Soil vanadium(V)-reducing related bacteria drive community response to vanadium pollution from a smelting plant over multiple gradients

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1. Introduction

Vanadium is the 20th most abundant element in the Earth’s crust, and is beneficial or essential for living organisms due to its unique roles in biological structures and functions (Crans et al., 2004; Zhang et al., 2015a). In modern industry, vanadium is mainly applied to steel to ameliorate the strength and corrosion resistance, and it is also employed in many other industries including pigments, catalysts, and pharmaceuticals (Liu et al., 2017; Schlesinger et al., 2017). Anthropogenic activities, such as the mining and smelting of navajoite, widely using of vanadium products, and fossil fuel combustion, have resulted in substantial vanadium entering into regional geological environments (Imtiaz et al., 2015; Xiao et al., 2017). Severe vanadium contamination in soils has been reported by many researchers (Cao et al., 2017; Imtiaz et al., 2015; Larsson et al., 2015). Excessive concentration of vanadium is harmful to both plants and animals and sometimes it even threatens human health (Hao et al., 2015; Mandiwana and Panichev, 2010; Rinklebe et al., 2019). Vanadium has adversely effects on phosphate metabolism (Zhang et al., 2012, 2014). High vanadium ingestion can result in serious diseases, including renal damage and potentially pulmonary tumors (Heinemann et al., 2000; Chen and Liu, 2017). The seedling leaves of soybean would become yellow and withered and there are significantly decreased yields of shoot and roots when the soil vanadium content is high (Wang and Liu, 1999). The background value of different metals in China was 61 mg kg$^{-1}$, 0.38 g kg$^{-1}$, 3.0 g kg$^{-1}$, 6.6 g kg$^{-1}$, 23 mg kg$^{-1}$, 74 mg kg$^{-1}$ and 82 mg kg$^{-1}$ for Cr, Ti, Fe, Al, Cu, Zn and V respectively (Wei et al., 1991). The concentration of vanadium in Chinese environmental quality standard for agriculture soil is 130 mg kg$^{-1}$ (GB15618-2009) (CMEP, 2009).

Microorganisms play an important role in Earth’s biogeochemical cycle, which can not only regulate ecosystem processes but also influence biogeochemical dynamics in geological environments (Xu et al., 2017; Das et al., 2019). Microbes are sensitive to environmental pollution and especially heavy metal pollution, and numerous studies have
demonstrated that heavy metal pollution may cause drastic changes in microbial community composition and activity, resulting in a decrease of microbial diversity and enrichment of tolerant species via the process of environmental filtering (Ma et al., 2019; Turpeinen et al., 2004; Wang et al., 2018; Wu et al., 2019). On the other hand, microbes may be actively involved in the immobilization, degradation and transformation of the toxic metals (Fierros-Romero et al., 2017; Gong et al., 2016; Luo et al., 2014), and play a crucial role in the in situ remediation of heavy metal pollution (Hansen et al., 2017). For example, the highly toxic vanadium(V) (V(V)) could be reduced to less toxic vanadium(IV) (V(IV)) by many bacterial species in vanadium polluted environment (hereafter referred to as V(V)-reducing related bacteria, VRB) (Hao et al., 2015; Qiu et al., 2017; Rivas-Castillo et al., 2017; Yelton et al., 2015; Zhang et al., 2015a). Similar as sulfate-reducing bacteria, which are microorganisms with the function of reducing sulfate to sulfide, VRB is a kind of community, which possess a common vanadium reduction function and might play a vital role in vanadium reducing process (Schoeffler et al., 2019).

