	30.5±1.1	51.1±5.1	0.7±0.1	1.0±0.2
Spartina alterniflora										
A2	04/2009	16.5	7.3	14.3	332.9	13	40.8±8.1	58.0±13.3	1.0±0.2	0.8±0.2
A2	12/2009	10.2	8.2	29.6	847.2	13	39.5±5.7	51.5±10.4	0.9±0.1	0.9±0.2
A2	08/2010	28.9	7.8	17.9	831.2	13	34.3±5.3	46.9±12.8	0.7±0.2	0.9±0.2
B2	12/2009	10.0	8.0	28.5	744.7	13	34.4±5.0	52.4±12.2	0.9±0.2	1.0±0.2
B2	08/2010	28.3	8.1	8.6	160.6	13	32.0±3.4	51.7±12.0	0.7±0.2	0.8±0.2
Scirpus mariqueter										
B3	10/2009	16.8	7.9	20.3	569.1	12	28.7±3.0	44.5±5.9	0.6±0.1	0.7±0.1
B3	12/2009	10.0	8.0	28.5	869.1	12	33.3±3.9	50.9±6.6	0.8±0.1	0.7±0.1
B3	08/2010	29.7	8.1	11.5	170.6	13	29.9±3.1	46.5±13.3	0.6±0.2	0.8±0.2

[a] Temperature compensated conductivity at 25 °C

AVS concentrations. In the upper layer (approximately 6 cm) of the *P. australis* rhizosphere, the AVS concentrations were obviously higher than those of lower depths (Fig. 2(a$_1$–a$_3$)), showing a sharply decreasing trend with increasing depth, with the exception of profile B1 in August (Fig. 2(a$_4$)). However, the profiles of the AVS concentrations were different in the rhizosphere of *S. alterniflora*. A particularly notable increasing trend in AVS with depth until 15 cm or deeper was observed, at which point AVS decreased; several peak values (15, 21, 30 and 33 cm) were identified. Similarly, the distribution of AVS concentrations in the rhizosphere of *S. mariquete* increased and then decreased with depth, with peak values at 24 and 30 cm. In addition, the AVS concentrations for each site were greater

during the warm months (April and August) than during the cold months (December) (Fig. 2), particularly in the rhizosphere of *S. alterniflora* (Fig. 2(b$_1$–b$_5$)).

The depth-integrated mean concentrations of the SEM exhibited slight seasonal variations at the different sites (Table 1). The profiles of the SEM concentrations also exhibited slight variations with depth across all profiles (Fig. 2). However, one-way ANOVA statistical analysis showed that there is significant higher SEM mean concentrations in the rhizosphere of *S. alterniflora* than those in the rhizosphere of *S. mariqueter* ($P<0.05$), suggesting that the phases of metals in the sediments could be significantly influenced by *S. alterniflora*. Although the AVS concentrations in the sediments were under-estimated, the SEM

Table 2 The characteristics of the surface salt marsh sediment used for microcosm experiments of incubation ($n=5$)

Grain size (%)	35 (<4 μm, clay)	63 (4–63 μm, slit)	2 (>63 μm, sand)
pH$_{CaCl2}$	7.6±0.2		
TOC (%)	1.1±0.0		
Sulfate (μmol g^{-1})	6.6±0.4		
SEM	1.13±0.06		
	Total concentration (μg g^{-1})	HCl extractable (μg g^{-1})	% of total
Al	79,647±3,129	3,343±91	4
Fe	46,621±3,565	6,500±299	14
Mn	922±79	664±34	72
Cu	45.5±4.6	23.4±1.5	51
Ni	39.9±2.5	5.8±0.4	14
Pb	45.9±4.0	16.0±0.7	35
Zn	126.4±10.2	38.2±2.5	30

