Stabilizing effects on a Cd polluted coastal wetland soil using calcium polysulphide

Chen Tu[a], Feng Guan[a,b], Yuhuan Sun[b], Pengpeng Guo[a], Ying Liu[a], Lianzhen Li[a], Kirk G. Scheckel[d], Yongming Luo[a,c,*]

[a] Key Laboratory of Coastal Environmental Processes and Ecological Remediation, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
[b] College of Environment and Safety Engineering, Qingdao University of Science and Technology, Qingdao 266042, China
[c] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[d] National Risk Management Research Laboratory, United States Environmental Protection Agency, Cincinnati 45224, USA

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ABSTRACT

In this study, different dosages of calcium polysulphide (CaS_x) were used as an amendment to investigate effects on the immobilizing of Cd in a wetland soil by pot experiment. In addition to chemical analysis (pH and bioavailable Cd concentration), changes in soil enzyme activities, microbial carbon utilization capacity, metabolic and community diversity were examined to assess dynamic impacts on soil environmental quality and toxicity of Cd resulting from ameliorant dosing. Soil pH increased immediately upon CaS_x amendment compared to the unamended control (CK), and then declined slowly to a level lower than CK. Diethylenetriamine pentaacetic acid (DTPA) extractable Cd concentration was determined to characterize the bioavailability of Cd in the soil. The CaS_x dose-dependent effect observed that with increasing CaS_x dosage, the immobilizing efficiency decreased. Soil urease and catalase activity assays and Biolog EcoPlate assay indicated that early stage addition of CaS_x significantly inhibited soil microbial activities. However, mid and late stage time periods showed the inhibition effects were alleviated, and the microbial activities could be recovered in 1% and 2% CaS_x treatments. Moreover, with increasing incubation time, microbial community diversity and richness were significantly recovered in 1% and 2% CaS_x treatments compared to the CK. No considerable changes were observed in the 5% CaS_x treatment. Conclusively, the 1% CaS_x amendment was an efficient and safe dosage for the stabilization of Cd contaminated wetland soil. This study contributes to the development of *in situ* remediation ameliorants and technologies for heavy metal polluted wetland soils.

1. Introduction

Coastal wetland is a transitional zone between the terrestrial and the marine ecosystem. It is strongly affected by global climate change and intensive anthropogenic activity. It provides essential ecosystem services to people and the environment, including providing production and living materials (such as food, raw materials and water resources), protecting biodiversity, degrading pollution and purifying the environment, regulating runoff floods and regional climate control, and serves as tourist and sightseeing destinations (Costa-Böddeker et al., 2017). In recent years, with the rapid development of industrialization, agricultural intensification and urbanization in the coastal zones, a large quantity of industrial wastes, agricultural fertilizers and pesticides, mining wastewaters, and municipal sewage have been emitted to the coastal wetland soils (Xu et al., 2017; Abad-Valle et al., 2016). Contaminants, such as heavy metals, could transfer from these potential waste streams into the coastal wetland soils causing pollution, and resulting human health and ecological risks to the coastal wetland soil ecosystem (Reddy et al., 2009; Bao et al., 2017). According to some investigations reviewed by Pan and Wang (2012), the average Cd concentration in estuarine and coastal environments in China varies from 0 to 9.7 mg kg^{-1}. However, in some particular sites adjacent to mining and smelling districts in the coastal zone of China, Cd concentrations can be as high as 200–400 mg kg^{-1} (Fan et al., 2006; Zhong et al., 2017). Therefore, it is of great urgency to develop green and sustainable remediation technologies for cost-effective remediation of

heavy metal polluted coastal wetland soils.

Unlike most organic contaminants, heavy metals in the soil cannot be totally degraded, but can be controlled and remediated through the following two *in-situ* strategies. One is to extract heavy metals from the polluted soil with technologies such as chemical/biological leaching and phytoextraction. The other is to decrease the mobility and bioavailability of heavy metals in the soil by adding natural or chemical/biological synthesized amendments, also known as solidification/stabilization (Lee et al., 2009; Koptsik, 2014). Due to its economic efficiency and environmental compatibility, stabilization has been widely and successfully applied in the remediation of the heavy metal-polluted soils in farmland, industrial sites, mining areas, as well as coastal wetlands. Common stabilization agents used in the heavy metal polluted soils include phosphates (such as apatite, hydroxyapatite, bone meal, *etc.*), organic substances (such as humic acid, chitosan, straw, organic fertilizer, biochar, *etc.*) and other minerals (such as zeolite, lime, red mud, bentonite, sepiolite, *etc.*) (Lee et al., 2009; Janoš et al., 2010; Koptsik, 2014; Maletić et al., 2015; Abad-Valle et al., 2016; Guemiza et al., 2017). Their stabilization mechanisms vary with respect to different types of stabilization agents, soil physicochemical properties, as well as soil microbial activities.