In the last several years, the release of vanadium and the resulting pollution to geological environments have attracted increasing attention (Gardner et al., 2017; Wright et al., 2014; Yang et al., 2017). A considerable amount of research has been conducted to investigate the environmental pollution of vanadium, including its concentration distribution, speciation analysis, mobility, bioaccumulation as well as microbial response (Cao et al., 2017; Teng et al., 2011; Xiao et al., 2015). Our previous study observed a significant influence of vanadium in shaping microbial community composition, structure and diversity in surface soil of Panzhihua mining and smelting area, China (Cao et al., 2017). However, the core microbiome responsible for mediating community response to vanadium pollution have not been identified and potential core role of VRB assemblage in the shift of community under vanadium oxidation stress has not been well explained. Moreover, previous studies on the modification of community by vanadium are mostly qualitative descriptions, while constructing the relatively quantitative relation between vanadium stress and core microbiome response is more meaningful for predicting the effect of vanadium pollution on microbial community and assessing the impact on the ecosystem.

In the present study, a comprehensive field survey was conducted to explore the profile of microbial community and VRB assemblage in soils around a vanadium smelting plant over horizontal and vertical gradients by Illumina high-throughput sequencing of 16S rRNA genes. Combining with the vanadium concentration, the influences of vanadium pollution on the microbial community and VRB assemblage were quantitatively investigated. Furthermore, a laboratory incubation experiment was conducted to simulate the community succession and validate the responses of community and VRB assemblage to vanadium observed in the field survey. The objectives of this study are to quantitatively establish the link between VRB assemblage and vanadium oxidation stress and elucidate the core role of VRB to drive the community response to vanadium pollution.

2. Materials and methods

2.1. Site description and sample collection

The studied vanadium smelting plant is located in Panzhihua City (South Sichuan Province, China), which is the most famous base of vanadium-bearing titanomagnetite in China. Extensive smelting activities have resulted in severe vanadium contamination in around soils (Cao et al., 2017). In April 2017, representative surface and profile soil samples over multiple gradients were collected in different directions and various distances from the smelting plant (Fig. 1). A total of 24 surface soil samples were collected in triplicate at a depth of 0–10 cm from the east direction (East Group) at a distance of 10 m, 50 m, 200 m, 500 m, 800 m and 1000 m (E1, E2, E3, E4, E5 and E6), south direction (South Group) at a distance of 10 m, 50 m, 500 m, 800 m, 1000 m and 1800 m (S1, S2, S3, S4, S5 and S6), west direction (West Group) at a distance of 10 m, 50 m, 200 m and 800 m (W1, W2, W3 and W4), north direction (North Group) at a distance of 10 m, 100 m, 200 m, 500 m, 800 m, 1000 m, 1500 m and 2000 m (N1, N2, N3, N4, N5, N6, N7 and N8). Profile samples were collected from five sites: one site was in the south direction at a distance of 800 m (VS), one site was in the east at a distance of 700 m (VE), and the other three sites were in the north at a distance of 200 m (VN1), 1000 m (VN2) and 2000 m (VN3), respectively. Profile samples at VS (VS1-VS6), VN1 (VN1_1-VN1_6), VN2 (VN2_1-VN2_6) and VN3 (VN3_1-VN3_6) were collected at vertical depths of 0–10, 20–30, 40–50, 60–70, 80–90 and 100–110 cm, and at VE site, samples were collected at depths of 0–10, 20–30, 40–50, 60–70, 80–90, 100–110, 120–130, 140–150 and 160–170 cm (VE1-VE9). All collected soil samples were divided into two parts depending on their usages. One part for physicochemical analyses and laboratory incubation experiment was kept in polyethylene bags and stored at 4 °C. The other part for microbial analysis was frozen in liquid nitrogen in the sampling site and stored in −80 °C freezer in the laboratory until use.

