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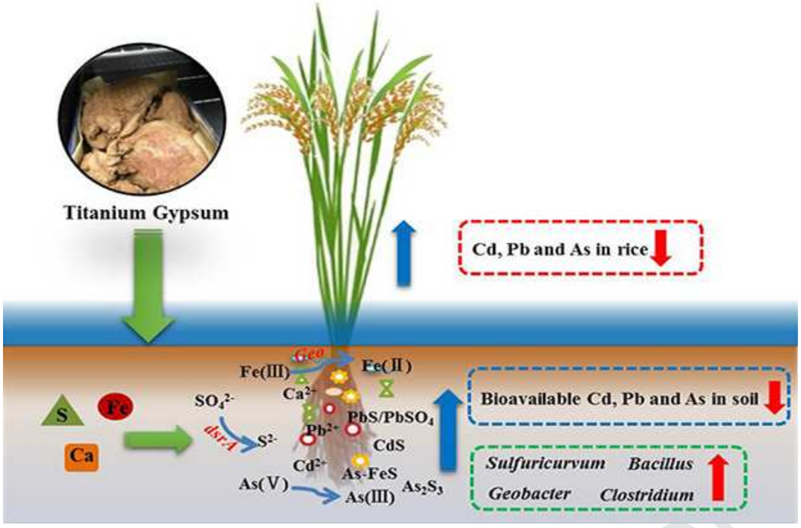
Titanium Gypsum
Cd, Pb and As in rice
S
Fe
Ca
Geo
Fe(III)
Fe(II)
Ca2+
SO4 2-
dsrA
S2-
Pb2+
PbS/PbSO4
CdS
Cd2+
As-FeS
As(V)
As(III)
As2S3
Bioavailable Cd, Pb and As in soil
Sulfuricurvum
Bacillus
Geobacter
Clostridium

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Simultaneous immobilization of the cadmium, lead and arsenic in paddy soils amended with titanium gypsum

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Abstract

In situ immobilization of heavy metals in contaminated soils using industrial by-products is an attractive remediation technique. In this work, titanium gypsum (TG) was applied at two levels (TG-L: 0.15% and TG-H: 0.30%) to simultaneously reduce the uptake of cadmium (Cd), lead (Pb) and arsenic (As) in rice grown in heavy metal contaminated paddy soils. The results showed that the addition of TG significantly decreased the pH and dissolved organic carbon (DOC) in the bulk soil. TG addition significantly improved the rice plants growth and reduced the bioavailability of Cd, Pb and As. Particularly, bioavailable Cd, Pb and As decreased by 35.2%, 38.1% and 38.0% in TG-H treatment during the tillering stage, respectively. Moreover, TG application significantly reduced the accumulation of Cd, Pb and As in brown rice. Real-time PCR analysis demonstrated that the relative abundance of sulfate-reducing bacteria increased with the TG application, but not for the iron-reducing bacteria. In addition, 16S rRNA sequencing analysis revealed that the relative abundances of heavy metal-resistant bacteria such as *Bacillus, Sulfuritalea, Clostridium, Sulfuricella, Geobacter, Nocardioides* and *Sulfuricurvum* at the genus level significantly increased with the TG addition. In conclusion, the present study implied that TG is a potential and effective amendment to immobilize metal(loid)s in soil and thereby reduce the exposure risk of metal(loid)s associated with rice consumption.

Capsule: Titanium gypsum is a potential and effective amendment to immobilize Cd, Pb and As in paddy soils.

Keywords: Titanium gypsum, Heavy metals, Immobilization, Paddy soil, Rice

Introduction

Heavy metal pollutants in soil are of growing concern worldwide. In China alone, a vast majority of farmlands are contaminated with toxic trace metal(loid)s such as Cd, Pb and As (Yang et al., 2018; Huang et al., 2018). According to the National Soil Pollution Survey Bulletin (2014), the concentration of Cd, Pb and As were 7.0%, 1.5%, 2.7% above the permissible standard, respectively. This poses an immediate threat to human health and food safety because a large number of these contaminated soils are from rice paddy fields. Rice is one of China's staple food crops and has been identified as the main source of dietary intake of toxic trace metal(loid)s (Xiao et al., 2018). Therefore, there is an urgent need to limit the transfer of toxic metal(loid)s from soil to grains.

One important way to prevent the transfer of heavy metal(loid) contaminates from soil to grains is to limit their bioavailability in the soil by in situ immobilization (Ok et al., 2011; Li et al., 2016). Currently, numerous organic and inorganic soil amendments as well as field management strategies such as irrigation have been widely used to immobilize and reduce the bioavailability of heavy metal(loid)s (Hou and Li, 2017; Sharma and Nagpal, 2017; Shaheen and Rinklebe, 2015; Irshad 2015; Tran et al., 2015; Clemente et al., 2015). The immobilization mechanisms include liming effect, which could reduce bioavailable Cd, Pb for plant uptake (Chen et al., 2016; Mahar et al., 2015); complexation with functional groups (McLaren and Cameron, 1996); formation of stable metal compounds containing phosphorus and calcium(Ca) (Rehman et al., 2015; Udeigwe et al., 2011); as well as sorption and precipitation. However, the use of some soil amendments may incur cost and in some cases lead to the generation of secondary pollutants (Hou et al., 2016; Song et al, 2018). In recent years, sustainability and green remediation materials have drawn increasing attention in the environmental remediation field (Hou and Al-Tabbaa, 2014; Zhang et al., 2018). Industrial by-product used for remediation is one aspect of green remediation that promotes sustainable practices for waste recycling. Titanium gypsum (TG) is a typpe of industrial by-product derived from the titanium dioxide industry and mainly composed of crystalline gypsum ($CaSO_4 \cdot 2H_2O$) and amorphous ferric hydroxide ($Fe(OH)_3$) (Gázquez et al., 2013).The utilization of TG can reduce the consumption of natural gypsum, save production costs, and bring good economic, environmental and social benefits. TG contains enough calcium (Ca) and sulfur (S) to provide the necessary nutrients for crop growth (Peacock et al., 2000). Moreover, the flocculation rate of TG in the soil is high, thus enabling TG to prevent soil loss to a certain extent (Fauziah et al., 1996).

