Temperature response of sulfide/ferrous oxidation and microbial community in anoxic sediments treated with calcium nitrate addition

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Abstract: Nitrate-driven sulfide oxidation has been proved a cost-effective way to control sediments odor which has long been a universal problem for urban rivers in south China areas. In this work, sediments treatment experiments under a dynamic variation of temperature from 5°C to 35°C with 3% of calcium nitrate added were conducted to reveal the influence of temperature variation on this process. The results showed that microbial community was remarkably restructured by temperature variation. *Pseudomonas* (15.56-29.31%), *Sulfurimonas* (26.81%) and *Thiobacillus* (37.99%) were dominant genus at temperature of $\leq 15^{\circ}\text{C}$, 25°C and 35°C respectively. It seemed that species enrichment occurring at different temperature gradient resulted in the distinct variation of microbial community structure and diversity. Moreover, nitrate-driven sulfide and ferrous oxidation were proportionally promoted only when temperature increased above 15°C . The dominant bacteria at high temperature stage were those genus that closely related to autotrophic nitrate-driven sulfide and ferrous oxidizing bacteria (e.g. *Thiobacillus*, *Sulfurimonas* and *Thermomonas*), revealing that promotion of sulfide/ferrous oxidation could be attributed to the change of dominant bacteria determined by temperature variation. Thus, a higher treatment efficiency by calcium nitrate addition for odor control would be achieved in summer than any other seasons in south China areas.

Keywords: Sediments, odor control, nitrate-driven sulfide/ferrous oxidation, temperature response, microbial community

1. Introduction

Calcium nitrate addition has been of great concern as a cost-effective approach for in situ sediments treatment since it was first introduced by Ripl in 1976 (Ripl, 1976). Numerous

researches carried out so far have focused on sediments odor control (Babin et al, 1999), organic matter degradation by denitrification (Murphy et al., 1995), phosphorus release control (Foy, 1986; Yamada et al., 2012; Lin et al., 2015), transfer and transformation of inorganic nitrogen (Liu et al., 2015), sulfide-driven nitrate reduction (Zhang et al., 2009; Shao et al., 2011; Yang et al., 2012; Hayakawa et al., 2013; Zhang et al., 2015; Dolejs et al., 2015), microbial community structure variation (King and Nedwell, 1984; Chen et al., 2013), risk assessment (Shao et al., 2009 Wang, 2016), nitrogen cycle associated functional gene analysis (Xu et al., 2014), nitrate-dependent iron sulfide and ferrous oxidation (Straub et al., 1996; Juncher Jørgensen, 2009; Vaclavkova, 2014; Robertson, 2016) and so on. In recent years, this technology attracted an unprecedented attention in China because of the poor quality of urban surface water environment (Chen, 2013; Xu, 2014, Zhang, 2015; Wang, 2016), and the local government regards the solution to the sediments black color and odor problem, a long-existing and universal pollution of urban rivers in south China areas, as its priority.

It is generally accepted that the reduction of ferric iron and sulfate in anaerobic sediments lead to the accumulation of acidified volatile sulfide (AVS) including H_2S and iron sulfide (FeS), which results in the sediment's black color and odor problem (Babin et al,1999). For odor control, nitrate can serve as electron acceptor and stimulate the oxidation of AVS by autotrophic denitrifier (Murphy, 1995; Babin et al, 2003; Zhang, 2009). Those bacteria participating in this process were known as nitrate reduction sulfur oxidizing bacteria (NR-SOB). *Thiobacillus denitrificans* and *Sulfurimonas denitrificans* were the two most commonly reported autotrophic NR-SOB (Shao, 2010). These bacteria could utilize S^{2-} , HS^- , S_2O_3 and/or S^0 as electron acceptor and ultimately produced sulfate (Shao, 2010). Besides sulfide, ferrous iron oxidation was usually observed in case of sediment treatment by calcium nitrate addition (Yamada et al., 2012). Iron sulfide oxidation was considered to be connected with the change of sediments color probably (Rickard, 2005). Oxidation of ferrous iron in the presence of nitrate has been recognized since the early 1990s (Straub et al., 1996). Jørgensen (2009) and Vaclavkova (2014) even confirmed the occurrence of microbial FeS_2 and FeS oxidation accompanied with nitrate reduction after adding nitrate in sediments, with sulfate and ferric hydroxide as end product. Many nitrate-dependent Fe(II) oxidizing bacteria had been identified, among which *Thiobacillus denitrificans* was first discovered capable of

oxidizing sulfide and ferrous (and pyrite) simultaneously (Straub et al., 1996; Jørgensen, 2009). Other understandings about NR-SOB and Fe(II) oxidizing bacteria have been summarized by Shao (2010) and Hedrich (2011). With the development of high throughput sequencing technology, many experiments on microbial community structure analysis have been carried out and more nitrate-reduction-related bacteria were reported (Bai et al., 2012; Chen et al., 2013). Relevant studies revealed that calcium nitrate injection could change the microbial community structure from sulfate reduction bacteria (SRB) to NRB including heterotrophic NRB and NR-SOB (Hubert, 2007), in which Proteobacteria was always reported to be the one that increased remarkably when AVS was removed (Chen, 2013). This was identical with the reports that most of NR-SOB and ferrous oxidizing bacteria belong to Proteobacteria (Shao, 2010; Hedrich, 2011). It showed a high correlation between AVS oxidation and a shift of microbial community structure due to the nitrate reduction (Chen, 2013).