2.2. Physicochemical analyses

Soil samples were air-dried at room temperature and sieved through 2 mm mesh for subsequent physicochemical analyses. pH was determined by a HI 3221 pH meter (Hanna Instruments Inc., USA). Oxidation-reduction potential (Eh) was analyzed using an Eh meter with a platinum electrode installed at 15 cm depth in each site (Honma et al., 2016). Total nitrogen (TN), organic matter (OM), and available phosphorus (AP) were measured based on reported methods (Cao et al., 2017). Total concentrations of vanadium and other metals in soils were measured by inductively coupled plasma-optical emission spectrometer (Prodigy XP, Leeman Labs, Inc., USA) after microwave digestion (MARS HF (1:1:2, v/v/v) (Liu et al., 2015). Precision and accuracy of heavy metal measurement were verified using standard reference materials from the National Research Center for Geoanalysis of China. Accepted recoveries ranged from 90% to 110%. Each sample was analyzed in triplicate. The relative deviation of the triplicate samples was < 10% in all batch measurements.

2.3. Incubation experiment

One low vanadium contaminated soil sample (E6) was selected to conduct the incubation experiment. Serum bottles of 50 mL equipped with rubber plugs were used for cultivation. Each bottle was filled with 2 g soils and the residual space was filled with simulated groundwater, which contained the following components (per liter): CaCl2 (0.2464 g); MgCl2·6H2O (1.0572 g); NH4Cl (0.1557 g); KH2PO4 (0.0299 g); KCl (0.0283 g); NaCl (0.4459 g) and NaHCO3 (0.8082 g) (Cao et al., 2017). At the start of incubation, two gradients of vanadate (1 mg L−1 and 10 mg L−1 as V(V)) as electron acceptor with corresponding carbon source of glucose (10 mg L−1 and 100 mg L−1 as COD) as electron donor were added to soils. During the incubation, the bottles were re-filled with new simulated groundwater every three days, and this microbeal cultivation lasted for three months to achieve a stable evolution. All experiments were carried out in duplicate and at room temperature (22 ± 2 °C). Incubated soil samples were collected for microbial community analysis when incubating for 30 d, 60 d and 90 d, respectively.

2.4. Microbial community analysis

All triplicate mixed soil samples and incubated soil samples were applied to microbial community analysis by Illumina MiSeq high-throughput sequencing of 16S rRNA gene technique. The genomic DNA in 0.5 g soil sample was extracted using a FastDNA Spin Kit for Soil
(Qiagen, CA, USA) according to the manufacturer's protocol. Bacterial 16S rRNA genes were amplified with PCR primers 338F (ACTCCTACGGGAGGCTACGAG) and 806R (GGACTACHVGGGTWTCTAAT) targeting the V4-V5 hyper variable regions (Liu et al., 2017). Each PCR reaction was performed in 20 μL reaction mixture, which consisted of 4 μL of 5 × FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase, and 10 ng of template DNA, using the following thermal cycling conditions: an initial denaturation at 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 57 °C for 30 s and 72 °C for 45 s, and a final extension at 72 °C for 10 min. The 16S rRNA gene amplicon sequencing was conducted on the Illumina MiSeq platform by Majorbio (Shanghai, China). Sequencing data including all field and incubated samples have been submitted to the NCBI Sequence Read Archive (accession No. SRP188735, SRP224622).

The obtained sequence data were processed and analyzed using FLASH and Trimmomatic (Sun et al., 2017; Wang et al., 2016). The low quality and chimeric sequences were filtered to get high quality sequences, which were then clustered into operational taxonomic units (OTUs) by UPARSE (version 7.0 http://drive5.com/uparse/) at the 97% similarity level. Taxonomic classification of the OTUs was determined using RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115) 16S rRNA database using a minimum confidence of 70% (Amato et al., 2013). The rarefaction curve was constructed by random sampling for all the sequences (Zhang et al., 2019a). Relative abundance (%) of individual taxa within each community was estimated by comparing the number of sequences assigned to a specific taxon versus the number of total sequences obtained for that sample. Alpha diversity analysis (i.e., Chao1/Ace richness estimator, and Simpson/Shannon diversity index) was conducted using the Mothur software (Zhang et al., 2020).

