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Effect of liming on sulfate transformation and sulfur gas emissions in degraded vegetable soil treated by reductive soil disinfestation

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ABSTRACT

Reductive soil disinfestation (RSD), namely amending organic materials and mulching or flooding to create strong reductive status, has been widely applied to improve degraded soils. However, there is little information available about sulfate (SO_4^{2-}) transformation and sulfur (S) gas emissions during RSD treatment to degraded vegetable soils, in which S is generally accumulated. To investigate the effects of liming on SO_4^{2-} transformation and S gas emissions, two SO_4^{2-} -accumulated vegetable soils (denoted as S1 and S2) were treated by RSD, and RSD plus lime, denoted as RSD₀ and RSD₁, respectively. The results showed that RSD₀ treatment reduced soil SO_4^{2-} by 51% and 61% in S1 and S2, respectively. The disappeared SO_4^{2-} was mainly transformed into the undissolved form. During RSD treatment, hydrogen sulfide (H_2S), carbonyl sulfide (COS), and dimethyl sulfide (DMS) were detected, but the total S gas emission accounted for <0.006% of total S in both soils. Compared to RSD₀, lime addition stimulated the conversion of SO_4^{2-} into undissolved form, reduced soil SO_4^{2-} by 81% in S1 and 84% in S2 and reduced total S gas emissions by 32% in S1 and 57% in S2, respectively. In addition to H_2S , COS and DMS, the emissions of carbon disulfide, methyl mercaptan, and dimethyl disulfide were also detected in RSD₁ treatment. The results indicated that RSD was an effective method to remove SO_4^{2-} , liming stimulates the conversion of dissolved SO_4^{2-} into undissolved form, probably due to the precipitation with calcium.

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Introduction

Due to the high economic benefit of vegetables, the cultivation area of greenhouse vegetables has continuously risen to 24.8 million ha, accounting for 14.5% of the farmland in China (Food and Agriculture Organization, FAO, 2013). It is known that intensive vegetable cultivation is characterized by multiple

cropping, frequent irrigations and high fertilizer application, which easily lead to soil degradation, such as acidification, salinization, nitrate (NO_3^-) and sulfate (SO_4^{2-}) accumulation, and occurrence of soil-borne diseases (Blok et al., 2000; Cao et al., 2004; Messiha et al., 2007; Zhu et al., 2012). Once the degradation of greenhouse vegetable soils occurs, vegetable yield and economy incomes of farmers decrease significantly. Therefore,

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effective methods for improving degraded vegetable soils are of great interests in China.

Recently, reductive soil disinfestation (RSD), which is also called biological soil disinfestation (BSD) or anaerobic soil disinfestation (ASD), has been developed to improve the degraded soil in USA, Netherlands and Japan (Messiha et al., 2007; Momma, 2008; Butler et al., 2012). RSD consists of three steps: (1) amending easily decomposable organic materials (e.g., crop straw, green manure, molasses), (2) irrigating field, and (3) covering the soil surface with plastic film (Momma, 2008), all of which create strong reductive condition to restrain soil-borne diseases and root-knot nematodes (Lamers et al., 2004; Butler et al., 2012). RSD can also effectively improve soil structure (Akhtara and Malik, 2000; Oka, 2010), decrease soil electrical conductivity (EC), raise pH and remove accumulated NO_3^- in soil (Zhu et al., 2012). For these advantages, RSD has been widely popularized as an environmentally friendly method applied to different degraded agricultural systems (e.g. vegetable and banana fields) (Goud et al., 2004; Mowlick et al., 2013; Huang et al., 2014).

In practical vegetable cultivation, large amount compound fertilizer or potassium fertilizer containing SO_4^{2-} are generally applied to fields, which easily lead to serious accumulation of SO_4^{2-} in soil. SO_4^{2-} accumulation not only causes soil acidification, salinization but also aggravates the toxic effects of activated aluminum and iron to crops. More importantly, the acidic-sulfate vegetable soils are suitable for pathogen growth (Koike et al., 2003). Thus, effectively lowering SO_4^{2-} content should be readily aroused increasing concern when applying RSD method to improve degraded vegetable soils in which SO_4^{2-} accumulated seriously. During RSD treatment, strong reductive condition is created and SO_4^{2-} can be converted into hydrogen sulfide (H_2S), which is greatly driven by sulfate reducing bacteria (SRB) (Yoda et al., 1987; O'Flaherty et al., 1998). In agricultural production, liming is a common practice to improve acid soil. Fortin et al. (1996) and Al-Zuhair et al. (2008) proposed that the growth of SRB was high in basic media (pH of 7–8), which could induce a significant drop in SO_4^{2-} concentration. Meanwhile, they found that the growth of SRB was not evident and SO_4^{2-} concentration decreased slightly in acid condition (pH < 7). Remarkably, as the end product of SO_4^{2-} reduction, H_2S has toxicity effect on microorganism and increase in H_2S emission might favor the toxicity to soil-borne pathogens (Momma, 2008). Therefore, it is possible that an increase in soil pH may not only promote the decrease in SO_4^{2-} content but also enhance H_2S production, both of which help to improve degraded vegetable soils.