Calcium polysulphide (CaS$_x$), also called lime sulfur, is a common soil bactericide and insecticide which has been widely applied in prevention and eradication of plant diseases and insect pests in agricultural production approved by the United States Department of Agriculture (USDA). Due to its strong reducibility and solidification of heavy metals, CaS$_x$ has been considered as an ideal stabilization agent, and has been applied in the control and remediation of chromium (Cr) contaminated soil and waste water (Yahikozawa et al., 1978; Chrysochoou et al., 2010; Chrysochoou and Ting, 2011; Kameswari et al., 2015). CaS$_x$ rapidly transformed hexavalent chromium (Cr(VI)) from a highly mobile and toxic species to the immobile and lower toxicological Cr(III) form, which caused the geochemical fixation of Cr (Fruchter, 2002). Chrysochoou et al. (2010) added CaS$_x$ to Cr(VI)-contaminated soils and found the solubility of transformed Cr(III) was continually decreased over one year of incubation, indicating high efficiency and long-term stabilization of Cr. Maletić et al. (2015) compared the stabilization effects of four ameliorants, including bone meal, activated carbon, bentonite and CaS$_x$ to the stabilization of Pb, Cu and Ni-contaminated soils. They found CaS$_x$ had better stabilization effects on the heavy metals than other ameliorants, and the stabilization effects increased with increasing dosages of CaS$_x$. However, to our knowledge, researches regarding on the remediation of Cd-contaminated soil by CaS$_x$ are very limited. Furthermore, the environmental impacts of CaS$_x$ application to the coastal wetland soil ecosystem remain unclear.

The aim of the present study was therefore to investigate the stabilization effects of CaS$_x$ on the remediation of Cd-contaminated wetland soil, and to clarify the dosage-dependent effects of CaS$_x$ application on soil enzyme activities and soil microbial metabolic and community diversity. The results of this study will be helpful to the development of an efficient, safe and long-acting stabilization soil amendment for the *in-situ* remediation of Cd-contaminated wetland soil.

2. Materials and methods

2.1. Physicochemical properties of the tested soil

The tested soil was collected from the surface layer of the coastal wetland in Shandong Province, China. The basic physicochemical properties were as follow: pH 7.85, soil organic matter 8.04 g kg^{-1}, EC 0.13 ms cm^{-1}, total N 0.56 g kg^{-1}, total P 1.09 g kg^{-1}, total K 15.36 g kg^{-1}, CEC 5.40 cmol kg^{-1}, Na 13.88 g kg^{-1}, Ca 14.81 g kg^{-1}, Cd 0.22 mg kg^{-1}, sandy loam. It belongs to Fluvisols according to the FAO soil classification system.

2.2. Preparation of the soil stabilization amendment CaS$_x$

The tested soil stabilization amendment CaS$_x$ was prepared according to the method modified from Levchenko et al. (2015). Briefly, CaO, S and distilled water were mixed (1:2:15) and heated at 100 °C for 2 h to produce the CaS$_x$ by the following reaction:

$$CaO + S + H_2O \rightarrow CaS_x + CaS_2O_3 + CaCO_3 \tag{1}$$
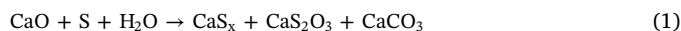
The reaction yields CaS$_4$ and CaS$_5$ (64–67%) and by-products of CaS$_2$O$_3$ (23–28%) and CaCO$_3$ (0.3–1.1%), with 5–11% unreacted sulfur remaining. The main active ingredient of CaS$_x$ are CaS$_x$ (x = 5–8) as well as other components such as HS$^-$, CaSO$_4$, CaO, S and Ca(OH)$_2$. The mixture was then filtered after it was cooled to room temperature. The filtered CaS$_x$ was a dark orange solution, while the pale-yellow precipitate was composed of CaS$_2$O$_3$, CaCO$_3$, and unreacted sulfur. Since CaS$_x$ can be easily oxidized when exposed to air, the filtered CaS$_x$ was sealed by liquid paraffin and stored in the bottle before using.

2.3. Soil stabilization remediation experiment design

The tested soil was disaggregated and sieved to a final size of < 2 mm after air dried. 5 kg of sieved soil was artificially spiked with 500 mL of Cd(NO$_3$)$_2$ stock solution (Cd concentration 2.1 g L^{-1}), and aged for 2 weeks to attain equilibrium. The final concentration of total Cd in the soil was 95 mg kg^{-1}. Soil moisture content was adjusted to 30% of the water holding capacity (WHC) before the pot experiment.

Soil stabilization remediation was conducted by pot experiment. Four treatments were designed with different CaS$_x$ amendment dosage: (i) Cd spiked soil without CaS$_x$ amendment (CK), (ii) Cd spiked soil with 1% (*v:m*) CaS$_x$ amendment (C1), (iii) Cd spiked soil with 2% (*v:m*) CaS$_x$ amendment (C2) and (iv) Cd spiked soil with 5% (*v:m*) CaS$_x$ amendment (C5). Each pot was filled with 300 g (dry weight) soil with the WHC maintained at 30%. All the treatments were conducted in the green house at room temperature, with 3 replicates. Soil samples were collected from all the treatments at 0 d (30 min), 3 days, 15 days, 30 days, 40 days and 55 days, respectively, for further analysis.

2.4. Soil pH and total Cd analysis

Soil pH was measured by pH meter (F2, Mettler Toledo, Switzerland) at a 1:2.5 (w:v) ratio of soil and deionized water. The total Cd content in the soil was analyzed using a HNO$_3$-HF-HClO$_4$ digestion followed by flame atomic absorption spectrometry (AA7000, Shimadzu, Japan).

2.5. Soil bioavailable Cd analysis

Diethylenetriamine pentaacetic acid (DTPA) is a one-step extracting agent that has been widely used to predict the bioavailability of heavy metals in soil (Udovic and Lestan, 2012; Luce et al., 2017). In this research, DTPA extractable Cd was adopted to characterize the bioavailability of Cd in the soil according to Kopittke et al. (2017). 10 mL of DTPA extracting agent was added into a 50-mL tube containing 5 g of air-dried soil passed through a 1 mm mesh. The mixture was oscillated at 180 r min^{-1}, 25 °C for 2 h, then filtered and diluted before the analysis of bioavailable Cd detected by atomic absorption spectrometry.