2.5. Data processing and statistical analyses

The potential ecological risk index (EI) was introduce to assess the degree of vanadium pollution in soils, which can be calculated using the following equation (Hakanson, 1980):

\[
EI = \frac{TRF \times C_{\text{obs}}}{C_0}
\]

where TRF (=2) is a given toxic factor response to vanadium (Pan et al., 2016), \(C_{\text{obs}}\) (mg kg\(^{-1}\)) is the measured concentration of vanadium in the soil samples and \(C_0\) (mg kg\(^{-1}\)) is the corresponding background value of China (CNEMC, 1990). This ecological risk assessment method has been widely used in heavy metal polluted soil (Pan et al., 2016; Yang et al., 2017; Sun et al., 2010). Based on obtained EI value, the ecological risk of vanadium can be categorized as low (< 40), moderate (40–80), considerable (80–160), high (160–320) and very high (> 320) levels (Hakanson, 1980; Pan et al., 2016).

Multivariate statistical methods were used to account for the collinearity between VRB assemblage and vanadium, other possible physicochemical properties or gradient factor. Redundancy analysis (RDA) was applied using the R software package (vegan 2.4.4) to present the relations between VRB assemblage and the significant variables and explain the effect of vanadium gradient on the VRB assemblage and the related bacteria. To prevent disproportionate effects of highly abundant taxa, all the species abundance values were standardized using correlation coefficients, and the inertial correlations were obtained. The hierarchical cluster analysis based on the relative abundance of top 30 abundant genera was conducted using average linkage between groups and Pearson correlation as measure interval by using Origin 9.0. Network analysis was carried out to illustrate the significant
correlations between core microbiome and soil microbial community, which was performed using software Cytoscape with input correlations calculated in the software local similarity analysis (LSA) using default settings (Sun et al., 2013). All the correlations between any two genera in this microbiome were calculated and then we chose the genera to structure network based on their significant relations to VRB (|r| > 0.8).

3. Results and discussion

3.1. Physiochemical properties and vanadium contents

The physiochemical properties of soil samples over horizontal and vertical gradients are summarized in Table S1. Overall, the surface soils in this area were alkaline with an average pH value of 8.18 ± 0.32, which is consistent with the previous observation by Cao et al. (2017). The average content of OM in the surface soils was 22.18 ± 0.53 g kg⁻¹, which is lower than the average value for soils in China (43.75 g kg⁻¹) (Wu and Cai, 2006). For the profile samples, there was a decreasing tendency of OM along the soil depth, which was also reported by Liang et al. (2019). The average contents of TN and AP in the surface soils were 0.66 ± 0.08 g kg⁻¹ and 12.01 ± 0.54 g kg⁻¹, respectively. The average contents of Cr (510.81 ± 0.21 mg kg⁻¹), Ti (15.05 ± 0.57 g kg⁻¹), Fe (26.71 ± 1.58 g kg⁻¹), Al (228.36 ± 0.61 mg kg⁻¹) and Zn (63.58 ± 0.11 mg kg⁻¹) in the surface soils were 3–40 times higher than the background values of China soils (Wei et al., 1991), suggesting a severe heavy metal contamination. An evident decreasing trend of concentrations of these heavy metals along the distance to vanadium smelter and soil depth was also observed.

Contents of vanadium as well as the ecological risk levels in soil samples are shown in Fig. 2. The average vanadium concentration in all collected surface soil samples was 1616.91 ± 12.35 mg kg⁻¹, which was much higher than the background level of vanadium in China soils (82 mg kg⁻¹) (Chen et al., 1991). This indicated that the soils around this area had been severely contaminated by vanadium. Remarkably, a decreasing tendency of vanadium with the distance away from the smelting plant had been observed (Fig. 2), which demonstrated the vanadium pollution in soils was mostly resulted from the pathway of atmospheric deposition of vanadium released from the vanadium smelter. Moreover, the vanadium concentrations in the soil profiles decreased over vertical gradient (Fig. 2). Vanadium concentration changes in different orientation were small, among which the soils in South Group were polluted more heavily than other directions relatively. There was more vanadium related industry in south direction comparing the mountain in north group, so soils in the south were contaminated more seriously. Even when soil depth reached to 170 cm, the vanadium concentration (109.65 ± 3.58 mg kg⁻¹) still exceeded the background value of soil vanadium in Sichuan Province (96 mg kg⁻¹) (CNEMC, 1994), indicating the vertical migration of vanadium contamination to the deeper soil. The ecological risk index indicated the vanadium in the soils near the smelting plant would induce considerable or moderate ecological risk.