Fig. 2 The vertical distributions of the AVS and SEM at the different rhizosphere profiles. a_1–a_4 *P. australis*. b_1–b_5 *S. alterniflora*. c_1–c_3 *S. mariqueter*
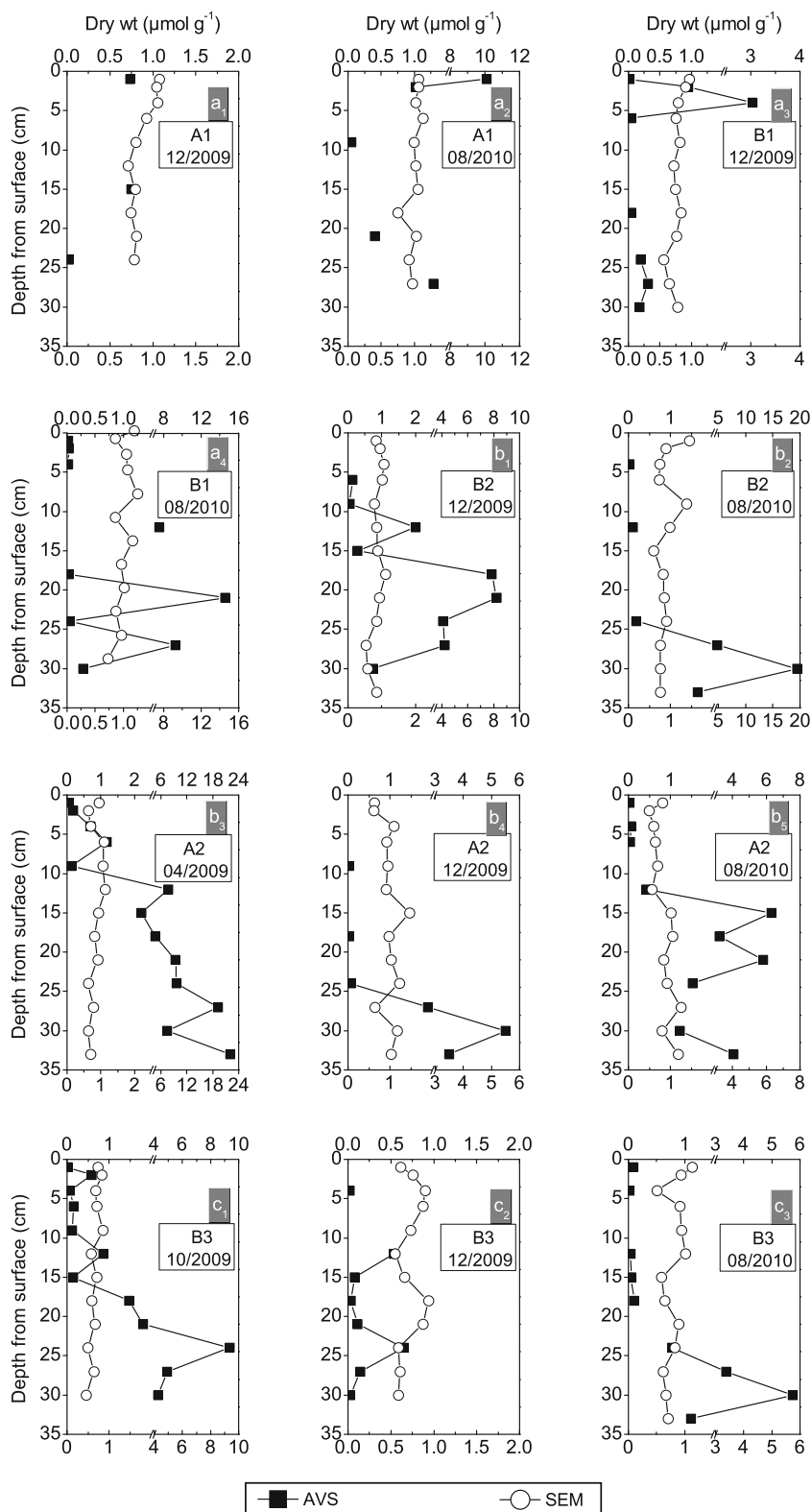
concentrations in the upper horizon (approximately 6 cm) of site A1 in August and site B1 in December were significantly less than the AVS concentrations (SEM/AVS of <1) (Fig. 2(a_2 and a_3)). The SEM concentrations in the deeper horizon (below 15 or 25 cm) of both *S. alterniflora* and *S. mariquete* were also significantly less than the AVS concentrations (Fig. 2(b_1–b_5, c_1 and c_3)). These results indicate that AVS could play a major role in the metal bioavailability of the upper rhizosphere of *P. australis* and the deeper rhizospheres of both *S. alterniflora* and *S. mariquete*. The results

also suggest that the ratio values of SEM/AVS exhibited a significant seasonal variation in the different rhizosphere sediments due to seasonal changes of AVS in the sediments.