2.6. Soil enzymatic activities assay

Soil urease activity was determined using the sodium hypochlorite-sodium phenate colorimetry assay with urea as the substrate, and was expressed as mg NH$_4$$^+$-N g^{-1} 24 h^{-1} (Bhaduri et al., 2016). Soil catalase activity was determined using the permanganimetric assay with hydrogen peroxide as the substrate, and was expressed as mL (0.1 mol L^{-1} KMnO$_4$) h^{-1} g^{-1} (Jorge-Mardomingo et al., 2013). Blank matrix control was set for each treatment, while blank sample and

matrix control were set during the whole experiment.

2.7. Soil microbial carbon utilization characteristics

Soil microbial carbon source utilization characteristics were analyzed using the Biolog EcoPlate assay (Jiang et al., 2017). The Biolog EcoPlate (Biolog Inc., Hayward, USA) contains 31 of the most useful carbon sources for soil community analysis, which belong to 6 categories including carbohydrates, carboxylic acids, polymers, amino acids, phenolic acids and amines. Soil suspensions (150 μL) were inoculated directly into Biolog EcoPlates and the microplates were incubated and analyzed at defined time intervals. Formation of purple color occurs when the microbes can utilize the carbon source and begin to respire. The community-level physiological profile is assessed by the rate of average well color development (AWCD) measured at OD_{590} on a microplate reader. The AWCD value is calculated according to formula (2) (Braun et al., 2010).

$$\text{AWCD value} = \sum (C_i - R)/31 \qquad (2)$$

Here, C_i is the OD_{590} value of the ith non-control well, R is the OD_{590} value of the control well, and 31 is the number of carbon sources in the Biolog EcoPlate.

2.8. Soil microbial community diversity

Soil microbial community diversity indexes, *i.e. Shannon* index (H) and *Simpson* index (D) are calculated using the following formulas according to Liu et al. (2017):

$$H = -\sum p_i (\ln p_i) \qquad (3)$$

$$\frac{1}{D} = \sum \frac{(n_i(n_i - 1))}{(N(N - 1))} \qquad (4)$$

Here, p_i is the ratio between the relative OD_{590} value ($C_i - R$) of the ith well and the sum of the entire plate, n_i is the relative OD_{590} value ($C_i - R$) of the ith well, N is the sum of OD_{590} value. Soil microbial community *Richness* index (R) refers to the total number of carbon sources used by soil microbes. It is expressed as the number of wells whose OD_{590} value ($C_i - R$) > 0.15 in each plate (Mahabadi et al., 2007).

2.9. Statistical analysis

Mean values and standard deviation (SD) of the data were calculated by Excel 2016. The statistical analysis was conducted using SPSS Statistics 21.0 software. The significant difference analysis was evaluated by the Duncan's test of difference analysis variance (ANOVA). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Dynamics of soil pH and DTPA extractable Cd concentration

Soil pH variation dynamics in Cd polluted wetland soil amended by different dosages of CaS_x is presented in Fig. 1. Within the entire remediation duration, soil pH in the control treatment (CK) without CaS_x application maintained in an alkalescent range (pH 7.86 ± 0.1), while soil pH in all the CaS_x amendment groups showed a significant increase relative to the control immediately after addition of CaS_x to the soil (30 min, 0 day). The soil pH in 1%, 2% and 5% CaS_x treatments were respectively 8.28, 8.61 and 9.16. The pH enhancement was directly correlated with the increasing of CaS_x dosage. However, as reaction time progressed, soil pH in all CaS_x treatments showed a rapid decrease. At the end of the experiment (Day 55), soil pH of all the treatments with CaS_x amendment were significantly lower than CK, and the extent of pH
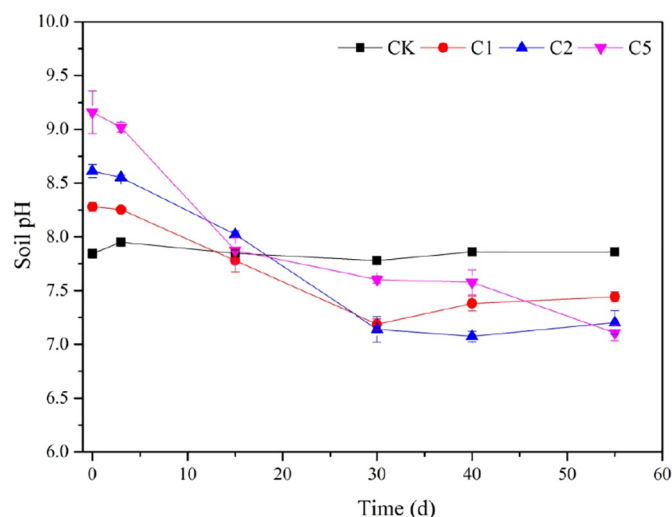