3.2. Microbial community composition and diversity

The high-throughput sequencing generated total 763,340 quality sequences across 33 soil samples including all the surface samples and the VE profile samples. A total of 7569 OTUs were identified, with a range of 1178 to 3341 for individual samples. The number of OTUs, Chao1/Ace richness estimator and Simpson/Shannon diversity index are shown in Table S2. Rarefaction curves of microbial communities in...
different groups of soils are shown in Fig. 3. High microbial diversity and richness was found in the samples with a moderate vanadium level (e.g., E2, S2, W3 and N2), whereas a low microbial diversity was found in the samples with either very high vanadium content or low vanadium content. It seems that appropriate vanadium level was beneficial for enhancement on the microbial diversity and richness, but excessive vanadium level would definitely pose an adverse impact.

The identified OTUs of bacterial microbiome across all soil samples can be assigned to 24 phyla, 56 classes, 117 orders, 230 families, and 421 genera. The dominant phyla were Actinobacteria (16.27–50.57%), Proteobacteria (4.29–41.85%), Chloroflexi (7.9–33.38%), Acidobacteria (0.39–23.43%) and Firmicutes (0.19–49.76%) (Fig. S2). Cao et al. (2017) has reported that Actinobacteria, Proteobacteria, Acidobacteria and Firmicutes were widely found in the soils around a vanadium smelting plant. In addition, Chloroflexi was also detected in a microbial fuel cell with V(V) as the electron acceptor (Zhang et al., 2015a). Remarkably, the richness of individual phylum changed a lot along the distance to the smelting plant and distinctly, the richness of individual phylum changed a lot along the vanadium smelting plant. In addition, Chloroflexi was also detected in a microbial fuel cell with V(V) as the electron acceptor (Zhang et al., 2015a). Remarkably, the richness of individual phylum changed a lot along the distance to the smelting plant and different directions, which may be affected by the disparity of vanadium concentration or other physico-chemical properties. With the increase of depth, Actinobacteria was observed to significantly increase in the profile soils, while Firmicutes showed a declining trend.

The dominant genera in all soil samples were Bacillus (6.61 ± 9.82%), Unclassified-c-Acidobacteria (5.58 ± 3.32%), Unclassified-f-Anaerolineaceae (3.73 ± 2.30%), Unclassified-o-Gaiellales (3.68 ± 4.65%) and Unclassified-o-JG30-KF-CM45 (3.41 ± 1.61%) (Fig. 3). The microbial compositions of surface soils were different from those of profile soils. Hierarchical cluster analysis based on the relative abundances of the top 30 genera showed that 33 genera could be organized into two groups: the first group contained surface soils and the other contained profile samples (Fig. 3). The dominant genera in surface soils included Unclassified-c-Acidobacteria (2.64–12.90%), Unclassified-f-Anaerolineaceae (0.58–10.03%), Unclassified-o-JG30-KF-CM45 (0.61–7.32%), Sphingomonas (0.65–7.23%) and RB41 (0.19–8.14%), while the dominant genera in profile soils included Bacillus (4.55–37.13%), Unclassified-o-Gaiellales (1.39–16.67%), Gaiella (1.26–7.21%), Unclassified-c-Actinobacteria (0.08–8.03%) and Unclassified-c-KD4-96 (1.14–5.88%).