Furthermore, the presence of the iron oxide in TG makes it more effective as a soil amendment rather than as a source of Ca and S fertilizer (Fauziah et al., 2011). TG has been used as a soil amendment to immobilize As, Cd, copper (Cu), and Pb in heavy metal contaminated soils (Lombi et al. 2004; Illera et al. 2004). Previous studies have shown that the content of Pb, zinc (Zn) and nickel (Ni) in the diethylenetriaminepentaacetic acid (DTPA) extracted portion were significantly reduced after the application of TG to the soil (Rodriguez-Jorda et al., 2009). Thus, it indicated that TG can effectively immobilize heavy metal(loid)s.

The mobility of Cd, Pb and As under different soil condition varies greatly. In fact the geochemical behavior of Cd and As are generally opposite, hence making it difficult for their simultaneous immobilization. For example, amending soil with agents such lime, fly ash and biochar can significantly decrease Cd and Pb mobility due to an increase in soil pH, however, this increase in soil pH increases As bioavailability (Tica et al. 2011). Moreover, soil redox condition is another important factor that affects the immobilization of heavy metal(loid)s. Cadmium tend to be immobilized under reducing conditions since soluble Cd^{2+} tends to precipitate as insoluble CdS. While on the other hand, anaerobic conditions favor the reduction of As(V) to As(III), which consequently increases As bioavailability (Bakhat et al., 2017).

It has also been reported that heavy metal(loid)s could be immobilized by iron plaque on the root surface and influence heavy metal(loid)s uptake by rice plants (Syu et al., 2013; Cao et al., 2018; Xu et al., 2018). In addition, biogeochemical processes in soil environments mediated by the microorganism largely influence the mobility and toxicity of these heavy metal(loid)s to plants. Therefore, understanding the soil microbial community compositional dynamics with environmental heavy metal gradients and plant uptake over time will introduce greater insight into the underlying processes (Sullivan et al., 2013).

Due to the difference in Cd, Pb and As geochemistry, few studies have been done on the simultaneous stabilization and regulation of Cd, Pb and As in the soil. Hence, alternative or complementary methods for Cd, Pb and As simultaneous immobilization to ensure the safety of agricultural production are required (Yao et al., 2017). Therefore, this study aims to test the feasibility of TG for the simultaneous immobilization of Cd, Pb and As in soil by carrying out pot trials. Furthermore, the change in microbial communities due to the amendment of TG was also investigated to discuss the possible mechanism of the simultaneous immobilization.

Materials and methods

Soil and TG characteristics

The paddy soil was sampled from a field located in Shangyu city of Zhejiang Province. The sample was collected from the top (0-20 cm) soil layer. The soil was air dried, sieved (mesh size, 2 mm) and stored at room temperature. The titanium gypsum (TG) was collected from Zhenjiang city of Jiangsu Province, then oven dried, ground and passed through a 20-mesh nylon mesh with particle size < 10 μm. The main chemical compositions of TG was calcium sulfate ($CaSO_4 \cdot 2H_2O$) and ferric oxide (Fe_2O_3) with a ratio of 7.4:1 (w/w). The contents of S, Ca, iron (Fe) were 11.2%, 13.2%, 2.1%, respectively. The Brunauer-Emmett-Teller (BET) surface area of TG was 39.9 m^2 g^{-1}. The physiochemical properties of the soil and TG are given in Table 1. The SEM and EDS images of TG were shown in Figure S1.

Pot trials

The rice seeds of cultivar Xiushui 03 were used in the pot trials. Two treatments 0.15% (TG-L) and 0.3% (TG-H) of TG contained about 150 mg S kg^{-1}, 32 mg Fe kg^{-1}, 200 mg Ca $kg^{-1}$and 300 mg S kg^{-1}, 64 mg Fe kg^{-1}, 400 mg Ca kg^{-1} respectively. The TG amendment which has a positive impact on rice plant growth was applied in each pot filled with 2.8 kg of contaminated soil. An application rate of 0.15% and 0.3% of TG was chosen because it was similar to rates used in the literature (i.e. Zhang, et al., 2019; Du et al., 2018). Furthermore, the 0.15% and 0.3% treatment rate was also not excessive or uneconomical on a field scale. TG was homogenized with all the soil in the pot. The control treatment received no TG amendment. Rice seeds were firstly sterilized in H_2O_2 solution (30%) for 15 min and washed with deionized water. Then rice seeds were placed on a fine nylon screen for germination before being transplanted into a nutrient solution (N：40 mg L^{-1}; P: 10 mg L^{-1}; K:40 mg L^{-1}; Ca: mg L^{-1}; Mg: 40 mg L^{-1}; Fe: 2 mg L^{-1}; Mn: 0.5 mg L^{-1}; Mo: 0.05 mg L^{-1}; B: 0.2 mg L^{-1}; Zn: 0.01 mg L^{-1}; Cu: 0.01 mg L^{-1}) as suggested by the International Rice Research Institute (Yoshida et al., 1971). Approximately 3-week-old rice seedlings were transplanted to each pot. After transplanting, the pots were flooded with water. The water management of the pot trials was performed following the convention that flooding be maintained during the tillering stage and heading stage, and drainage during the maturing stage. At the beginning of the experiment, the soil was amended with basal fertilizers (KH_2PO_4· at 0.72 g kg^{-1}) and urea (0.32 g kg^{-1}). Each treatment contains three replicates and all pots were randomly arranged in a greenhouse with 60–70% relative humidity, 14/10-h day/night duration, and 30/28 °C day/night temperature.

Sample preparation and analysis

Bulk soil samples (0-20 cm) were collected during the tillering and maturing stages. At the maturing stage, rhizosphere and bulk soil were carefully sampled as previous illustrated by Chen et al. (2015). Soil pH was measured with ultrapure water (2.5:1, w/v) using a potentiometer (Mettler Toledo S220-K, Greifensee, Switzerland). DOC and dissolved organic nitrogen (DON) were determined by Multi N/C analysis apparatus (multi N/C 3100, Jena, Germany). Bioavailable As in the soil was extracted using 0.05 M $NH_4H_2PO_4$, with a soil to solution ratio of 1:25 (Drahota , et al，2014). Bioavailable Cd and Pb were extracted by 0.10 M $CaCl_2$ using a soil to solution ratio of 1:20 and shaking for 4 h at 20 °C (Meng et al, 2018).