Fig. 1. Dynamic changes of soil pH under different doses of CaS_x amendment.
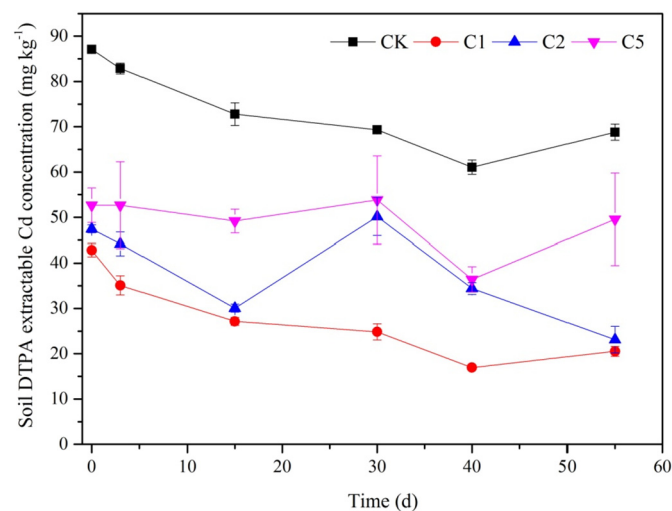

Fig. 2. Dynamic changes of soil DTPA extractable Cd concentration under different doses of CaS_x amendment.

decrease was positively correlated with dosage level of CaS_x ($p < 0.05$).

Dynamics of soil DTPA extractable Cd concentration in different treatments is shown in Fig. 2. DTPA extractable Cd concentrations in 1%, 2% and 5% CaS_x treatments were 42.84, 47.47 and 52.69 mg kg^{-1}, respectively, which were significantly decreased compared with CK (87.10 mg kg^{-1}) immediately after the addition of CaS_x (30 min, 0 day). Upon aging, DTPA extractable Cd concentration in C1 group kept decreasing within the entire experiment period and was markedly lower than that in C2 and C5. At the end of the experiment (Day 55), DTPA extractable Cd concentrations in CK, C1, C2 and C5 were 61.06, 20.53, 23.10 and 49.59 mg kg^{-1}, respectively. For all the treatments with CaS_x amendment, DTPA extractable Cd was significantly lower than that of the CK. The stabilization effect of CaS_x on soil Cd was not positively correlated with the dosage of CaS_x. On the contrary, the most efficient treatment for Cd immobilization was C1 with the lowest CaS_x amendment.

3.2. Dynamics of soil urease and catalase activities

Urease and catalase are two types of typical soil enzymes that are widely used for the evaluation of soil environmental quality and microbial activities. Dynamics of soil urease and catalase activities in the
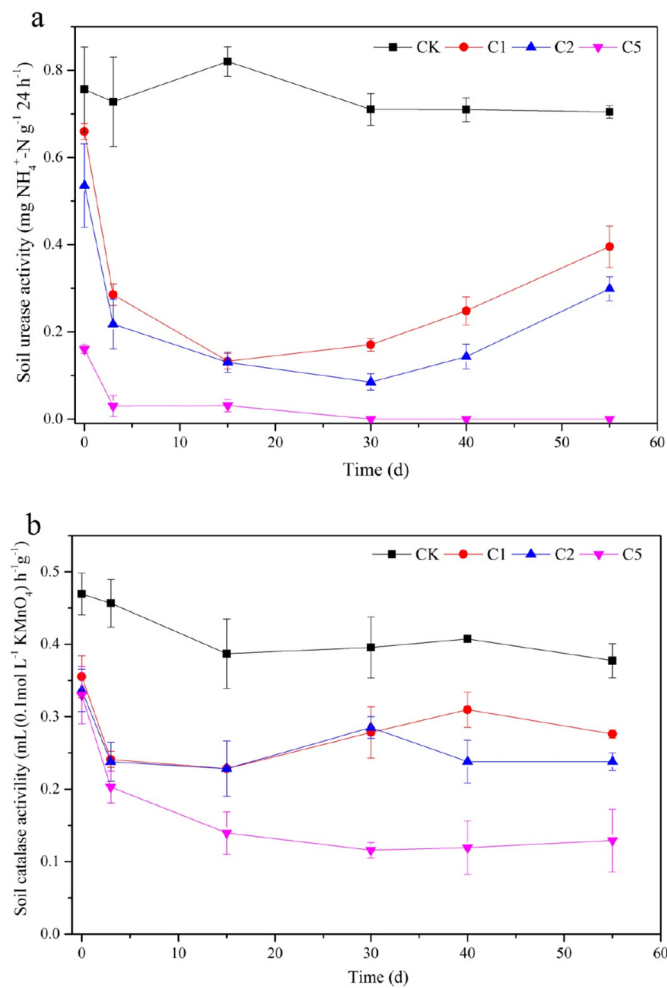
Fig. 3. Dynamic changes of soil enzyme activities under different doses of CaS$_x$ amendment (a: urease activity, b: catalase activity).

Cd polluted soil amended by CaS$_x$ are presented in Fig. 3. Soil urease activity decreased significantly ($p < 0.05$) in all treatments immediately after CaS$_x$ was added to the soil. At Day 0 (30 min), soil urease activities in 1%, 2% and 5% CaS$_x$ treatments were reduced by 12.74%, 18.79% and 70.03% compared to the CK. As reaction time increased, urease activity in C1 kept declining to 0.13 mg NH$_4^+$ N g^{-1} 24 h^{-1} during the first 15 days, then it slowly recovered, increasing to 0.40 mg NH$_4^+$ N g^{-1} 24 h^{-1} at the end of experiment (Day 55). Urease activity in C2 was similar to C1, except the recovery inflexion starts later (Day 30) than C1, and the soil urease activity in C2 (0.30 mg NH$_4^+$ N g^{-1} 24 h^{-1}) was significantly lower than C1 at Day 55. However, urease activity in C5 was totally different from that of C1 and C2. Through Day 3, soil urease activity in C5 decreased rapidly to 0.03 mg NH$_4^+$ N g^{-1} 24 h^{-1}, and maintained at a very low level around the detection limit for the duration of the study. There was no significant change for soil urease activity in the CK group during the whole experiment.