3.3. VRB assemblage and influence of vanadium pollution

Many bacteria, some archaea and fungi have been reported to be able to reduce vanadium (Table S3). In the present study, five genera from phyla of Proteobacteria and Firmicutes, which had been reported having the ability to reduce vanadium and possessing the potential vanadium reduction activity in this local environment, were detected in the collected soil samples, including Bacillus, Clostridium, Comamonadaceae, Geobacter, and Pseudomonas. There must be more unidentified bacteria having the similar vanadium reducing functions and features belong to VRB, which have relationship to vanadium reduction process. However, due to limitation of microbial culture, their vanadium reducing functions have not been explored yet. In this study, in order to explore the distribution VRB assemblage and its interaction with the environment, the five detected known VRB genera were considered as the surrogate of VRB assemblage. When VRB was discussed as assemblage, VRB abundance was calculated by summing the relative abundance of these five genera. This kind of assemblage abundance has also been used in analyzing sulfate-reducing bacteria (Jia et al., 2018). The distribution of these VRB genera in different spatial gradients are shown in Fig. 4. Notably, Bacillus occupied the overwhelming majority of the VRB assemblage, which suggested this genus might play a critical role in V(V)-reducing process. Rivas-Castillo et al. (2017) have also reported that some strains of Bacillus megaterium present not only high vanadium resistance, but also excellent removal capability of

![Hierarchical cluster analysis of microbial communities in all soil samples based on the top 30 abundant genera.](Image 50x75 to 546x389)

Fig. 3. Hierarchical cluster analysis of microbial communities in all soil samples based on the top 30 abundant genera. The relative abundance of genera was indicated by color intensity.
vanadium. Considering the severe vanadium contamination in local area, vanadium is undoubtedly a main factor influencing the soil microbial community. Besides, the Monte Carlo permutation test ($n = 499$) and correlation analysis (Table S4) identified Eh, TN, AP, Al and Cu as the important explanatory soil properties for VRB assembly. To explore the relations between VRB assemblage and environmental factors, RDA was conducted by taking vanadium as the main factor and Eh, TN, AP, Al and Cu as the co-factors. The ordination diagram of RDA illustrated genus *Bacillus* had extremely similar distribution with total VRB

Fig. 4. Relative abundance of VRB assemblage (*Bacillus*, *Geobacter*, *Clostridium*, *Pseudomonas* and *Comamonadaceae*) across all analyzed soils: (a) East surface soil, (b) south surface soil, (c) west surface soil, (d) north surface soils, (e) profile soil and (f) cultivated soil.
Fig. 5. Redundancy analysis of the effect of environmental factors on VRB assemblage. The red arrow is the main factor, the grey arrows are the co-factors, and the blue arrows are VRB. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Fig. 5), which was resulted from the dominating of Bacillus in VRB assemblage. The distribution of Clostridium also resembled with that of Bacillus or total VRB, while the arrows of Comamonadaceae and Pseudomonas lay on the opposite direction of Bacillus and Clostridium. It implied that the different bacteria of VRB would compete with each other for the limited living space and resources to support growth (Xu et al., 2017). For the relation between VRB and vanadium concentration, a negative and weak correlation was observed ($r = -0.307$, $p = 0.082$, $n = 33$), indicating there was no linear relation between vanadium and VRB. Furthermore, it was found the sample distribution presented a unimodal pattern along with the vanadium (Fig. 5), which might be described by the Gaussian distribution model (discussed below). Other co-factors generally exhibited linear relations to VRB (Fig. 5). Eh, TN, AP and Al showed significantly positive correlations ($r = 0.445–0.623$, $p < 0.01$, $n = 33$), while Cu had a significantly negative correlation ($r = -0.541$, $p < 0.01$, $n = 33$), resulting in relatively longer lengths of their arrows.