Plant height was measured from the soil surface to the upper leaf edge or the top of the panicle, then the rice plants were harvested and washed with deionized water. The washed plants were oven at 105 °C for 1 h and then dried at 60 °C 72 h. The plants were divided into four parts, roots, stems, leaves and whole grains for further analysis. Stems, leaves and whole grains were classified as the above-ground parts of rice plant. The iron plaques were extracted by dipping the fresh rice roots into a DCB solution (0.03 M sodiumcitrate + 0.125 M sodium bicarbonate + 0.015 g mL^{-1} sodiumdithionite) (Liu et al. 2004). Dried plant tissue samples were milled into homogenized powders by a grinding miller and stored in a vacuum drier before analysis. Prior to analysis 0.5 g of rice plant sample was weighed into 50 mL polypropylene digestion tube , then 10 ml 1:1 ratio of HNO_3 and H_2O_2 were added (Liu et al., 2013). After adding the HNO_3 and H_2O_2, the samples were digested using a microwave digester (MARS6, CEM Microwave Technology Ltd., USA). After digestion, the samples were filtered and diluted with deionized water before measuring the total metals using the ICP-MS (NEXION300XX, PerkinElmer, Inc., USA). A rice reference (GBW(E)080684) purchased from the National Research Centre of China was used to determine the accuracy of the analytical method. The plant sample recoveries of total heavy metal were within the range of 94.5-106.5% (n=3).

DNA extraction and Real-time qPCR analysis

Bulk soil samples (n=3) (Controls, TG-L, and TG-H) of tillering and maturing stage were taken for molecular analysis. Total genomic DNA was extracted directly from these samples using the FastDNA® spin kit (MP bio, Santa Ana, California, USA) following the manufacturer's instruction. DNA concentration was estimated using a spectrophotometer (NanoDrop ND-2000, USA) then stored at −80 °C for subsequent analyses. Real-time PCR (qPCR) was used to determine the quantitative distribution of sulfate reducing bacteria (*dsrA*) and iron reducing bacteria (*Geobacteraceae*). Bacterial 16S rRNA gene was targeted to quantify the total bacterial population and normalize the abundance of *dsrA* and *Geobacteraceae* genes in the samples. Information about primer pairs and the PCR thermal programs used were done according to previous studies (Cummings et al., 2003; Spence et al., 2008; Suzuki et al., 2000).

High-throughput Sequencing

High-throughput sequencing was performed using Illumina Miseq sequencing platform (Miseq, Illumina Inc., USA) at Zhejiang Institute of Microbiology, China. Output sequences were quality checked by filtering low quality reads using PRINSEQ (v. 0.19.5) and chimeric sequences using UCHIME package with default settings.

After the removal of barcode and primers, the remaining sequences were clustered into OTUs at 97% sequence identity by UCLUST (v1.1.579). Taxonomic assignments were performed with the ribosomal database project (RDP) classifier with a confidence cutoff at 0.8. Sequencing coverage, Shannon-Wiener index, Chao1, and ACE indices were calculated using MOTHUR (v 1.27).

Statistical Analysis

One-way analysis of variance (ANOVA) followed by Duncan's test were used to study the differences between multiple samples. All statistical analyses were performed using Windows-based SPSS 18.0.0.

Results and discussion

Effects of TG on rice growth and physico-chemical properties of soil

The addition of TG significantly improved the rice plants growth. Compared to the control, the plants total dry mass of TG-L and TG-H treatments increased by 139.8% and 118.8%, respectively (Table 2). Similarly, the rice plant height also significantly increased by 18.7% and 10.9%, respectively in TG-L and TG-H treatments. However, the addition of TG, regardless of application rate, did not have a significant effect on the dry mass of root. Amending the soil with 0.15% (TG-L) and 0.30% (TG-H) TG significantly ($p< 0.05$) increased the dry mass of grain by 258.2% and 146.8% compared with the control, respectively. However, there was no significant difference between TG-L and TG-H. The results obtained here suggested that the addition of TG could significantly improve the rice plants growth, while the high dose of TG had a lower growth stimulating effect than the low TG dose. This may have occurred because TG contains the required S which is an essential element for crop growth and excessive S may result in H_2S poison on rice growth. These results were consistent with that of Zhang et al. (2019) and Yang et al. (2016). Previous study has shown that gypsum application significantly increased the sulfur concentration in rice plants, and sulfhydryl containing enzymes is an important component of protein synthesis (Yang et al., 2014, Sun et al., 2017). Moreover, our previous study showed that $FeSO_4$ supply significantly increased glutathione (GSH) concentration in leaves, which could support the present results (Zou et al., 2018a). Moreover, the addition of SO_4^{2-} has also been shown to significantly increase plant height and biomass in Pb polluted soil and S could alleviate Pb stress on rice (Hu et al. 2007).

The physico-chemical properties of the rhizosphere (RS) and bulk soils (BS) are summarized in Table 3. Compared with the control soil, the application of TG treatment had no significant effect on the pH of the RS and BS. However, the pH of the RS was significantly lower than that of the BS, which may be due to the organic acids from root exudates and CO_2 produced by root and rhizospheric microorganisms (Song et al, 2012). Furthermore, compared to the control soil, the addiction of TG had little effect on the DOC contents of RS but significantly reduced the DOC content in the BS. This may have occurred since the clay particles and soil organic matter could combine by calcium cations (Ca^{2+}) (Chan and Heenan, 1999), which can promote the coagulation of DOC (Bolan et al., 2003). In addition, the addition of TG significantly reduced the content of dissolved organic nitrogen (DON) in the BS, but had less impact on the RS, which is closely related to the rhizospheric microorganisms and enzymatic activity (Wang et al, 2016).

Effects of TG on Cd, Pb and As bioavailability in soil

The bioavailable concentrations of Cd, Pb and As of soil during the tillering and maturing stage are presented in Fig.1. In the tillering stage, the bioavailable concentrations of Cd ranged between 0.03-0.05 mg kg^{-1}. Compared to the control, the addition of TG (TG-L and TG-H) has no significantly effect on decreasing the bioavailable concentrations of Cd in soil. While, the bioavailable concentrations of Pb significantly decreased by 61.9% and 38.1% in TG-L and TG-H during the tillering stage compared to the control, respectively. Moreover, no significant difference in Pb bioavailable concentration was observed between the different TG amendments. The addition of TG decreased the bioavailable concentrations of Pb due to excessive S resulted in PbS/$PbSO_4$ formation and precipitation (Kashem et al. 2001; Yang et al. 2016). Similarly, the TG-L and TG-H treatments significantly decreased the bioavailable concentrations of As by 36.7% and 38.0% respectively during the tillering stage. This decrease in As bioavailability may have occurred due to the formation of As-sulfide-like species. During the tillering stage, the flooded soil conditions promoted anaerobic conditions that consequently led to the production of microbial-derived sulfide. The newly form sulfide could react with As in the soil porewater to form insoluble arsenic-sulfide species such as orpiment and realgar, consequently reducing As bioavailability (Hashimoto and Kanke, 2018). During the maturing stage, the bioavailable concentrations of Cd and Pb showed a statistically significant increase when compared with that of the tillering stage. These variations may be related to the differences in pH, Eh and microbial activity during different stages of rice growth (Yang et al., 2015). Drainage of paddy fields in the maturing stage results in the oxidation of CdS/PbS to Cd^{2+}/Pb^{2+} and SO_4^{2-}, which has a much higher solubility than CdS/PbS formed under flooding conditions, resulting the availability of Cd and Pb for rice uptake was increased. While under aerobic conditions as a result of field drainage, the bioavailable concentrations of As significantly decrease. This is because As(III) was oxidized to As(V), which is strongly sorbed to soil mineral soil components such as Fe and Al (hydr)oxides (Beiyuan et al. 2017; Yu et al. 2017).