The general dynamics of soil catalase activities were similar to the urease activities (Fig. 3b). Compared to CK, soil catalase activities decreased significantly ($p < 0.05$) in all treatments immediately after CaS$_x$ was added to the soil. For 1% and 2% CaS$_x$ treatments, soil catalase activities kept declining in the first 15 days and started to rise afterward. At the end of the experiment (Day 55), soil catalase activities in C1 and C2 had recovered to 0.27 and 0.24 mL (0.1 mol L^{-1} KMnO$_4$) h^{-1} g^{-1}, respectively, which were still significantly lower than that in CK ($p < 0.05$). Dynamics of soil catalase activity in C5 was different from C1 and C2. It kept declining in the first 30 days to the
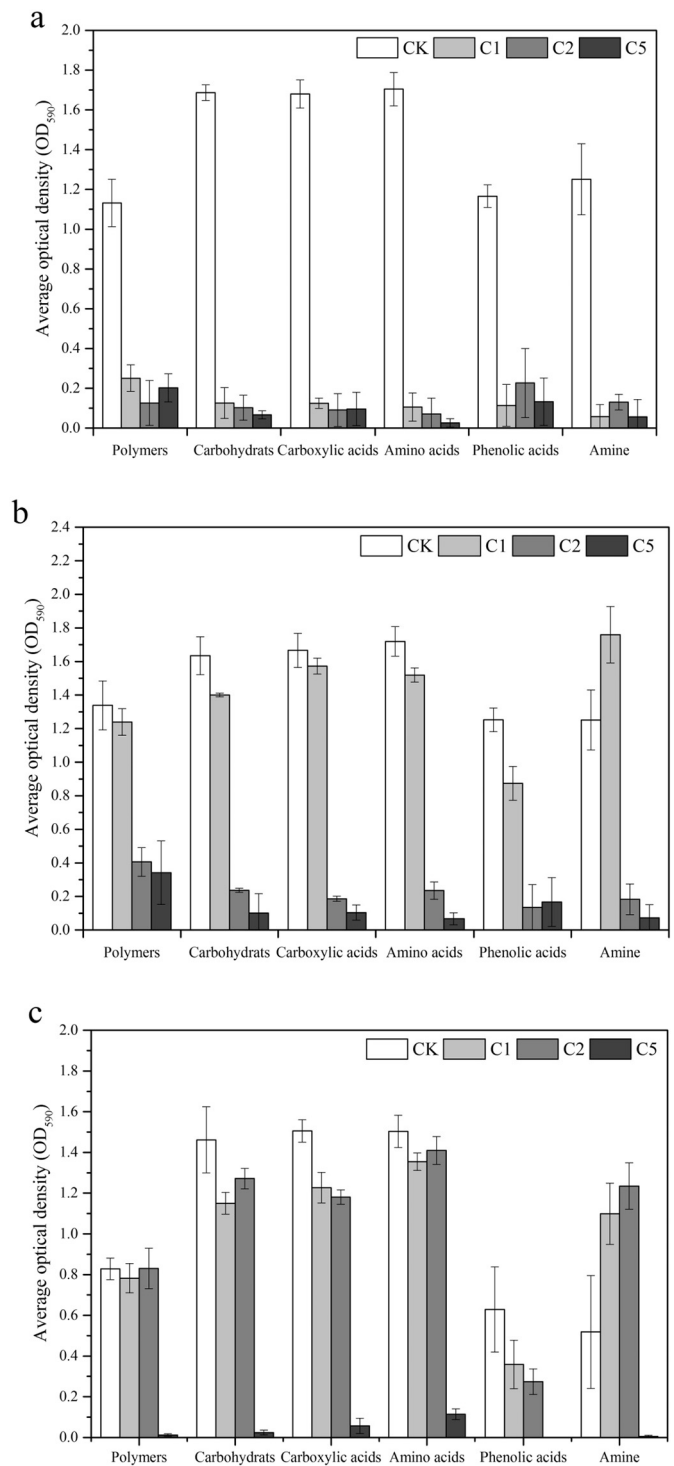

Fig. 4. Dynamic changes of soil microbial carbon utilization capacity under different doses of CaS$_x$ amendment (a: 3 days, b: 30 days, c: 55 days).

minimum of 0.12 mL (0.1 mol L^{-1} KMnO$_4$) h^{-1} g^{-1}, and remained stable at this level until the end of the experiment without any recovery.