The concentration of AVS in the sediment is a product of the equilibrium between AVS generation and loss due to oxidation and diffusion (Fang et al. 2005). Temperature change, degradation of labile organic matter and activity of sulphate-reducing bacteria (SRB) are regarded as major controlling processes for the AVS concentrations in the sediments (Choi et al. 2006; Koresky et al. 2007, 2008b). The AVS concentrations were higher during the warm months than during the cold months and were supported statistically by using one-way ANOVA statistical analysis ($P<0.05$). This difference might stem from the higher temperatures, which have a very strong influence on the dynamics of microbial soil reduction, and the increased bacterial activity during the warm months. The bacterial sulphate reduction was consistent with the depth-dependent redox conditions in the rhizospheres of both *S. alterniflora* and *S. mariquete* but not in the rhizosphere of *P. australis*. This finding could possibly be explained by the fact that organic matter decomposition promotes bacterial sulphate reduction, and a significant quantity of the labile organic matter produced by *P. australis* exits with the surface sediments of the rhizosphere. The AVS concentrations in the upper horizon of the rhizospheres of both *S. alterniflora* and *S. mariquete* were commonly lower than those in the deeper horizon, which could be a result of the intense oxidising action of O_2 via tidal pumping and diffusion and the decreased decomposition of labile organic matter.

Although, using one-way ANOVA statistical analysis, the results show that the depth-integrated AVS mean concentrations are not statistically different at all sties ($P<0.05$). Note that during the warm months, more accumulation of AVS was observed at the deeper horizons in rhizosphere of *S. alterniflora* compared with the native plants *P. australis* and *S. mariquete* (Fig. 2), which is likely the result of more labile organic matter being released by *S. alterniflora* roots and the increased relative abundance of sulphate reduction bacteria in the root zone of *S. alterniflora*. Studies have found that invasive *S. alterniflora* has a significantly higher net photosynthetic rate, greater litter input and longer growing season than those of native *P. australis* and *S. mariqueter* on Jiuduansha Island (one of the islands in the Yangtze River Estuary) (Liao et al. 2008; Li et al. 2009). Several studies have found that increased sulphate reduction rates occurred during spring (April and May) in the root zone of *S. alterniflora* (Hines et al. 1999; Koretsky et al. 2005). They postulated that this was due to enhanced availability of labile organic carbon during the period when *S. alterniflora* growth was most rapid. Edgcomb et al. (1999) has found that the relative abundance of SRB reach its peak values after August. Nie et al. (2009) has also found that the

higher richness and abundance of SRB occurred in the rhizosphere of *S. alterniflora* during late growing season. However, several other studies have suggested that *S. alterniflora* can lead more oxidized sediments by pumping oxygen into the rhizosphere (Lee et al. 1999; Koretsky et al. 2008a, b). These results suggest that *S. alterniflora* could have various effects to sulphate reduction and accumulation of AVS in sediments. Further studies are needed to investigate the complex roles of the radial loss of oxygen, the availability of reduced organic, microbial communities interaction and other localized conditions in the salt marsh sediments.

HCl extraction removes a variety of Fe and Mn phases, including Fe and Mn (hydr)oxide, sulphide and others (Poulton and Canfield 2005). Pearson correlation coefficients were calculated separately for the simultaneously extracted metals Al, Fe, Mn, Cu, Pb, Ni and Zn. The results in Table 3 show that Cu and Ni are correlated well with Fe; Pb is correlated well with Mn and Cu; Zn, Fe, Mn and Al are all correlated. These results suggest that Cu and Ni could be typically associated with Fe (hydr)oxides, Pb could be typically associated with Mn (hydr)oxides and Zn could be typically associated with Fe and Mn (hydr)oxides, especially under oxic conditions.

Table 3 Pearson correlation coefficient between SEM-Al, SEM-Fe, SEM-Mn, SEM-Cu, SEM-Pb, SEM-Ni and SEM-Zn in the rhizosphere sediments

|	Cu	Fe	Mn	Ni	Pb	Zn
Phragmites australis						
Al	0.63[a]	0.88*	0.28	0.83*	0.14	0.56*
Cu		0.75*	0.65*	0.63*	0.73*	0.45*
Fe			0.49*	0.88*	0.31	0.53*
Mn				0.23	0.60*	0.40*
Ni					0.20	0.41*
Pb						0.29
Spartina alterniflora						
Al	0.56*	0.91*	0.41*	0.82*	0.25**	0.46*
Cu		0.63*	0.83*	0.34*	0.87*	0.57*
Fe			0.52*	0.84*	0.38*	0.45*
Mn				0.15	0.85*	0.58*
Ni					0.04	0.16
Pb						0.53*
Scirpus mariqueter						
Al	0.80*	0.83*	0.71*	0.86*	0.72*	0.57*
Cu		0.76*	0.84*	0.71*	0.88*	0.71*
Fe			0.76*	0.88*	0.64*	0.41*
Mn				0.68*	0.82*	0.68*
Ni					0.60*	0.41*
Pb						0.77*