Effect of TG on the concentration of Cd, Pb and As in different tissues of Rice

The Cd, Pb and As accumulation in roots, straw, and whole grains were analyzed. The concentrations of Cd, Pb, and As in roots were significantly higher than those in straw and brown rice (Table 4) indicating that Cd, Pb, and As was primarily accumulated in the roots, which was consistent with previous studies (Liu et al., 2015; Yu et al., 2017). The concentrations of Cd, Pb and As in the roots treated with TG have no significant difference compared to that of the control. The application of TG significantly limited the accumulation of Cd, Pb and As in brown rice. The concentration of Cd and As were reduced by 96.9% and 46.9%, respectively in TG-H treatment. Additionally, there was no accumulation of Pb in brown rice with the application of TG irrespective of does. While, the TG-L showed the high reduction in Cd, Pb and As accumulation in the rice straw. The decreased in Cd, Pb, and As in rice grains could be explained by the limited dissolved Cd, Pb and As in the soil during the tilling stage. Previous study has suggested that the uptake of trace metals by rice plants was strongly dependent on the bioavailability of these trace metals (Gustave et al., 2019). In our study, the application of TG significantly decreased the bioavailability Cd, Pb and As during the tilling stage. Since, rice grows actively from tillering stage to filling stage, reducing the bioavailable concentrations of Cd, Pb and As in the tillering stage was suggested to be the key to decrease the accumulation of Cd, Pb and As in rice with the addition of TG.

As shown in Table 5, the TG-L treatment had no significant effect on the concentration of Fe, Mn, Cd, Pb and As in the iron plaque on the root surface. TG-H treatment significantly increased the concentration of Mn in the iron plaque, and significantly decreased the concentration of Cd and Pb on the roots surface. However, the addition of TG-H had no significant effect on the increase of Fe concentration and the decrease of As concentration in rice root. Although Fe addition promotes the formation of iron plaque (Fresno et al.,2016), no significant increase was observed for the DCB-extracted Fe with the addition of TG. This may be due to the fact that rice roots have lower activity and ability to secrete oxygen during the maturation stage thereby limiting the formation of the Fe plaques at this stage (Yu et al., 2017). Furthermore, the bioavailable concentrations of Cd, Pb and As of bulk soil decrease in TG-H treatment, and SO_4^{2-} may enhance the desorption of arsenate from iron plaque (Hu et al., 2007), which contributed to the decrease of DCB-extracted Cd, Pb and As.

Effects of TG on relative gene abundances of *Geobacteraceae* and *dsrA*

In order to analyze the effects of the application of TG to the functional genes of specific micro-organism in the rhizosphere soil, we performed qPCR on *dsrA* and *Geobacteraceae* (*Geo*) genes. The copy ratio of *dsrA* to16S rRNA and *Geo* to 16S rRNA are presented in Fig.2. The application of TG-L and TG-H highly affected the relative abundance of the *dsrA* gene in soil. However, the application of TG had no clear effect on the relative abundance of *Geo* gene. Under aerobic conditions, As is mainly absorbed onto the surface of Fe oxides in paddy soil. On the contrary, in the anaerobic conditions *Geobacteraceae* family is able to use DOC as an electron donor to reduce both Fe(III) and As(V) (Ohtsuka et al., 2013), resulting in the release of As(V) and/or the reduction of As(V) to As(III) by As(V)-reducing microorganisms (Yamaguchi et al., 2011). Since the enhancement of iron-reducing bacteria favors the Fe oxide reduction and As mobility (Gustave et al., 2018; Yamamura et al., 2018), the decrease in the abundance of *Geo* gene relative abundance during the mature stage could have resulted in the decrease in As bioavailability. The microbial reduction of Fe oxide results increase of dissolved Fe(II) and As. Similar, the direction transformation of As(V) to As(III) from the surface of mineral oxide can enhance As bioavailability. This occurs because As(V) is less mobile than As(III) and tend to easily precipitated with mineral oxide. Moreover, As(III) has a lower affinity for metal oxides and tend to be more bioavailable to rice plants. In addition, under anoxic conditions microbially-derived sulfide can react with Fe and As to produce Fe sulfide minerals and As-sulfide-like species, resulting As precipitation and insolubilization (Burton et al., 2011; Hashimoto and Kanke, 2018). Furthermore, the increase in the *dsrA* gene could have played a role in limiting Cd bioavailability. Sulfate-reducing bacteria can enhance sulfate reduction, which consequently could have promoted Cd precipitation as CdS (Qiao et al., 2017). Jiang and Fan (2010) reported that sulfate-reducing bacteria can reduce 70% of the exchangeable fraction Cd in Cd-contaminated soils.