3.3. Dynamics of soil microbial carbon utilizing capability

Thirty-one of the commonly used carbon sources in the Biolog EcoPlates were classified into 6 categories including carbohydrates, carboxylic acids, polymers, amino acids, phenolic acids and amines. Kinetic effects of different CaS$_x$ dosages on soil microbial carbon utilization capability are shown in Fig. 4. On Day 3, microbial utilization of
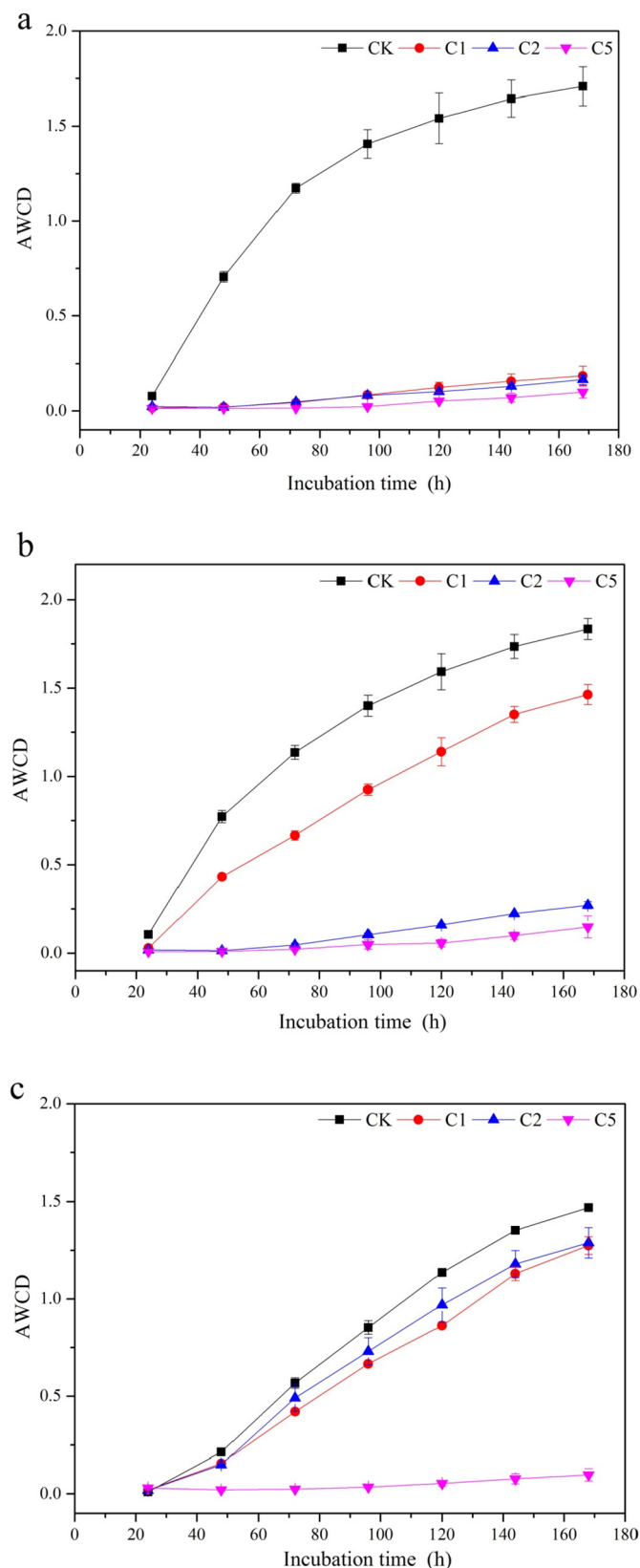
a

b

c

Fig. 5. Dynamic changes of AWCD value of soil microbial community under different doses of CaS_x amendment (a: 3 days, b: 30 days, c: 55 days).

all the six carbon substrate categories was significantly inhibited in all the CaS_x treatments relative to CK (Fig. 4a). With increased incubation time, microbial utilization of all six carbon substrate categories in the

1% CaS_x treatment started to show a significant increase on Day 30, while 2% and 5% CaS_x treatments had no obvious recovery (Fig. 4b). On Day 55, all the categories of carbon substrates except phenolic acid significantly recovery in both 1% and 2% CaS_x treatments, but not in the 5% CaS_x treatment.

3.4. Dynamics of soil microbial community diversity

AWCD of Biolog EcoPlates was used as an indicator of soil microbial metabolic activity. Variation of AWCD with incubation time is presented in Fig. 5. Three days after CaS_x application, AWCD in all the CaS_x amended groups decreased significantly (Fig. 5a). On Day 30, AWCD in C1 showed an obvious enhancement relative to C2 and C5, but still significantly lower than CK (Fig. 5b). On Day 55 of CaS_x amendment, AWCD in both 1% and 2% CaS_x treatments increased significantly to near the level of CK, while AWCD in C5 had no obvious change during the incubation (Fig. 5c).
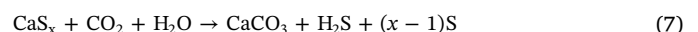
The effects of different CaS_x dosages on soil microbial community diversity, as reflected by *Shannon*, *Simpson* and *Richness* indices, are listed in Table 1. As shown in Table 1, the *Shannon*, *Simpson* and *Richness* indices decreased significantly 3 days after CaS_x amendment, and the differences of these indices among various CaS_x dosages were not considerable. On Day 30, the *Shannon*, *Simpson* and *Richness* indices in 1% and 2% CaS_x treatments increased significantly compared to Day 3, but still lower than CK. On Day 55, the *Shannon*, *Simpson* and *Richness* indices in 1% and 2% CaS_x treatments had recovered to the level of CK ($p < 0.05$), while none of these indices in 5% CaS_x treatment showed any enhancement during the incubation.

4. Discussion

The migration and transformation of heavy metals in polluted soil and sediment can be hindered by stabilization techniques based on amendments of suitable immobilizing agents. Adsorption of heavy metals on mineral surfaces, formation of stable complexes with organic ligands, surface precipitation and ion-exchange are identified as the main mechanisms responsible for the reduction of the metal mobility, leachability and bioavailability. The key to the control and remediation of heavy metal polluted wetland soil is the screening and characterizing of proper stabilization agents, including clay minerals, lime, red mud, biochar and CaS_x, *etc.* (Bradl, 2004; Garau et al., 2007; Luce et al., 2017; Qiao et al., 2017).