$P<0.01$; **$P<0.05$, significance level

Solution dynamics in microcosm incubation experiments

Sulphate dynamics

The redox transformations were monitored in solution during microcosm incubation experiments. The pH decreased within 3 days and then increased slightly in two types of batch experiments, but the pH remained at 8 and 7 after 20 days in the first and second batch, respectively (Fig. 3a). This result suggests that acetate, propionate and inorganic carbon were most likely produced by microbial respiration, which could generate a large amount of H^+, and then the acetate, propionate and inorganic carbon reacted with Fe(III) and Mn(IV/III) (hydr)oxides and sulphate and were consumed (Weber et al. 2009a). The dissolved oxygen decreased within 3 days and then increased, with the most noticeable results in the first batch. However, the concentration of the dissolved oxygen remained lower in the second batch because of the isolation and decomposition of the external carbon source (Fig. 3b). The increase in dissolved oxygen in the second batch indicates that the plastic centrifuge tube was not fully sealed, which allowed oxygen to infiltrate the solution. In the first batch, the dissolved sulphate decreased within 20 days but then increased, which corresponded with the increase and decrease of AVS (Fig. 3c, d). In the second batch, the dissolved sulphate decreased over the course of 50 days, which corresponded with the increase of AVS, particularly within 10 days (Fig. 3c, d). Although the dissolved oxygen increased from 3 to 10 days in the first batch, the AVS concentrations increased approximately 30-fold, while in the second batch during the same time, the AVS concentrations increased approximately 90-fold. This result indicates that AVS generation overwhelms AVS loss and

Fig. 3 Aqueous dynamics of sulphate, Fe, Mn and major chalcophile metals during sediment incubation: **a** pore water pH, **b** dissolved oxygen, **c**, **d** sulphate and soil AVS (soil dynamics), **e** dissolved Fe, **f** dissolved Mn, **g** dissolved Ni, **h** dissolved Zn, **i** dissolved Cu and **j** dissolved Pb. *Error bars* represent one standard deviation of the triplicate samples
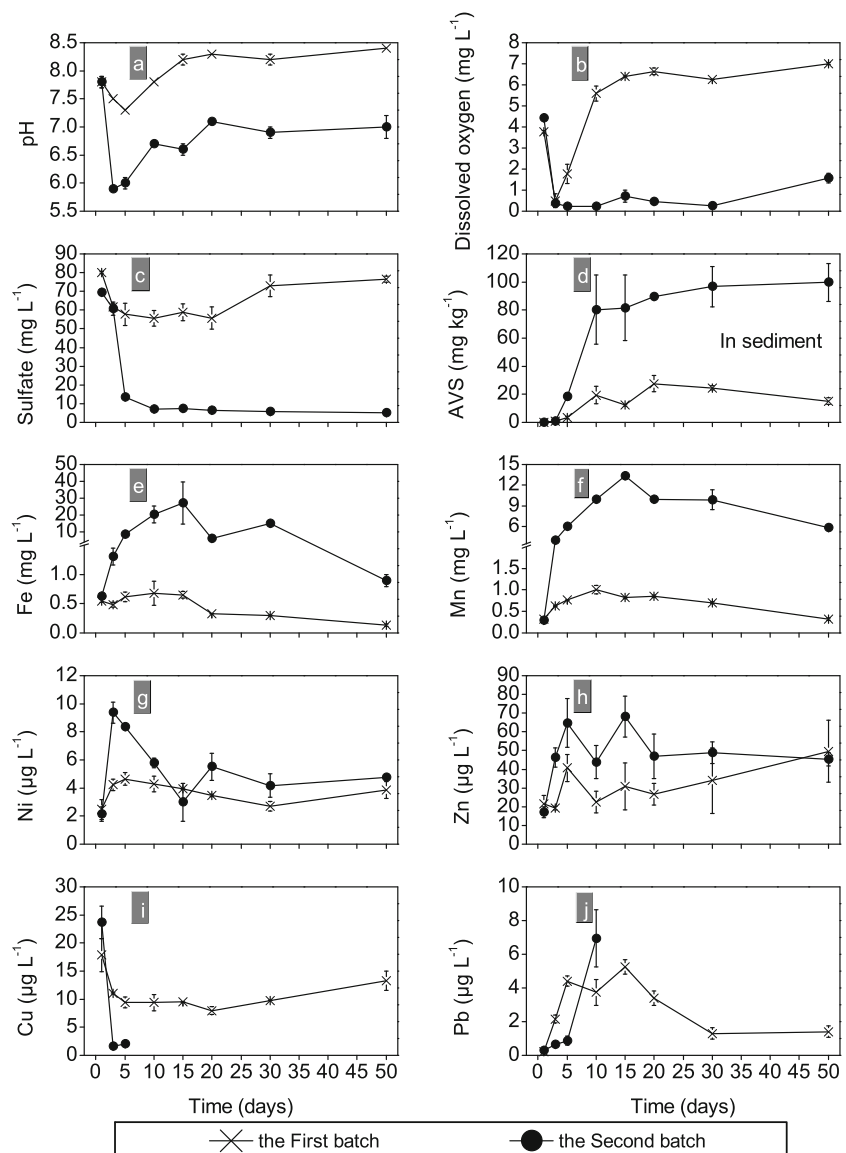
ultimately leads to accumulation of AVS in sediment, implying that the anaerobic respiration is overwhelming even if the second batch experiment is not isolated. We infer that more labile organic matter supplied may account for more sulphate reduction.