Effects of TG on microbial community structure

The alpha diversity indices of the soil bacterial 16S rRNA gene are shown in Fig. 3. Chao1, ACE, Shannon and Simpson diversity indices in the tillering stage were significantly ($p < 0.05$) different and higher compared to the maturing stage, suggesting high abundance and diversity of microbial community structure during tillering stage. However, the alpha diversity of TG-L in tillering stage were significantly ($p < 0.05$) higher than TG-H, which indicated that the presence of the 0.15% TG could increase the richness and diversity of species as to elemental S has been reported to be related to the abundances and diversity of microbial community. Microbial diversity has been reported to play a crucial role in maintaining the stability and functionality of heavy metal contaminated soils (Zheng et al., 2016; Zou et al., 2018b). However, results of several studies have shown that the heavy metal concentrations could influence microbial community structure by an increase in the abundance of metal-tolerant strains (Hui et al. 2012; Xie et al., 2016). Taxonomic information indicated that the soil bacterial community composition was modified by the amendments of TG. At the phylum level (Fig.4A), it showed that the most dominant bacterial phyla in all soils were *Proteobacteria*, followed by *Bacteroidetes*, *Firmicutes*, *Epsilonbacteraeota* and *Patescibacteria*. In the maturing stage, the relative abundance of *Proteobacteria* significantly increased. The application of different amount of TG had a certain effect on the bacterial community. The significant change was that the relative gene abundance of *Proteobacteria* was significantly reduced with the addition of TG-L and TG-H, especially in the maturing stage, which decreased by 23.7% and 28.8%. *Proteobacteria* was reported to be in heavy metal contaminated areas (Yong et al., 2018; Gang et al., 2018) and was the predominant phylum in different mines (Gupta et al., 2017). The addition of TG also decreased the relative abundance of *Bacteroidetes* while increased the relative abundance of *Firmicutes* in the maturing stage. *Epsilonbacteraeota* was detected at low relative abundances in the tillering stage, and it showed a significant increase in TG-L and TG-H as compared to control in the maturing stage. At the genera level, the more abundant groups in TG-L and TG-H samples compared to control samples in the tillering stage were *Sulfuritalea*, *Bacillus*, *Fonticella*, *Clostridium_sensu_stricto_1* and *Clostridium_sensu_stricto_10*, whereas *Sulfuricella*, *Geobacter*, *Nocardioides* and *Sulfuricurvum* were more abundant in TG-L and TG-H in the maturing stage as shown in Fig.4B. These results revealed that amendments of TG could decreased the abundance of other microorganisms sensitive to heavy metals, and increase the relative abundances of some microorganisms, which have a potential tolerance to heavy metal. For example, *Sulfuricurvum* was mainly related to sulfate oxidation (Kodama, 2004). *Geobacter* is a dissimilatory iron reducing bacteria (Dang et al., 2017; Ohtsuka et al., 2013) and plays an important role in As transformation. *Bacillus* is also a dissimilatory iron reducing bacteria and is capable of a tolerance to As (Yamamura et al., 2007).

Conclusions

This study successfully evaluated the effect of TG on the immobilization of Cd, Pb and As and their subsequent uptake by rice with two application rates. The results showed that the application of TG significantly improved the growth of rice plants, and decreased the soil bioavailable Cd, Pb, and As concentrations. Furthermore, the accumulation of Cd, Pb and As in rice grain significantly decreased with the TG application. In addition, TG addition could increase the relative abundance of sulfate-reducing bacteria and heavy metal-resistant bacteria, which might contribute to the decrease of Cd, Pb and As bioavailability in soil. Overall, TG might be a potential and promising agricultural soil amendment because it is an effective immobilizing agent to simultaneously reduce the uptake of Cd, Pb, and As by rice. Nonetheless, further studies are required to verify the results under field conditions and for a wider variety of soil types.

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Table 1 Physical, chemical properties of soil and TG

Table 2 Effect of TG on total dry weight of rice plant, dry weight of grain, root and straw, and plant height

Table 3 Effect of TG on the pH, DOC and DON concentration of rhizosphere/bulk soils after harvest of the rice

Table 4 Effect of TG on Cd, Pb and As concentration (mg kg^{-1}) in rice tissues

Table 5 Effect of TG on DCB-extractable Fe, Mn, Cd, Pb and As concentrations in Fe and Mn plaque on root surface

Fig.1. Effects of TG on bioavailable concentrations of Cd(A), Pb(B), As(C) in soil. The different lowercase letters indicate a significant difference at $p < 0.05$ (Duncan's test), (Control: no addition; TG-L: 0.15%; TG-H: 0.30%). Different capital letters indicate a significant difference at $p < 0.05$ in the same treatments.

Fig.2. Relative gene abundances of *dsrA* (A) and *Geo*(B) under different treatments at rice tillering stage and maturing stage. Different lowercase indicates significant differences of the different treatments between Control, TG-L and TG-H ($p < 0.05$). The capital letter indicates significant differences between tillering stage and maturing stage of the same treatments.

Fig.3. Effects of TG on soil prokaryotic α-diversity in different treatments. (Control: no addition; TG-L: 0.15%; TG-H: 0.30%; TS: Tillering Stage; MS: Maturing Stage)

Fig.4. Relative abundance of microbial phyla (A) and genus (B) in different soil samples. (Control: no addition; TG-L: 0.15%; TG-H: 0.30%; TS: Tillering Stage; MS: Maturing Stage)

Table1 Physical, chemical properties of soil and TG

	pH	EC	CEC	OM	Total N	Available P	Available K	DOC	DON	Clay	Sand	Silt	Cd	Pb	As
		(us cm^{-1})	(cmol kg^{-1})	(g kg^{-1})	(mg kg^{-1})					(%)			(mg kg^{-1})		
Soil	6.07	322.3	5.4	43.3	2.7	19.5	85.3	343.5	45.6	15	37	48	0.5	359.9	105.1
	pH	Chemical compositions (wt, %)								Particle Size	$CaSO_4 \cdot 2H_2O$ /Fe_2O_3（w/w）	BET Surface Area(m^2 g^{-1})	Cd	Pb	As
		O	S	Ca	Fe	C	Ti	Al	Si				(mg kg^{-1})		
TG	8.58	54.1%	11.2%	13.2%	2.1%	18.8%	0.4%	0.1%	0.1%	<10μm	7.4	39.9	0.1	28.6	163.3

TG: titanium gypsum; EC: Electrical Conductivity; OM: Organic Matter; Total N: Total Nitrogen; Available P: Available Phosphorus; Available K: Available Kalium; DOC: Dissolved Organic Carbon; DON: Dissolved Organic Nitrogen.

Table 2 Effect of TG on total dry weight of rice plant, dry weight of grain, root and straw, and plant height

Treatment	Total D.W. of plant (g pot^{-1})	D.W. of grain (g pot^{-1})	D.W. of root (g pot^{-1})	D.W. of straw (g pot^{-1})	Plant height (cm)
Control	21.86±4.68b	4.47±3.69b	2.26±0.27a	15.07±1.24b	80.00±4.07b
TG-L	52.42±7.24a	16.01±6.23a	3.11±0.62a	33.30±8.84a	94.93±7.81a
TG-H	47.82±6.92a	11.03±3.66ab	2.93±0.36a	33.87±4.35a	88.73±7.46ab

Control: no addition; TG-L: 0.15%; TG-H: 0.30%; D.W.: Dry Weight. Values are mean ± standard deviation. Different lowercase letters indicate a significant difference between Control, TG-L, and TG-H treatments at $p < 0.05$ (Duncan's test).