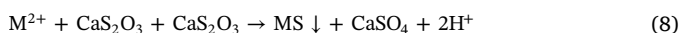### 4.1. Effects of CaS_x on soil pH and metal bioavailability

In this study, within the first 15 days after different doses (1%, 2% and 5%) of CaS_x addition, soil pH was significantly enhanced compared to the CK, and the level of pH increase showed a positive correlation with the dosage of CaS_x (Fig. 1). This is mainly due to the original physico-chemical features of CaS_x synthesized in this research. CaS_x is a dark orange solution, with an alkaline pH (pH = 11.0). It may contain various chain species including hepta-, octa-, and nano-sulphide with a pH ranging from 6.0 to 11.0 (Gun et al., 2004; Kamyshny et al., 2007). CaS_x solution can enhance soil pH rapidly and significantly as soon as it interacts with the soil solid or liquid components. On the other hand, CaS_x solution is unstable in soil and water environments containing O_2 and CO_2. With increased incubation time, CaS_x decomposes rapidly in the soil with the participation of O_2 and CO_2, producing H_2S, $CaCO_3$, $CaSO_4$, and solid sulfur particles. The reaction equations are listed as follows:

$$CaS_x + 3/2O_2 \rightarrow CaS_2O_3 + (x - 2)S \tag{6}$$

$$CaS_x + CO_2 + H_2O \rightarrow CaCO_3 + H_2S + (x - 1)S \tag{7}$$

In soil or wastewater containing heavy metals, the immobilization reactions can be explained as:

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Table 1
Dynamic changes of soil microbial community diversity under different doses of CaS$_x$ amendments.

Sampling time(d)	Treatment	*Shannon* index(H)	*Simpson* index(D)	*Richness* index(R)
3	CK	3.30 ± 0.04 a	26.30 ± 0.85 a	28.00 ± 1.00 a
	C1	2.53 ± 0.17 b	10.75 ± 2.06 b	9.67 ± 3.21 b
	C2	1.97 ± 0.60 b	6.25 ± 4.46 b	6.00 ± 2.65 bc
	C5	2.30 ± 0.13 b	7.59 ± 1.34 b	4.67 ± 2.31 c
30	CK	3.32 ± 0.35 a	26.52 ± 0.84 a	29.00 ± 1.73 a
	C1	3.19 ± 0.01 ab	22.29 ± 0.38 b	25.33 ± 0.58 a
	C2	2.81 ± 0.15 b	14.40 ± 2.57 c	13.67 ± 2.89 b
	C5	2.21 ± 0.40 c	7.39 ± 2.85 d	7.00 ± 3.61 c
55	CK	3.22 ± 0.04 a	23.67 ± 1.21 a	25.00 ± 1.00 a
	C1	3.22 ± 0.05 a	23.49 ± 1.19 a	26.67 ± 1.53 a
	C2	3.16 ± 0.03 a	22.04 ± 0.79 a	24.33 ± 0.58 a
	C5	2.11 ± 0.16 b	6.20 ± 0.75 b	5.33 ± 0.58 b

Different letters in the table indicate significant differences in the data in each column ($p < 0.05$).

$$M^{2+} + CaS_2O_3 + CaS_2O_3 \rightarrow MS \downarrow + CaSO_4 + 2H^+ \qquad (8)$$

$$M^{2+} + H_2S \rightarrow MS \downarrow + 2H^+ \qquad (9)$$

Here, M represents the heavy metal cations, MS represents the precipitated metal sulphides (Dahlawi and Siddiqui, 2017). As the reaction goes on, heavy metals in the soil are stabilized in the form of MS precipitation, while H$^+$ also accumulated in the soil, causing decreasing soil pH at equilibrium. Our results are in line with Chrysochoou et al. (2010), who have reported that within the first 60 days after CaS$_x$ applied to Cr (VI) polluted soil, soil pH was initially enhanced from 6.0 to 11.0 and gradually declined to 8.0 to 8.5 within 1 year.

In this research, after CaS$_x$ amendment to the soil, DTPA extractable Cd concentration in the soil decreased significantly compared to CK. Moreover, the decreasing level of DTPA extractable Cd concentration in the soil was inversely correlated with the CaS$_x$ dosage (Fig. 2). According to the literatures, appropriate dosage of CaS$_x$ amendment could chemically react with heavy metals in the soil, and produces stable metal sulfide or precipitation of hydroxide, which can reduce the mobility of heavy metal (Aratani et al., 1979; Chrysochoou et al., 2010; Maletić et al., 2015; Dahlawi and Siddiqui, 2017). However, excessive dosage of CaS$_x$ amendment may initiate the formation of a soluble metal ion complex $[MS_x]^{n-}$, thus causing the dissolution and re-mobilization of metal cations from the precipitated compounds (Li, 2014). In this research, 1% CaS$_x$ amendment was deemed as the appropriate dosage, which brings a continuous stabilization effect on Cd in the soil. Higher concentration (2% and 5%) of CaS$_x$ amendment resulted in significantly weaker Cd stabilization effects than 1% CaS$_x$ treatment. The excessive CaS$_x$ may react with Cd and produce soluble complexes of $[CdS_2]^{2-}$, which enhances the mobilization and migration of Cd in the wetland soil. However, further analysis for Cd speciation in the soil is needed, with the utilization of synchrotron-based μXRD, μXRF and μXANES techniques. Likewise, investigation of lower (< 1%) CaS$_x$ amendment levels or multiple lower CaS$_x$ amendment levels on Cd immobilization would help clarify the appropriate soil dosage.