Except for being reduced into H_2S , SO_4^{2-} may also be transformed into other sulfur (S) forms during RSD treatment. For example, SO_4^{2-} is easily converted into organic S forms (e.g. ester-S and carbon-bound S) when soil SO_4^{2-} content is high, especially in organic carbon abundant soils (Saggar et al., 1981; Goh and Gregg, 1982). Furthermore, SO_4^{2-} can be reduced into sulfide and leached under flooding condition. Noticeably, except for H_2S , other volatile S gases, such as carbonyl sulfide (COS), carbon disulfide (CS_2), methyl mercaptan (CH_3SH), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) are also emitted from the soils (Minami, 1982). Due to the negative environmental impacts, e.g., air pollution, climate effects and precipitation chemistry (Jørgensen and Okholm-Hansen, 1985; Staubes et al.,

1989; Howarth et al., 1992), the volatile S gases have attracted more attention. However, no relevant studies have been conducted when soils are treated by RSD. Considering the negative environmental impacts of SO_4^{2-} in soil and water and S gases in the atmosphere, the conversion of SO_4^{2-} and the S gas emissions from soil are of particular importance during RSD treatment to degraded vegetable soils.

In this study, two severely degraded vegetable soils with different SO_4^{2-} contents were chosen and treated by RSD approach (soils were amended by alfalfa and flooded). Lime was also added to two soils to investigate the effect of pH on SO_4^{2-} content, the products transformed and six volatile S gas emissions (i.e., H_2S , COS, CS_2 , CH_3SH , DMS and DMDS) during RSD treatment. We hypothesized that lime addition could accelerate SO_4^{2-} removal and increase volatile S gas emissions.

1. Materials and methods

1.1. Site description and sample collection

Both soils used in this study were sampled from two severely degraded greenhouse vegetable field with high SO_4^{2-} content as compared to adjacent rice fields in Wujiang village, suburban Hexian (31°71'N and 118°37'E), Anhui province, China. These vegetable fields have been uniformly cultivated for approximately 10 years and two or three vegetables were planted per year. The dominant vegetables were hot pepper (*Capsicum annuum* L.), tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), muskmelon (*Cucumis sativus* L.) and kidney bean (*Phaseolus vulgaris* L.). The two vegetable soils were noted as S1 with relatively high SO_4^{2-} content (917 mg S/kg) and S2 with relatively low SO_4^{2-} content (425 mg S/kg), respectively, which was mainly attributed to the difference in fertilization type. Compared to S2, farmer usually applied much more potassium fertilizer containing SO_4^{2-} to S1, which directly lead to SO_4^{2-} content in S1 higher than that in S2.

After harvesting tomato, soil samples were randomly collected from the 0–20 cm layer in the two fields on 10 June 2013. After vegetable residues and stones were removed with tweezers, fresh soil was sieved (<2 mm) and immediately stored at 4°C before the incubation experiment. Total carbon (TC) and total nitrogen (TN) contents were 15.9 and 2.08 g/kg in S1 and 16.0 and 2.08 g/kg in S2, respectively. The other properties of both soils were listed in Table 1. Compared to the pH of adjacent rice soils (around 5.8), the two vegetable soils were seriously acidified.

The powder of alfalfa passed through a 0.25 mm sieve was used as easily decomposable organic matter for RSD treatment. The alfalfa was characterized to be TC of 549 g C/kg; TN of 20.3 g N/kg; TS of 2.33 g S/kg.

1.2. Experimental design

The degraded vegetable soils were treated according to RSD method (Zhu et al., 2012). There were two treatments: flooding + alfalfa (RSD₀) and flooding + alfalfa + lime (RSD₁). The application rates of lime were 5.28 g/kg in S1 and 4.37 g/kg in S2, respectively, both of which adjusted soil pH to 8.4. The application rate of alfalfa was 4.67 g/kg (dry soil), equivalent to

Table 1 – Changes in soil properties in different treatments after the incubation.

Soil	Treatment	pH	EC	NH ₄ ⁺	NO ₃ ⁻	TS	DOS	SO ₄ ²⁻ soi ^a	US	SO ₄ ²⁻ lea ^b
			mS/cm	mg N/kg	mg N/kg	mg S/kg	mg S/kg	mg S/kg	mg S/kg	mg S/kg
S1	Initial	4.4 ± 0.0c	0.74 ± 0.01a	28.9 ± 0.1c	147 ± 5a	1159a	228a	917a	13.6c	
	RSD ₀	5.9 ± 0.1b	0.39 ± 0.03b	29.7 ± 0.4b	0.66 ± 0.50b	1059 ± 37b	138 ± 1b	449 ± 96b	472 ± 79b	188 ± 63a
	RSD ₁	7.6 ± 0.0a	0.30 ± 0.04c	32.6 ± 0.2a	0.10 ± 0.06b	1126 ± 3a	177 ± 13c	178 ± 16c	771 ± 13a	62.4 ± 15.7a
S2	Initial	4.8 ± 0.0c	0.65 ± 0.02a	18.6 ± 0.2c	295 ± 8a	703a	103a	425a	175b	
	RSD ₀	6.4 ± 0.0b	0.21 ± 0.07b	27.1 ± 0.1b	0.86 ± 0.45b	475 ± 126b	70.7 ± 22.9a	164 ± 120b	240 ± 100ab	132 ± 69a
	RSD ₁	7.6 ± 0.0a	0.24 ± 0.01b	30.5 ± 0.4a	0.68 ± 0.11b	563 ± 124ab	97.7 ± 18.8a	67 ± 15b	399 ± 92a	6.6 ± 3.7b

Data are presented as data represent mean ± SD (n = 3).