681 **Table 3** Effect of TG on the pH, DOC and DON concentration of rhizosphere/bulk soils after harvest of the rice

Treatment	pH		DOC（mg kg^{-1}）		DON（mg kg^{-1}）	
	RS	BS	RS	BS	RS	BS
Control	6.50±0.37aA	7.00±0.22aA	130.62±5.72aA	178.36±18.21aB	48.66±7.10aA	19.14±2.95aB
TG-L	6.01±0.22aA	6.71±0.18aB	124.38±19.28aA	140.49±13.21bA	23.60±3.80bA	19.52±5.05aA
TG-H	6.07±0.24aA	6.78±0.16aB	129.96±20.22aA	141.14±16.00bA	23.89±5.24bA	20.25±2.61aA

682 Control: no addition; TG-L: 0.15%; TG-H: 0.30%; RS：Rhizosphere Soil; BS：Bulk Soil; DOC: Dissolved Organic Carbon; DON: Dissolved
683 Organic Nitrogen; Values are mean ± standard deviation. The different lowercase letters indicate a significant difference between Control, TG-L,
684 and TG-H treatments at $p < 0.05$ (Duncan's test). Different capital letters indicate a significant difference at $p < 0.05$ between RS and BS. .

Table 4 Effect of TG on Cd, Pb and As concentration (mg kg^{-1}) in rice tissues

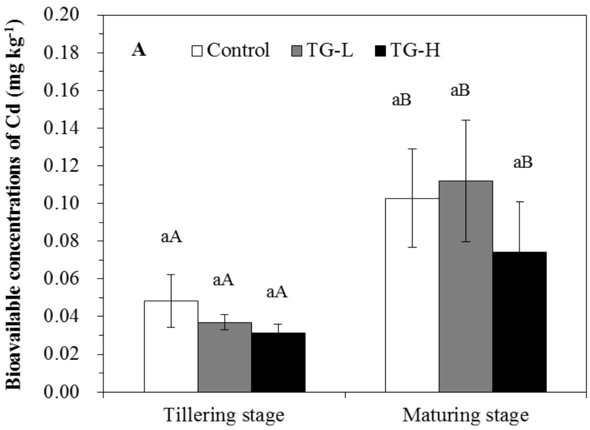
	Treatments	root	straw	brown rice
Cd	Control	1.65±0.54a	0.22±0.05a	0.064±0.042a
	TG-L	1.51±0.81a	0.05±0.01b	0.011±0.007ab
	TG-H	0.83±0.32a	0.13±0.10ab	0.002±0.002b
Pb	Control	753.53±111.54a	2.90±1.39a	0.38±0.18
	TG-L	729.29±182.31a	0.90±0.22a	ND
	TG-H	682.00±243.85a	1.32±0.23a	ND
As	Control	1374.58±152.81a	20.88±2.64a	0.96±0.02a
	TG-L	1318.86±373.0 a	14.93±0.40b	0.56±0.24ab
	TG-H	1125.79±172.14a	18.18±0.34ab	0.49±0.12b

Control: no addition; TG-L: 0.15%; TG-H: 0.30%; ND: Not detected. Values are mean ± standard deviation. Different lowercase letters indicate a significant difference between Control, TG-L, and TG-H treatments at $p < 0.05$ (Duncan’s test).

Table 5 Effect of TG on DCB-extractable Fe, Mn, Cd, Pb and As concentrations in Fe and Mn plaque on root surface

Treatments	DCB-Fe (g kg^{-1})	DCB-Mn (mg kg^{-1})	DCB-Cd (mg kg^{-1})	DCB-Pb (mg kg^{-1})	DCB-As (mg kg^{-1})
Control	49.62±3.47a	527.93±69.99b	0.33±0.06a	153.11±7.01a	1066.39±96.51a
TG-L	51.16±0.49a	665.87±124.36b	0.39±0.05a	145.77±15.12a	1008.34±80.40a
TG-H	52.84±2.91a	1022.00±349.31a	0.16±0.10b	95.90±26.39b	975.98±110.17a

Control: no addition; TG-L: 0.15%; TG-H: 0.30%; Values are mean ± standard deviation. Different lowercase letters indicate a significant difference between Control, TG-L, and TG-H treatments at $p < 0.05$ (Duncan's test).



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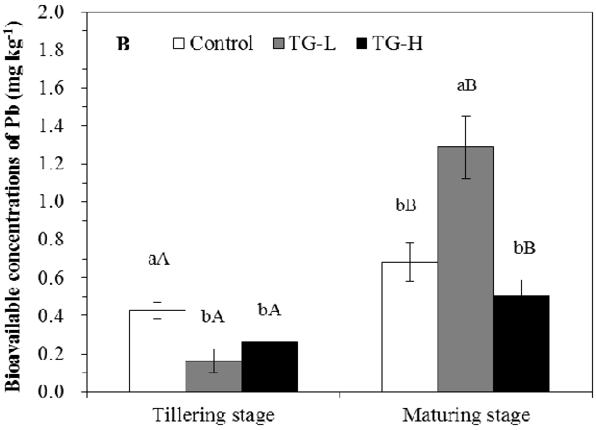

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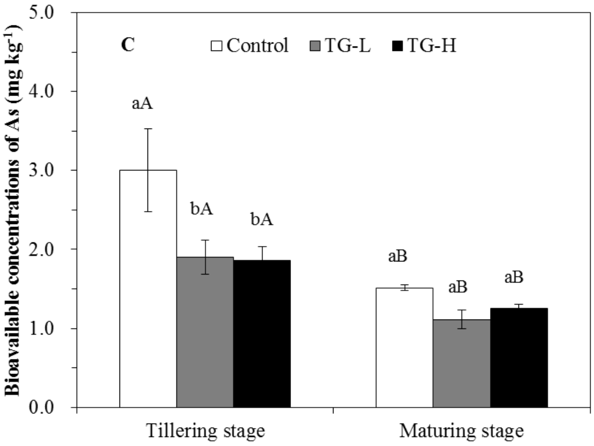