Heavy metal dynamics

In the second batch, a rapid increase in dissolved Fe and Mn was observed within 15 days, followed by a gradual decrease, indicating that the reductive dissolution of Fe(III) and Mn(IV/III) (hydr)oxides quickly resulted in elevated concentrations of dissolved Fe and Mn (Fig. 3e, f). The formation of solid secondary minerals of Fe and Mn could have led to the decrease in dissolved Fe and Mn from 15 to 30 days. Changes in the opacity of the plastic tube and the growth of a light yellowish-brown matter on the tube walls were observed after 30 days of incubation; these observations indicate that the dissolved Fe and Mn were oxidised by oxygen, which also resulted in the precipitation of the Fe and Mn (hydr)oxides and the decrease in the dissolved Fe and Mn. Following the rapid increase in the dissolved Fe and Mn, the dissolved Pb, Ni and Zn concentrations increased within 3, 5 and 10 days, respectively (Fig. 3g–i), suggesting that the reductive dissolution of Fe(III) and Mn (IV/III) (hydr)oxides could encourage the release of Pb, Ni and Zn into solution (Zachara et al. 2001; Koretsky et al. 2007; Weber et al. 2009a). A decrease in dissolved Ni and Zn and a depletion of dissolved Cu and Pb accompanied the sulphate reduction. The difference between the two groups is that the dissolved Ni and Zn concentrations remained relatively constant at approximately 5 and 50 $\mu g\ L^{-1}$ after 20 days, respectively (Fig. 3g, h), implying that the dissolved Ni and Zn are not completely controlled by the metal sulphide formation and that they are not likely to be partly incorporated in carbonate precipitates or other phases (Weber et al. 2009a; Voegelin and Kretzschmar 2005). However, the dissolved Pb levels, which decreased to undetectable levels within 10 days (Fig. 3j), and the dissolved Cu levels, which were depleted within 5 days (Fig. 3i), suggest that the dissolved Pb and Cu could be almost completely sequestered in the metal sulphide phases under sulphate-reducing conditions (Weber et al. 2009a, b). In the first batch, the dissolved heavy metals exhibited somewhat similar changes with the second batch. The dissolved Ni, Zn and Cu slightly increased after 20 days (Fig. 3g–i), accompanying AVS concentration decrease in the sediment (Fig. 3d). This finding suggests that the sulphides of Ni, Zn and Cu could be oxidised and remobilised.

The ratio values of SEM/AVS of >1 persisted during the entire course of the incubation of the first batch. However, ratio values of SEM/AVS of >1 were only maintained for the first 5 days in the second batch. From 20 to 50 days, the ratios were less than 1 (approximately 0.5), but during the same time, the concentrations of dissolved Ni and Zn remained relatively constant (Fig. 3g, h). This result suggests that even though the ratio values of SEM/AVS of <1, some dissolved heavy metals, such as Zn and Ni, are not sequestered in sulphides. Previous investigations have also found that Zn and Ni are rarely sequestered in sulphides in freshwater floodplain soil (Weber et al. 2009a) and that the metals are removed from pore water by the formation of mono-sulphide phases, although only partially for Zn and Mn and not at all for Pb (O'day et al. 2000). Thus, the ratio values of the SEM/AVS method may not provide appropriate measurements of all of the chalcophile metal bioavailability.