RSD₀: flooding and amending alfalfa; RSD₁: flooding and amending alfalfa in combined with lime. TS: total sulfur; DOS: dissolved organic sulfur in soils; US: undissolved sulfur; EC: electrical conductivity.

Different letters within a column indicate significant differences between the means (p < 0.05).

^a SO₄²⁻ content in soil.

^b SO₄²⁻ concentration in leaching solution.

9.8 ton/ha. The additional S in alfalfa was 10.9 mg S/kg (dry soil).

Fresh soil (210 g dry weight) was thoroughly mixed with alfalfa powder and lime as designed, and then was packed into PVC cylinder cores (5.0 cm diameter × 15 cm length) according to the bulk density. Thin polytetrafluoroethene (PTFE) film covered the inner wall of cylinder cores to avoid S gases adsorption. The depth of soil layer was 10 cm. Distilled water was then added to achieve the 1:1 (m/m) soil/water ratio, forming one centimeter water layer. All cores were incubated at 35°C for 21 days and water lost by evaporation was compensated. The emission rate of S gases and Eh in each treatment was measured at days 1, 2, 3, 4, 5, 6, 7, 8, 9, 13, 17 and 21 (three cores of each treatment as replications each time). Before S gas sampling, PVC cylinder cores were ventilated by air for 15 min, and then resealed by lid fitted with butyl rubber septa for 4 hr at 35°C. The inner wall of lid was also covered by thin PTFE film. The headspace gas was collected using 25 mL specialized syringe which prevented S gas adsorption and measured immediately. The soil (three soil cores of each treatment) was collected at 21 day after incubation. Before sampling, flooded water in core was leached out through the valve fitted in the bottom of PVC cylinder for 10 min. The leaching solution was collected in 150 mL plastic bottle for analyses of SO₄²⁻ concentrations. After draining water off, the soils in cores were well mixed. A part of soils was used to determine soil moisture content, another part (fresh soil) was used to determine pH, EC, NH₄⁺, NO₃⁻, SO₄²⁻, total S, dissolved S and undissolved S contents. The volume of leaching solution was measured, and then filtered through a qualitative filter paper and stored at 4°C until analysis of SO₄²⁻ concentrations. The exact SO₄²⁻ content in soil was the sum of SO₄²⁻ content in leaching and residual SO₄²⁻ content in soil after leaching.

Total S or dissolved S was the sum of SO₄²⁻ content in leaching and residual total S or residual dissolved S content in soil after leaching.

1.3. Analyses

Soil properties were determined on the basis of the soil agrochemical analysis procedures (Lu, 2000). Soil pH was measured in a 1:2.5 (m/m) soil to water ratio using a pH detector

(S220, pH Electrode LE438, Mettler, Greifensee, Switzerland). Soil Eh was measured directly in soil using an ORP detector (S220, ORP Electrode LE501, Mettler, Greifensee, Switzerland). Soil EC was measured in a 1:5 (m/m) soil to water ratio using a specific conductivity meter (KangYi Corp., Shanghai, China). TC and TN of soil and alfalfa were analyzed using an automated nitrogen-carbon analyzer coupled to a 20/20 isotope ratio mass spectrometer (20/20, SerCon Ltd., Crewe, UK).

Soil NH₄⁺ and NO₃⁻ were extracted with 2 mol/L KCl at a 1:5 (m/m) soil to solution ratio by shaking soil for 1 hr at 300 r/min and 25°C. The extracts were filtered through a qualitative filter paper and stored at 4°C until analysis. The concentrations of NH₄⁺ and NO₃⁻ in both soil extractions were determined with a continuous flow analyzer (Skalar San⁺⁺, Breda, Netherlands). NH₄⁺ and NO₃⁻ concentration in leaching solution was measured directly.

Soil SO₄²⁻ and dissolved S were extracted with 0.016 mol/L KH₂PO₄ at a 1:5 (m/m) soil to solution ratio by shaking at 300 r/min and 25°C for 1 hour. The extract was passed through 0.45 μm filter paper and stored at 4°C until analysis. The concentration of SO₄²⁻ in soil extraction and leaching solution were determined using an ion chromatograph (Dionex ICS-1100, Thermo, Waltham, USA). The concentration of dissolved S in extracts was determined with inductively coupled plasma atomic emission spectrometry (ICP-AES) (Prodigy, Leeman, New York, USA). The content of total S in soil and alfalfa was firstly completed digested and then determined with ICP-AES (Prodigy, Leeman, New York, USA).

Six species of S gases (H₂S, COS, CH₃SH, DMS, DMDS, CS₂) were analyzed by using an Agilent 7890A gas chromatograph with a sulfur chemiluminescence detector (SCD) (Wasson-EGE, Fort Collins, Colorado, USA). A CP8575 capillary column (60 m × 0.32 mm × 0.39 mm, Agilent Technologies, Santa Clara, California, USA) was used, and the GC oven temperature was programmed initially at 40°C, holding for 11 min, firstly to 80°C at 40°C/min, holding for 5 min, and then to 150°C at 70°C/min, holding for 7 min. Under these conditions, H₂S, COS, CH₃SH, DMS, DMDS and CS₂ were readily separated. The calibration was carried out after dilution of standard six S gases mixture (H₂S, 51.3 μg/L; COS, 66.5 μg/L; CH₃SH, 66.9 μg/L; DMS, 65.5 μg/L; DMDS, 132.7 μg/L; CS₂, 131.3 μg/L, filling gas was nitrogen, Dalian-DT, Ltd., Shenyang, China). Calibration

curves (dose-area) was obtained by on-line diluting standard gas with helium (He), and the dilution ratios were 1, 8.2, 17.2, 39.4, 66.3 and 96.9. The correlation coefficients of calibration curves were H_2S , 0.9998; COS , 0.9998; CH_3SH , 0.9999; DMS , 0.9995; DMDS , 0.9999; CS_2 , 0.9987. The relative precision of the measurement was <4%, based on the reproducibility of consecutive standard sample ($n = 9$).