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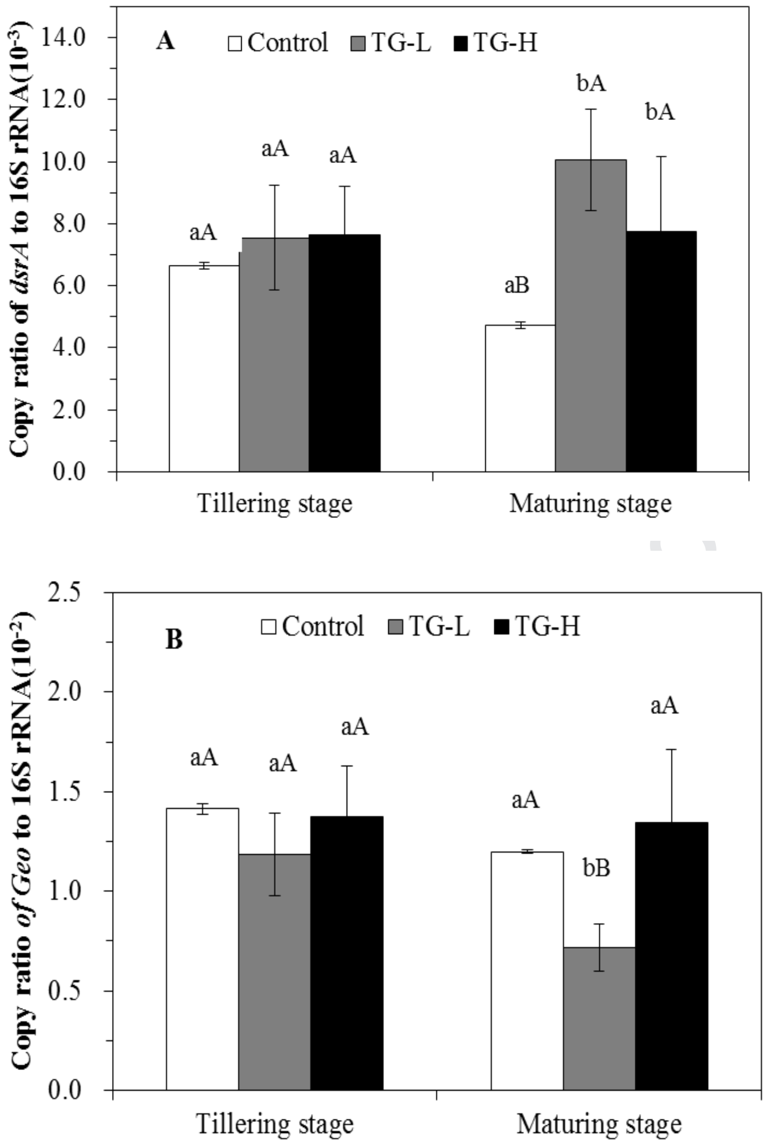
695 **Fig.1.** Effects of TG on bioavailable concentrations of Cd(A), Pb(B), As(C) in soil. 696 The different lowercase letters indicate a significant difference at $p < 0.05$ (Duncan's 697 test), (Control: no addition; TG-L: 0.15%; TG-H: 0.30%). Different capital letters 698 indicate a significant difference at $p < 0.05$ in the same treatments.



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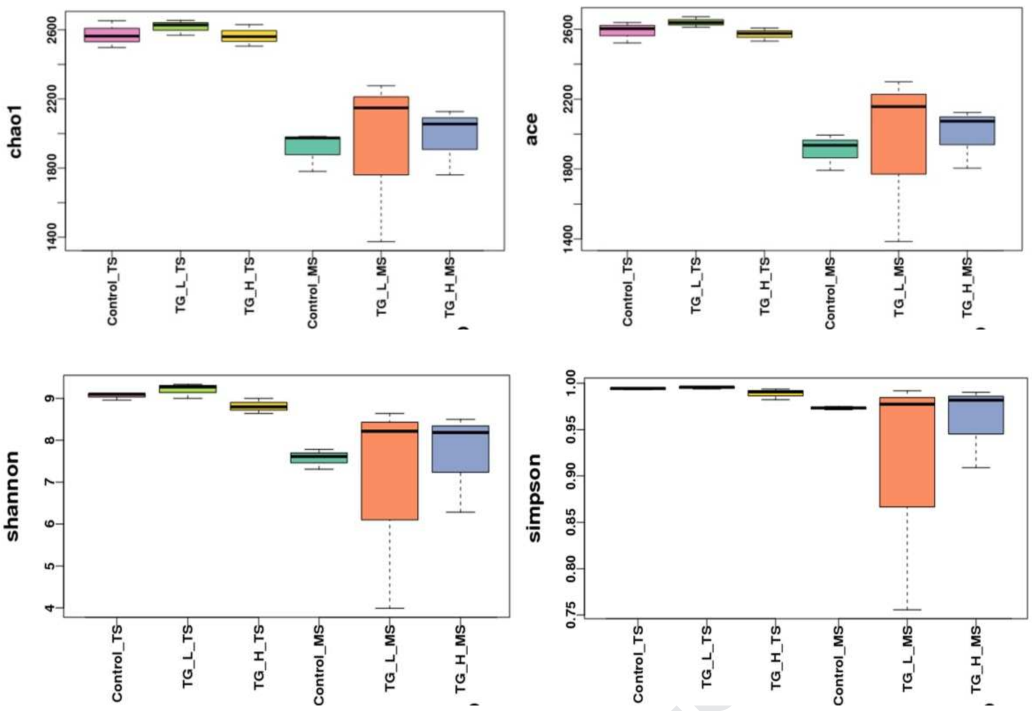