As discussed above, vanadium was a main factor influencing the soil microbial community, and the relation between vanadium and VRB was nonlinear. To empirically and mathematically model the relation, we constructed the function of VRB abundance versus log concentration of vanadium, and found the relation could be well described by Gaussian equation ($R^2 = 0.653$, $p < 0.001$) as follows (Fig. 6):

$$y = 3.43 + 43.41e^{-61.73(x-2.61)^2}$$

(2)

Here, $x$ (mg kg$^{-1}$) is the vanadium concentration in soils and $y$ (%) is abundance of VRB in the microbial community. The abundance of VRB increased with the vanadium content when the vanadium concentration was at a low level. However, when the vanadium was aggrandized constantly, the richness of VRB significantly decreased. Thus, our results indicated that low level of vanadium could promote the growth of VRB and the high content of vanadium would inhibit the multiplication of VRB.

3.4. Role of VRB assemblage to drive the community shift

According to ecological theory, the microbial community is shaped by deterministic factors such as species competition and niche differentiation (e.g., environmental filtering) (Wu et al., 2019). The present study has demonstrated vanadium pollution was a critical factor influencing the soil microbial community, and the VRB was the responsive microbial assemblage. To evaluate the contribution of VRB change on the shift of soil microbial communities, we forward selected regression model to analyze the relation between the relative abundance of VRB assemblage and the richness and diversity of the whole community. Ace/Chao1 richness estimator had slightly negatively effective relation with VRB (Fig. S3a and b) (Ace: $r = -0.458$, $p < 0.05$; Chao1: $r = -0.457$, $p < 0.05$), which meant the richness of microbial community would decrease with the increase of VRB abundance. Noticeably, Shannon/Simpson diversity index significantly correlated with VRB abundance (Fig. S3c and d) (Ace: $r = -0.772$, $p < 0.05$; Chao1: $r = 0.915$, $p < 0.05$), indicating the enhancement of VRB assemblage would remarkably impact the diversity and evenness of the whole microbial community.

To furtherly explore how VRB drove the community shift, a network analysis was conducted (Fig. 7). All the correlations between any two genera in this microbiome were calculated and then we chose the genera to structure network based on their significant relations to VRB ($|r| > 0.8$) (Banerjee et al., 2016). This network consisted of 49 genera (first level interaction) that had direct relationship with VRB and 29 genera (second level interaction) that were closely associated with the first level interaction genera and could be influenced indirectly by VRB. In the first level, many genera were identified to be metal-resistant or metal-reducing genera, such as Acidobacterium (Stroud et al., 2014), Alcanivorax (Baltar et al., 2018), Rhodotherax (Ma et al., 2015), Thauera (Zhang et al., 2019b), Rhodanobacter (Hemme et al., 2016), Fictibacillus (Zheng et al., 2017), Janibacter (Vetrovsky and Baldrian, 2015), Leucobacter (Sturm et al., 2018), Dyella (McGee et al., 2018), and Granulicella (Falagan et al., 2017). These genera positively correlated to VRB, revealing the existence of co-occurring patterns between VRB and the first level interaction genera. In the second level, the genera could connect with VRB indirectly through the first level interaction genera. Totally, the bacteria of the first and second levels as well as VRB accounted for 1.32–52.77% of the whole community. Therefore, VRB might drive the change of such a large group of bacteria under the vanadium pressure, resulting in shift at the community level. Moreover, the network analysis was only based on the five genera of VRB surrogate, more unidentified VRB were likely to exist in community, and the influence coverage of VRB assemblage might be even larger.

3.5. Validation from the laboratory incubation test

The laboratory incubation test indicated the exposure of vanadium could significantly change the soil microbial community. Principal coordinates analysis (PCoA) on the community structures of the original and cultivated soil samples shown that bacteria converged to similar structure at lower vanadium loading, while the structure of microbial
community held distinct alteration during the cultivation process at a higher vanadium level (Fig. S4). This implied the soil microorganisms could adapt to the environment containing low level of vanadium and evolve a stable structure; however, the high concentration of vanadium would dramatically impact the balance of microbial community, which was difficult to restore stability. The rarefaction curves showed the diversity of soil microbial communities was significantly reduced after exposing vanadate and in a dose-dependent pattern (Fig. S5), which was also reflected by the alpha diversity estimator/index (Table S5). Higher vanadate concentration (10 mg L$^{-1}$) induced a greater impact on the community diversity. This is consistent with the observation of field survey (Figs. 2 and S1; Table S2).