1.4. Statistical analyses and calculations

The content of SO_4^{2-} , dissolved S or total S in soil was calculated as the following equation:

$$C(S) = V \times L(S)/w + C(S)_R \quad (1)$$

where, $C(S)$ (mg S/kg) is the content of SO_4^{2-} , dissolved S or total S in soil; V (L) is the volume of leaching solution; $L(S)$ (mg S/L) is the concentration of SO_4^{2-} in leaching solution; w (kg) is the soil weight in PVC cylinder cores; and $C(S)_R$ (mg S/kg) is the content of SO_4^{2-} , dissolved S or total S in soil after leaching.

The content of dissolved organic S was calculated by deducting SO_4^{2-} from dissolved S. The content of undissolved S was calculated by deducting dissolved S from total S. Cumulative S gas emissions were calculated by summing the amounts over all the sampling intervals during the incubation period. The amount of each sampling interval was calculated by multiplying the average emission rate by the number of days of the interval. Average emission rate was calculated as the mean value of the two measurements spanning the interval.

The relationships were analyzed using Pearson's correlation test in SPSS 17.0 software. Soil characteristics and the cumulative S gas emissions were compared with LSD test at $p = 0.05$.

2. Results

2.1. Changes in soil pH, EC and Eh

Before the RSD treatment, pH increased uniformly to 8.4 from initial 4.4 in S1 and 4.8 in S2. The changes of soil pH in lime-amended (RSD_1) and lime-unamended (RSD_0) treatments showed an opposite tendency during the incubation. At the end of the incubation, pH in RSD_0 treatment increased to 5.9 and 6.4 in S1 and S2, respectively, while declined to 7.6 in both soils in RSD_1 treatment (Table 1). Compared to the initial values (0.74 and 0.65 mS/cm in S1 and S2, respectively), soil EC was significantly decreased in both soils treated by RSD, irrespective of with or without lime addition (Table 1). In S1, soil EC dropped to 0.39 and 0.30 mS/cm in RSD_0 and RSD_1 treatments, respectively. In S2, soil EC decreased to 0.21 and 0.24 mS/cm in RSD_0 and RSD_1 treatments, respectively. Amending alfalfa and flooding significantly lowered soil Eh, more observably in lime-amended soils. Soil Eh in RSD_0 treatment continuously decreased and reached the lowest values (–150 and –154 mV for S1 and S2, respectively) on 13 days (Fig. 1). Soil Eh in RSD_1 treatment quickly dropped to the lowest values (–254 and –261 mV in S1 and S2, respectively) on day 4, but it had a great raise after day 4 and then kept relatively stable (Fig. 1). At the end of incubation, soil Eh reached –112 and –136 mV in RSD_0 treatment and –173 and –179 mV in RSD_1 treatment in S1 and S2, respectively.

2.2. Changes in N and S pools

Soil NO_3^- content significantly decreased from 147 mg N/kg in S1 and 295 mg N/kg in S2 to less than 1 mg N/kg after the incubation (Table 1). In comparison to NO_3^- , soil NH_4^+ content at the end of the incubation increased observably from 28.9 mg N/kg to 29.7 and 32.6 mg N/kg in RSD_0 and RSD_1 treatments in S1 and from 18.6 mg N/kg to 27.1 and 30.5 mg N/kg in RSD_0 and RSD_1 treatments in S2, respectively.

Under flooding condition, SO_4^{2-} content was greatly decreased in both soils (Table 1). After the incubation, SO_4^{2-} content for RSD_0 treatment significantly decreased from 917 and 425 mg S/kg to 449 and 178 mg S/kg in S1 and S2, respectively, reduced by 51% in S1 and 61% in S2. Lime addition accelerated the removal of SO_4^{2-} and SO_4^{2-} content reached to 178 and 67 mg S/kg in S1 and S2, respectively, reduced by 81% in S1 and 84% in S2. Similar to the changes of SO_4^{2-} content, dissolved organic S (DOS) content in soils declined at the end of incubation, which reduced by 4.7%–39% of initial DOS content in both soils. Undissolved S (US) content in soil, calculated by total S minus dissolved S content, greatly increased from 13.6 and 175 mg S/kg to 472 and 240 mg S/kg in S1 and S2 for RSD_0 treatment, respectively. The increase in US content in both soils became more obvious in RSD_1 treatments, which is up to 771 and 399 mg S/kg in S1 and S2, respectively. The total S content at the end of incubation significantly decreased in both soils treated by RSD_0 , and the reduction degree was higher in RSD_0 than RSD_1 in both soils. Observably, approximately 188 (S1) and 132 (S2) mg S kg^{-1} of SO_4^{2-} was found in leaching solution for RSD_0 treatment, however, lime addition reduced the SO_4^{2-} content in leaching solution, which amounted to 62.4 and 6.6 mg S/kg in S1 and S2, respectively.