4.2. Effects of CaS$_x$ on soil microbial enzymatic activities

Although a considerable body of work exists concerning the effects of CaS$_x$ on the stabilization of heavy metals in soil and wastewater, there are few reports regarding the impacts of CaS$_x$ on soil environmental quality and ecosystem functions. Soil enzymatic activity is an important indicator for accessing the status of soil environmental quality. For example, urease is an essential soil enzyme that catalyzes urea hydrolysis into ammonia and carbonic acid. It plays an important role in the soil nitrogen cycling driven by soil microorganisms (Garau et al., 2007; Bhaduri et al., 2016). Catalase is an anti-oxidative enzyme, which directly converts H_2O_2 to H_2O and O_2 (Willekens et al., 1997). Changes of catalase activity in the soil indicates the occurrence of

oxidative stress induced by various environmental stresses, including soil contamination by heavy metals and other hazardous pollutants (Shrestha et al., 2013; Wyrwicka and Urbaniak, 2016). In this research, within the early stage (0–15 day) after CaS$_x$ addition (Fig. 3a), soil urease and catalase activities in all CaS$_x$ treatments were significantly declined compared to CK, indicating considerable acute toxicity of CaS$_x$ to soil microbial activities in N cycling and oxidative stress. Eqs. (6) and (7) demonstrate that after CaS$_x$ was amended to the soil, H$_2$S and S^0 were produced concurrently with the stabilizing of heavy metals in the soil. However, H$_2$S and S^0 may also inhibit soil microbial activities and soil ecosystem functions to some extent (Aratani et al., 1979; Dahlawi and Siddiqui, 2017). Dorman et al. (2002) reported that H$_2$S could directly inhibit the activity of cytochrome oxidase which is a key enzyme in mitochondrial respiration, while S^0 could oxidize cytochrome b and produce H$_2$S, causing further cell oxidative damage. Moreover, the inhibited enzyme activity shows a significant dosage-dependent effect. In this research, at the early stage after CaS$_x$ addition (0–15 day), the decreasing level of soil urease and catalase was positively correlated with the increasing of CaS$_x$ dosage. As the remediation time increased, urease and catalase activities in 1% and 2% CaS$_x$ treatments showed significant increase, indicating the inhibition to soil microbial activity had been alleviated. However, urease and catalase activities in 5% CaS$_x$ treatment showed no recovery from the initial exposure, indicating an irreversible damage to microbial activities (Fig. 3b & 3c).

4.3. Effects of CaS$_x$ on soil microbial metabolic and community diversity

Carbon source utilization capability has been shown to be a satisfactory indicator of describing microbial metabolic profile and soil environmental quality, because it reflects the potential of microbes to respond promptly to environmental changes (Garland and Mills, 1991; Yao et al., 2003; Guo et al., 2015). Biolog EcoPlate is a quick, effective, and inexpensive method used to detect the dynamic variation of microbial metabolic and community diversity in soil, especially in contaminated soil (Sprocati et al., 2014; Guo et al., 2015). Although many studies have found that CaS$_x$ had high-efficiency on the stabilization of heavy metals (Cr, Pb, Cu and Ni) in soil, few researches are available on the environmental effects of CaS$_x$ amendments to coastal wetland soils. According to a USEPA toxicity report for direct human exposure, CaS$_x$ has a high pH around 11.5, which may cause irreversible damage to the eyes and light corrosion to the skin upon contact (USEPA, 2005). In this study, we report for the first time the soil microbial carbon utilization capability (Fig. 4), microbial metabolic diversity indicated by AWCD (Fig. 5), and microbial community diversity indicated by *Shannon*, *Simpson* and Richness indices (Table 1) were significantly decreased in the CaS$_x$ treatments compared to CK at the initial stage of the incubation. The above decreasing levels are closely related to the CaS$_x$ dosage. Bailey et al. (2012) also reported that CaS$_x$ could significantly inhibit the growth of specific bacterial strain *Shewanella oneidensis*, and a dose

dependent effect was also observed. However, in the mid and later stage of incubation, the adverse effects of CaS$_x$ amendment are alleviated, as evident by the recovery of soil microbial metabolic and community diversity from Day 30. This result is consistent with Maletić et al. (2015), who reported that the addition of CaS$_x$ could not only effectively stabilize metal contaminated soils, but also reduce the microbial toxicity of heavy metal in the pore water of soil. Considering cost feasibility, the price of commercially available CaS$_x$ (500 $/t) is slightly more expensive than biochar (300 $/t); however, the recommended effective dose for biochar is 5%, which is 5-fold greater than the effective CaS$_x$ recommended amendment rate of 1%. Taking cost and efficiency into account, CaS$_x$ can be considered as an economically and environmentally friendly amendment for the *in-situ* stabilization of Cd contaminated wetland soil.

5. Conclusions

The stabilizing effects and environmental impacts of CaS$_x$ amendments in Cd polluted wetland soil were investigated individually in this study. CaS$_x$ could significantly decrease the bioavailability and microbial toxicity of Cd in the soil, thus reduced the environmental risk of the Cd in the wetland soil ecosystem. The levels of bioavailable Cd, soil urease and catalase, microbial carbon utilization ability, and microbial metabolic and community diversity in the wetland soil were closely related to the CaS$_x$ dose. CaS$_x$ amendment of 1% was an efficient and safe dosage for the stabilization of Cd in the wetland soil. To our knowledge, this is the first report that assessing the Cd stabilizing efficiency and environmental impacts in wetland soil using CaS$_x$. Results from this study should provide useful scientific evidence for the development of *in-situ* remediation ameliorants and technologies for heavy metal polluted wetland soils.

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