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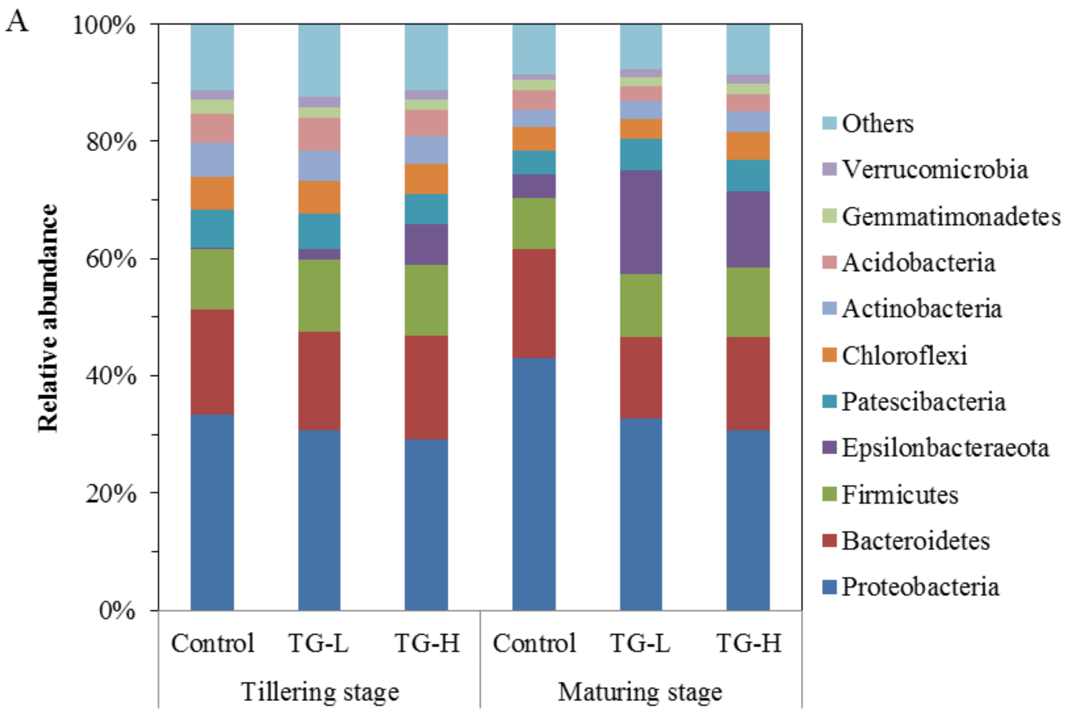
A
100%
80%
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Control
TG-L
TG-H
Tillering stage
Maturing stage
Others
Verrucomicrobia
Gemmatimonadetes
Acidobacteria
Actinobacteria
Chloroflexi
Patescibacteria
Epsilonbacteraeota
Firmicutes
Bacteroidetes
Proteobacteria

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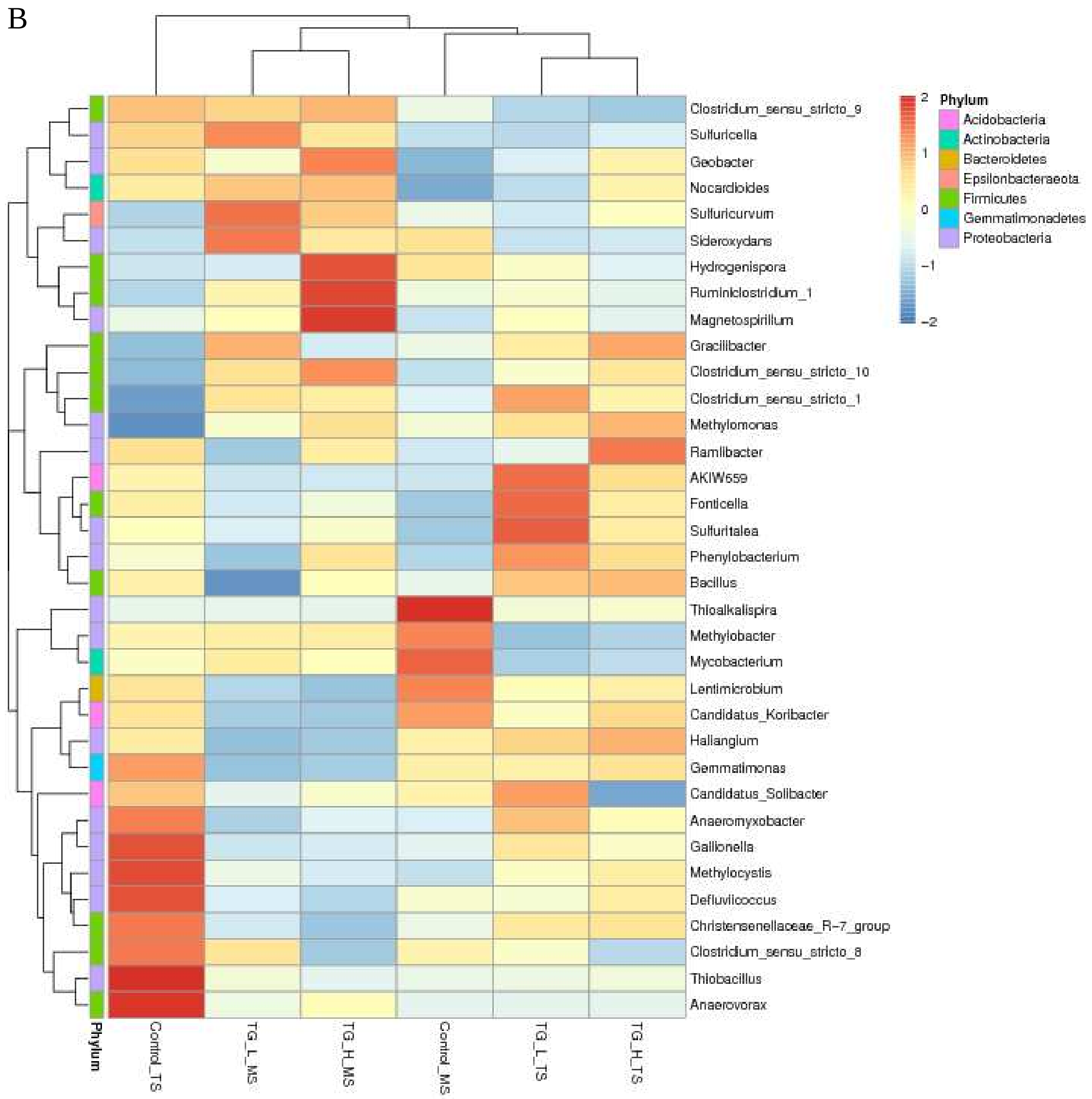

Fig.4. Relative abundance of microbial phyla (A) and genus (B) in different soil samples. (Control: no addition; TG-L: 0.15%; TG-H: 0.30%; TS: Tillering Stage; MS: Maturing Stage).

- Titanium gypsum addition decreased the bioavailability of Cd, Pb and As in soil
- Titanium gypsum addition reduced the accumulation of Cd, Pb and As in brown rice
- Titanium gypsum addition enhanced the abundance of sulfate-reducing bacteria and metal-resistant bacteria in soil
- Titanium gypsum is a potential and effective amendment to immobilize metals in soil

Author Statement

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.