2.3. Soil sulfurous gas emission

Various S gas emissions were shown in Figs. 2 and 3. Only three S gases (H_2S , COS and DMS) were detected in RSD_0 treatment, and all six S gases, except for CH_3SH in S2, were detected in RSD_1 treatment in both soils (Figs. 2 and 3). During

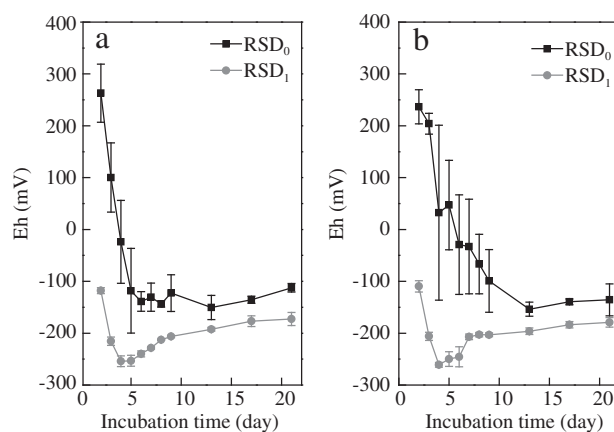


Fig. 1 – Changes in Eh in soils with different treatments during 21-day incubation period in soil 1 (a) and soil 2 (b). RSD_0 : flooding and amending alfalfa; RSD_1 : RSD_0 and lime adjusting soil pH to 8.4. Bars refer to standard deviation. RSD: reductive soil disinfestation.

the entire incubation, COS and DMS were detected all the time in both treatments for two soils. There was no obvious peak value of COS or DMS emission appeared during the incubation, in which the emission rates of COS ranged from 9.1 to 35.0 ng S/(kg-hr) and the emission rates of DMS were around 5 ng S/(kg-hr) in both treatments (Figs. 2 and 3). The emission rate of H₂S varied greatly and had an obvious peak value during the incubation. At the beginning of the incubation, H₂S was not detected. With time prolonged, H₂S was produced and the peak value of H₂S emission (184–242 ng S/(kg-hr)) for both soils in RSD₀ treatment appeared at day 9, and thereafter declined quickly and leveled off in S1. But it was not the case in S2, in which H₂S rapidly declined after 9 days of incubation and then increased sharply to 150 ng S/(kg-hr) at the end of incubation. In RSD₁ treatment, the peak value of H₂S emission rate (42.3–61.1 ng S/(kg-hr)) for both soils occurred before day 9, thereafter went downhill and then increased slightly in both soils. The other S gases (CH₃SH, CS₂ and DMDS) were detected only in RSD₁ treatment. The emission rates of CH₃SH, CS₂ and DMDS increased slowly or

kept relatively stable during the incubation. In S1, the emission rates of CS₂ ranged from 0.33 to 5.26 ng S/(kg-hr), and the emission rates of CH₃SH and DMDS were relatively small (0.14–0.56 ng S/(kg-hr)). In S2, CH₃SH was not detected during the entire incubation, and the emission rates of CS₂ and DMDS (0.11–0.65 ng S/(kg-hr)) were less than the rates in S1.

In RSD₀ treatment, the emitted total S gases were 38.1 and 40.8 μg S/kg, which account for only 0.003% and 0.006% of total S in S1 and S2, respectively, during the entire incubation. H₂S was the major emitted S gas (28.2–32.9 μg S/kg), which constituted 73.9%–80.7% of the cumulative emission of total S gases, and the ratios of COS (7.37–8.35 μg S/kg) and DMS (0.5–1.59 μg S/kg) were 18.1%–21.9% and 1.2%–4.2% in both soils, respectively (Table 2). Lime addition cuts down the total S gas emission to 25.8 μg S/kg in S1 and to 17.4 μg S/kg in S2, and changed the S gas constituents in both soils. The cumulative COS emission (9.26–10.5 μg S/kg), which contributed to 35.9%–59.9% of the cumulative emission of total S gases, was higher than H₂S (5.11–9.03 μg S/kg) in RSD₁ treatment (Table 2). In S1, the ratio of cumulative CS₂ (5.26 μg S/kg) emission to total S