The dynamics of heavy metal mobility and transformation in the salt marsh sediments

The results from Fig. 2 reveal that both temperature change and organic decomposition have a significant influence on the dynamics of sulphate reduction. The incubation results from Fig. 3 reveal that providing more labile organic matter results in the release of more Fe and Mn into solution and that more sulphate is reduced in the flooded sediments in a short time. These results suggest that the processes of labile and reactive organic material decomposition in anoxic conditions are critical for the reductive dissolution of the (hydr)oxides of Fe(III) and Mn(IV/III) and for bacterial sulphate reduction. The dissolved Cu, Pb, Ni and Zn could be initially mobilised by the reductive dissolution of the metal-bearing (hydr)oxides of Fe and Mn (Weber et al. 2009a). Subsequently, bacterial sulphate reduction could cause the dissolved Cu and Pb to sequester in sulphide phases, although this sequestration does not occur to the same extent for Zn and Ni.

The results allow us to refine our understanding of heavy metal mobility and transformation in salt marsh sediments of the Dongtan wetland relative to seasonal changes. During the warmer months (April, August and October), the enhanced decomposition of organic matter initially mobilises more trace heavy metals through the reductive dissolution of the (hydr)oxides of Fe(III) and Mn(IV/III). Subsequently, more sulphides (e.g., the sulphides of Cu and Pb) could be formed by the increased SRB activity. However, Zn and Ni might rarely be sequestered in sulphides. During the colder months (December), the microbial reduction activity is reduced. The rates of the abiotic and biogeochemical oxidation processes are driven by O_2 to overcome the reduction, and they result in the rapid oxidation of Fe(II) and Mn(II) (Rose and Waite 2002; Santana-Casiano et al. 2005). The precipitation of the amorphous or crystal forms of Fe and Mn (hydr)oxides may efficiently capture the dissolved trace heavy metals (Lee et al. 2002; Weber et al. 2009a; Borch et

al. 2010). Thus, when the dissolved trace heavy metals in the pore water diffuse into the oxide horizon or when the oxygenated surface water permeates to the deeper sediment horizon, the dissolved trace metals will be captured by the amorphous or crystalline Fe and Mn (hydr)oxides again.

Furthermore, the salt marsh plants are likely to play an important role in the mobility and transformation of Cu, Pb, Ni and Zn, especially the invasive exotic species *S. alterniflora*. Our results demonstrate that more sulphate is reduced in the rhizosphere of *S. alterniflora* than in the native species *P. australis* and *S. mariquete* during the warm seasons, implying that the variation of redox conditions will be enhanced by *S. alterniflora* throughout the growing seasons, and hence may significantly affect the dynamics of the Cu, Pb, Ni and Zn bio-geochemical processes in the Dongtan wetland.

Conclusions

We conclude that there is a significant seasonal variation in redox conditions in subsurface salt marsh sediments of Dontan wetland. Following the growth of plans, higher amounts of labile and reactive organic materials are provided, and these organic matters are decomposed and leaded to more reductive conditions in the sediments, suggesting that the degradation of labile and reactive organic materials could be key to understanding the seasonal variability of AVS and SEM, particularly in the rhizosphere of *S. alterniflora*. Furthermore, combined with the results of the mobility of heavy metals and accumulation of AVS in the sediments of microcosm incubation experiments during the changing redox conditions, we infer that when the subsurface conditions change from oxidised to progressively more reduced in the salt marsh sediments, Cu, Pb, Ni and Zn were initially mobilised by the reductive dissolution of the Fe(III) and Mn(IV/III) (hydr)oxides. Subsequently, the bacterial sulphate respiration sequestered Cu and Pb in sulphide phases, but Ni and Zn were rarely, if at all, sequestered in sulphide precipitates. The rate of these reactions could be enhanced during summer and autumn, and more reducing conditions could release Zn and Ni from the sediments to the pore water and surface water, which could cause potentially toxic to benthic bio-communities and aquatic life in the salt marsh of the Yangtze River Estuary.

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