Furthermore, the evolutions of various phylum and VRB were investigated during the incubation process (Fig. S2f, Fig. 4f). The dominant phylum Acidobacteria decreased from 10.49% in E6 to 3.78% in E6_3M1 and to 2.83% in E6_3M10. Firmicutes had an increase tendency in the process of incubation (Fig. S2f). Like the observation of field survey, Bacillus spp. was still predominant in VRB during the whole cultivation process. Abundance of total VRB increased consecutively from 5.88% (E6) to 2.45% (E6_1M10) after cultivating for 30 days, which is probably due to the strong toxicity of high level of vanadium to microbes (Zhang et al., 2015b) and the new incubation environment. But as the cultivation proceeded, VRB seemed to accommodate the high vanadium content and incubation environment, and the total abundance dramatically increased to 47.20% (E6_3M10) 90 days later. The species of VRB had the intrinsic capacity to remove vanadium by reducing the highly toxic V(V) to less toxic V(IV) and possessed a wide range of resistance mechanisms to metals, which could counteract the toxic effect of vanadium under the oxidative stress (Ding et al., 2014; Rivas-Castillo et al., 2017). Moreover, in some cases, vanadium could be involved in nitrogen fixation as a growth factor and promote the growth of VRB, e.g., Bacillus (Hao et al., 2018). Vanadium(V) reduction is also an energy-conserving process, which could result in proton translocation and promote the growth of microorganisms in vanadium reduction process (Carpentier et al., 2005). In addition, we calculated the bacteria closely related to VRB ($|r| > 0.8$), and found the abundance of VRB and the associated bacteria increased from 10.26% in E6 to 15.13% in E6_3M1 and 47.66% in E6_3M10 during the cultivation process, suggesting a predominant drive of community change caused by VRB under vanadium pressure. In general, the laboratory incubation experiment validated the conclusion of field survey that the abundance of VRB increased at low vanadium concentration but decreased at high vanadium level; VRB played the core role to drive community response to vanadium pressure.
4. Implications and limitations

This study showed that the smelting plant caused heavy vanadium pollution in local area, which posed a serious impact on soil microorganisms over horizontal and vertical gradients. Combing field survey and laboratory incubation experiment, we found the VRB assemblage played the core role to drive community response to vanadium pollution. More importantly, the link between VRB and vanadium level was quantitatively established with a nonlinear Gaussian equation. To our best knowledge, the core role of VRB assemblage to drive community response and quantitative link between VRB and vanadium level have been reported for the first time. These findings should have major implications in the ecological risk assessment of vanadium pollution, especially for the regional geographical environments with active activities of navajoite mining and smelting. However, the present study used five genera of VRB as the surrogate by 16s RNA gene sequencing, which is relatively cursory. Future studies should further identify the VRB assemblage to species level with “omics” technologies such as metageneomics; the vanadium reducing activity could be confirmed by metatranscriptomics. What’s more, bacteria isolation experiment could be conducted from vanadium contaminated soil to show their reducing capabilities and to verify how many bacteria belong to VRB. Moreover, community functional response to vanadium pollution should also be explored, which will lead to the understanding of the ecological effects from the basis of microbial metabolism.

CRediT authorship contribution statement

Song Wang: Investigation, Validation, Writing - original draft. Baogang Zhang: Conceptualization, Project administration, Supervision. Tingting Li: Methodology, Software. Zongyan Li: Formal analysis, Resources. Jie Fu: Conceptualization, Writing - review & editing, Supervision.

Declaration of Competing Interest

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105630.

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