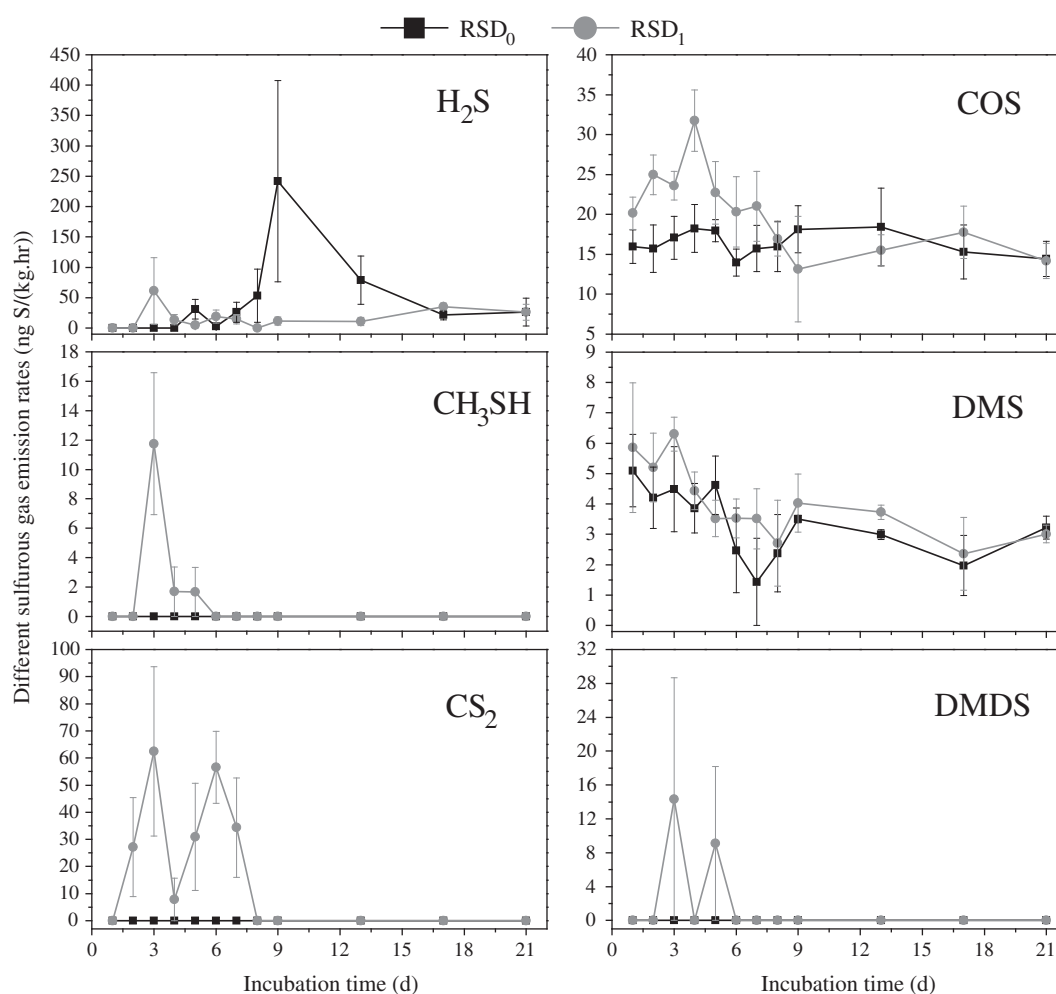


Fig. 2 – Average sulfurous gas emission rates in RSD₀ and RSD₁ treatments during 21 days of incubation period in soil 1. RSD₀: flooding and amending alfalfa; RSD₁: flooding and amending alfalfa and lime adjusting soil pH to 8.4. H₂S: hydrogen sulfide; COS: carbonyl sulfide; CH₃SH: methyl mercaptan; DMS: dimethyl sulfide; CS₂: carbon disulfide; DMDS: dimethyl disulfide; RSD: reductive soil disinfestation. Bars refer to standard error.

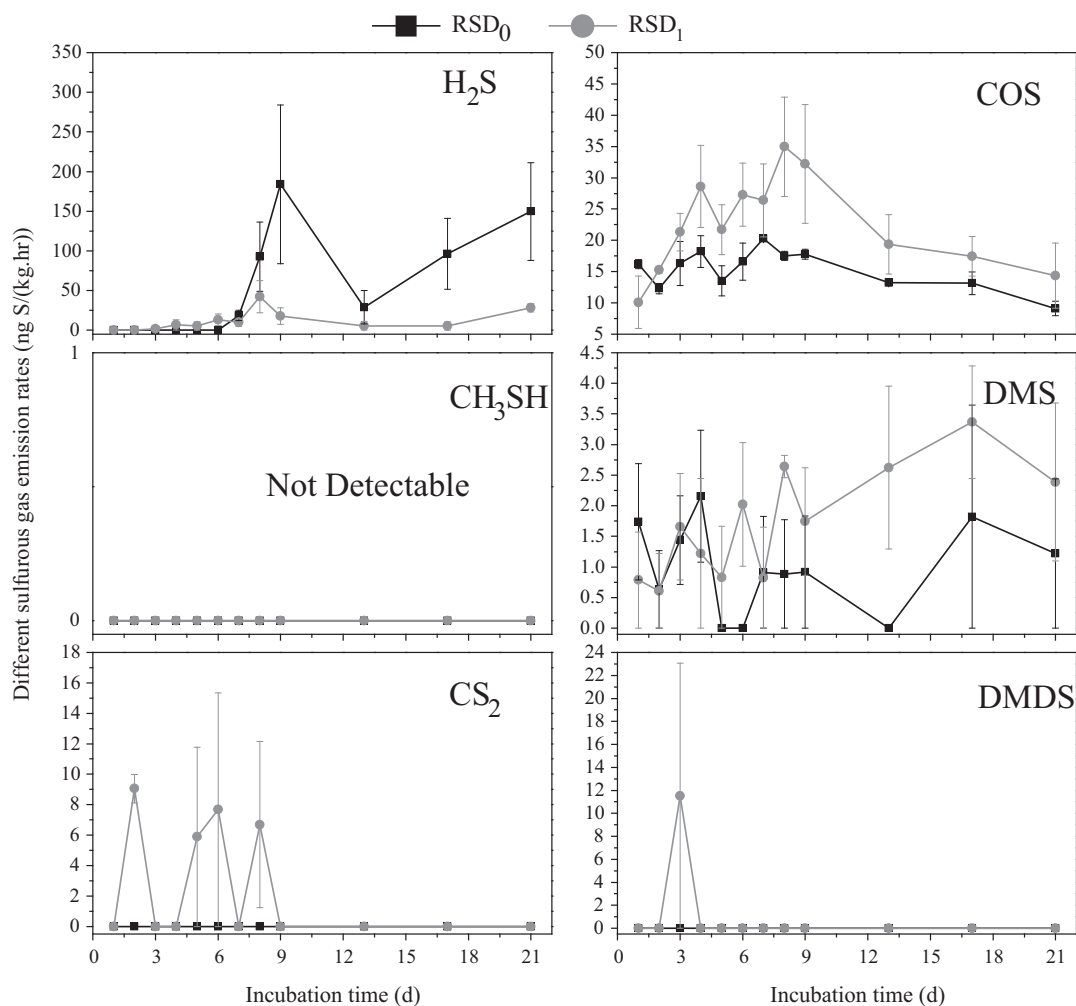


Fig. 3 – Average sulfurous gas emission rates in RSD₀ and RSD₁ treatments during 21 days incubation period in soil 2. RSD₀: flooding and amending alfalfa; RSD₁: flooding and amending alfalfa and lime adjusting soil pH to 8.4. H₂S: hydrogen sulfide; COS: carbonyl sulfide; CH₃SH: methyl mercaptan; DMS: dimethyl sulfide; CS₂: carbon disulfide; DMDS: dimethyl disulfide; RSD: reductive soil disinfestation. Bars refer to standard error.

gases was 20.4%, and the value of the rest S gases (CH₃SH, DMS and DMDS) was <10%. In S2, besides H₂S and COS, the rest of the S gas emission (CS₂, DMS and DMDS) only constituted <10% of the cumulative production of total S gases.

3. Discussion

3.1. Lime addition increases the emissions of COS, CH₃SH, DMS, CS₂ and DMDS but decreases H₂S emission

In flooded and alfalfa-amended soils, only H₂S, COS and DMS were detected. When lime is applied to soil, CH₃SH, CS₂ and DMDS were also produced, which might be attributed to the decrease in Eh. The similar result was reported by Devai and DeLaune (1995) that reduced S gas emissions (H₂S, COS, CH₃SH, DMS and CS₂) increased with decreasing redox potential, and only COS, DMS and CS₂ were detected when redox potential was relatively high. Due to faster decomposition rate of organic material in higher soil pH (Nyborg and Hoyt, 1978; Curtin et al.,

1998), more active C source as electron donor can be supplied through accelerating the decomposition of alfalfa by lime addition, and thus resulted in lower Eh as compared to only flooding treatment without alfalfa-amendment. Besides, large OH⁻ caused by lime could react with metal ion (e.g., Fe³⁺, Mn³⁺) and form precipitation (Francis and Tebo, 1999; Johnson and Hallberg, 2005), which can directly reduce the electron acceptors in soil. In stronger reductive condition, therefore, more forms of organic S gases are produced, possibly from the biodegradation of organic S forms and/or the methylation of inorganic S (e.g., sulfide) (Banwart and Bremner, 1975; Drotar et al., 1987; Howarth et al., 1992). Observably, the peaks of various S gas emissions occurred in different times during the entire incubation and responded differently to changes in Eh and pH (Figs. 2 and 3), indicating that the specific microbe responsible for various S gas productions may adapt differently to variable environmental condition and substrate (e.g., SO₄²⁻, pH and Eh).

Although kinds of S gas productions increased in lime-amended soils during RSD treatment, the total S gas production decreased, which might be mainly attributed to the content

Table 2 – Cumulative sulfurous gas production in different treatments in both soils (unit: $\mu\text{g S/kg}$).

Soil	Treatment	H ₂ S	COS	CH ₃ SH	DMS	CS ₂	DMDS	Total S gas
S1	RSD ₀	28.2 ± 23.7a	8.4 ± 2.5a	0.0 ± 0.0b	1.6 ± 0.2a	0.0 ± 0.0b	0.0 ± 0.0b	38.1 ± 25.4a
	RSD ₁	9.1 ± 2.8a	9.3 ± 1.1a	0.36 ± 0.33a	1.8 ± 0.4a	5.7 ± 2.9a	0.56 ± 0.52a	25.8 ± 5.8a
S2	RSD ₀	32.9 ± 21.9a	7.4 ± 0.9a	0.0 ± 0.0a	0.50 ± 0.24a	0.0 ± 0.0a	0.0 ± 0.0a	40.8 ± 21.6a
	RSD ₁	5.0 ± 1.6a	10.5 ± 4.0a	0.0 ± 0.0a	1.0 ± 0.7a	0.65 ± 0.72a	0.28 ± 0.48a	17.5 ± 4.0a

H₂S: hydrogen sulfide; COS: carbonyl sulfide; CH₃SH: methyl mercaptan; DMS: dimethyl sulfide; CS₂: carbon disulfide; DMDS: dimethyl disulfide. Data are represented as mean ± SD, n = 3, in $\mu\text{g S/kg}$.

Different letters within a column indicate significant differences between the means in the same soil ($p < 0.05$).

changes of various S gas productions (Table 2). Noticeably, lime addition significantly reduced the cumulative H₂S production by 68% in S1 and 85% in S2, but generally increased the other five volatile S gases productions. Even though the increases in other five volatile S gases, they were not sufficient to counter-balance the inhibitory effects of lime addition on H₂S production, thus resulted in the lower total S gas productions and higher ratios of COS, CH₃SH, DMS, CS₂ and DMDS to total S gases in lime-amended soils as compared to those in lime-unamended soils. This decrease in H₂S production in lime-amended soils during RSD treatment was not in agreement with our hypothesis that strong reductive and alkaline condition was a benefit for the reduction of SO₄²⁻ and H₂S production. Lime addition directly reduces the content of SO₄²⁻, which is the substrate for H₂S production, through reacting with Ca²⁺ and forming CaSO₄ precipitation, and thereafter possibly lowers H₂S emission. At low pH the produced H₂S exists in undissociated form and it dissociates into HS⁻ and S²⁻ with increasing pH (Perry et al., 1984; Al-Zuhair et al., 2008). Thus, it was possibly that a part of the produced H₂S in lime-amended soils stayed in soil as HS⁻ and S²⁻ because pH was still as high as 7.6 at the end of the incubation. Therefore, even if more H₂S was produced in alkaline reductive condition, the emitted H₂S might not increase. It was noteworthy that the cumulative total S gas productions accounted to 17.5–40.8 $\mu\text{g S/kg}$, which were less than 0.006% of total S, suggesting that S gas production was not an important process for S removal in vegetable soils treated by RSD.

3.2. Lime addition accelerates the conversion of SO₄²⁻ to other S forms

In this study, amending alfalfa and flooding significantly decreased EC values in vegetable soils, which further validated the previous study that RSD can effectively lower soil salinization (Zhu et al., 2012). As we hypothesized, SO₄²⁻ content greatly decreased when vegetable soils were treated by RSD, more obviously in lime-amended soils. SO₄²⁻ content reduced by 51%–61% in RSD₀ treatments compared to 81%–84% in RSD₁ treatments. The consumption processes of soil SO₄²⁻ mainly included crop absorption, conversion to other S forms, soil denudation, leaching and gases losses (McLaren et al., 1993; Eriksen et al., 1998). Under our experimental conditions, only the internal conversions of SO₄²⁻ to other S forms and gas losses were considered. As discussed above, the total productions of S gases during RSD treatment were very low, and the total S loss through S gas emissions could be ignored. Therefore, the reduction in soil SO₄²⁻ at the end of RSD treatment (day 21) was attributed to

the conversion to other S form under the conditions without leaching.

Based on our results, we could clearly see that undissolved S (US) in soil was significantly increased in both soils as amended with alfalfa under flooding condition (Table 1). Lime addition increased this conversion of SO₄²⁻ to undissolved S. The underlying mechanisms for increased undissolved S content in soil may be complex in reductive condition. It is likely that microbe assimilate SO₄²⁻ into organic S form (Goh and Gregg, 1982), especially in C-rich soil (Saggar et al., 1981). Wu et al. (1995) provided direct evidence that amending glucose, rape leaves and straw increased SO₄²⁻ immobilization. When easily decomposable organic materials are homogeneously incorporated into SO₄²⁻-accumulated soils, sufficient organic C stimulates the activities of microorganism for immobilizing a part of SO₄²⁻. In addition, the reductive products of SO₄²⁻ (e.g., S²⁻ and S⁻) under strong reductive condition react with Fe²⁺, Mn²⁺ and subsequently form insoluble inorganic S forms, such as FeS₂, MnS₂, FeS and MnS. Possibly, the direct microbial immobilization, the reduction and reaction of SO₄²⁻ with cations simultaneously contribute to the decrease in SO₄²⁻ in RSD-treated soils. Noticeably, calcium ion can directly react with SO₄²⁻ and form precipitation. Lime addition can significantly increase the magnitude of reaction of SO₄²⁻ with Ca²⁺, which is validated by the obvious reduction in SO₄²⁻ content in water leachate from 132 to 188 mg S/kg in RSD₀ treatment low to 6.6–62 mg S/kg in RSD₁ treatment (Table 1). Except for the changes of SO₄²⁻ in KH₂PO₄-extractable solution, dissolved organic S (DOS) content also significantly decreased in vegetable soils treated by RSD treatment, which might be due to the fact that a part of DOS was lost in water leachate, which was not determined. Somehow, DOS content in lime-amended soils was higher than that in lime-unamended soils, which was contrary to changes of SO₄²⁻ in soils amended with lime. It has been showed that liming significantly increases the solubility of organic matter in soil in the short-term (Riffaldi et al., 1996; Curtin et al., 1998; Tyler and Olsson, 2001). Thus, lime addition promotes undissolved organic S dissolved in water. Noticeably, the total S contents at the end of 21 days incubation were less than the initial values in both soils and approximately 32.6–100 mg S/kg in S1 and 140–228 mg S/kg in S2 were not recovered. These disappeared S might be attributed to DOS in water leachate, which was not determined in this study. Due to the various S forms in soil, it is difficult to elucidate the actual S transformation and exactly qualifying the distribution of SO₄²⁻ to other S pools (e.g., Ester sulfate, C-bound S, sulfide and precipitation with calcium) only through determining net changes of S forms. Based on S tracer techniques and microbial techniques, further studies should be

conducted to evaluate the underlying mechanisms of S transformation and the main regulating factors under flooding condition.

4. Conclusions

RSD method could effectively lower SO_4^{2-} content in vegetable soil, which was mainly transformed into undissolved S in soils. Lime addition significantly promoted the transformation of SO_4^{2-} content into undissolved S. In addition, lime addition stimulated the emissions of COS, CH_3SH , DMS, CS_2 and DMDS but inhibited H_2S emission. Noticeably, total S gases production during RSD treatment of vegetable soil contributed to less than 0.006% of the total S in soils during 21 days incubation irrespectively of with or without lime addition.

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