Though effects of nitrate on sulfide oxidation and microbial community structure have been fully studied, the effect of another important factor, temperature (seasonal or elevational), on sulfide/ferrous removal and microbial diversity was not yet clear. Researches on the arctic-alpine ecosystem (Liu et al., 2013; Lazzaro et al., 2015) or high-temperature ecosystems such as hot springs (Roeselers et al., 2007; Wang et al., 2014) had confirmed that community diversity and seasonal temperature have a significant correlation. Elevational variation also exerted great effect on the change of microbial community structure and diversity (Sakami, 2008). King and Nedwell (1984) and Ogilvie et al., (1997) had reported an obviously influence of temperature on the variation of heterotrophic bacteria during the nitrate reduction process, yet the process of autotrophic denitrification was not mentioned at that time due to the limitation of microbial community analysis method. Our previous experiment revealed that when the room temperature ranged from 10°C to 21°C, sulfide removal rate kept at a low level until temperature raised above 15°C (Wang, 2015). In cities of southern China, Etc. Guangzhou city, as shown in Fig. S1 (Temperature change in 2015 in Guangzhou City), the average annual temperature was about 21°C, but temperatures in winter were below 15°C. The lowest temperature in some years could even drop to 0°C. Obviously, the effect of seasonal change on the sediments treatment by calcium nitrate injection can not

be ignored in this area. Moreover, as we all know, temperature will affect the growth and activity of sulfur-oxidizing bacteria. For instance, it was reported that the optimum growth temperature of *Sulfurimonas denitrificans* was 22°C, while optimum growth temperature of *Thiobacillus denitrificans* ranged from 28°C to 30°C (Shao,2010). Thus it makes sense to infer that dominant bacteria in treated sediments may be different in each temperature grade, which would further influence the rate of sulfide oxidation. Therefore, the relationship among sulfide/ferrous oxidation, temperature variation and microbial community structure deserves further research to determine a proper season for the application of calcium nitrate addition, which was also the main purpose of this work.

2. Materials and Methods

2.1. Sediments

Surface sediments (0-30cm) were sampled from the tributary inlet (113.4°E, 23.1°N) of the Chebei River to the Pearl River. Prior to experiments, sediment samples were homogenized, sieved to remove the detritus and stored in a plastic tank covered with river water collected from the same location. At the beginning of the experiment, sediments were stored in a series of 500mL serum bottles. All bottles were sealed with rubber cap and stored in an incubator with a constant temperature of 5°C for 24h, and the physic-chemical characteristics of the sediment sample was measured as the control value of initial sediments, which was displayed in Table 1.

Table 1 Physic-chemical characteristics of initial sediments

Parameters	Mean±SD
Sediments	
AVS (mg·kg ⁻¹)	3842.37±744.52
Ferrous(mg·kg ⁻¹)	9493.40±1290.8
Ferric(mg·kg ⁻¹)	590.56±245.66
OPR (mV)	- 156.00±34.5
pH	7.15±0.03
Water content(%)	58.60±1.4
Density(g·cm ⁻³)	0.24±0.02
Interstitial water	
Sulfate(mg·L ⁻¹)	123.86±22.8
Nitrate(mg·L ⁻¹)	1.79±0.25
Nitrite(mg·L ⁻¹)	0.37±0.01

2.2. Microcosm systems and experiments

Experiment was carried out in a series of 500mL serum bottles and set up in six parallel systems. In each bottle, 250 mL homogenized sediments (with mean moisture content of 55.5%) was added, mixed with 250 mL water. In each bottle, based on our preliminary experimental results, 3% (M/M, dry-weight sediments) of calcium nitrate, which could completely oxidized AVS in this sediment sample, was injected (using syringe) and completely mixed with initial sediments. Then the treated sediments was sampled immediately and the sediment properties were detected, total DNA was extracted for microbial community structure analysis as well. All these results were described as index of "5 °C Beginning" in this paper. Next, five temperature gradient (5 °C, 10 °C, 15 °C, 25 °C and 35 °C) was set up with a time period of 3 days, within which the oxidation efficiency of AVS would be well reflected at various temperature stage according to our previous work(Wang, 2016). At the end of each temperature stage (the third day), treated sediments was sampled and sediment properties were measured and total DNA was extracted (shown as indexes of 5 °C End, 15 °C End, 25 °C End, 35 °C End in figures and tables). Simultaneously, another three 250 mL serum bottle (with 125 mL sediments, 125 mL water and 3% calcium nitrate) were used for the measurement of ORP with probes being fixed in the bottles. When sampling, 10 mL of sediment-water mixture was taken each time using a syringe, which pierced the rubber cap of serum bottle carefully to avoid the impact of oxygen.

2.3. Chemical analysis

Analyses of acid volatile sulfide (AVS) in sediments were performed according to the method described in our previous research (Wang, 2016). Fe^{2+} and Fe^{3+} analyses were conducted with the following procedure: about 2.5mL of sediment-water mixture was firstly extracted moderately from serum bottle, then 125 mL 0.75 mol/L HCL was added in, followed by 60 min extraction in 150 r/min oscillation, the leaching liquid was measured according to national standard methods for aqueous Fe^{2+} and Fe^{3+} detection (SEPA, 2002). Finally, the unit of AVS, Fe^{2+} and Fe^{3+} were converted to mg/kg(drw base) by calculating with the ratio of sediments water content. The water content of sediments were measured by weight loss of a known amount of wet sediments dried at 105°C to a constant value.

Due to the high solubility, NO_3^- -N, NO_2^- -N and SO_4^{2-} were sampled from sediment interstitial water and analyzed according to national standard methods for water and wastewater monitor (SEPA, 2002). The pH and oxidation reduction potential (ORP) were measured with meters.

2.4. DNA extraction and sequencing analysis

Seven sediments sample (including one initial sediment sample and six treated sediment samples under controlled temperatures ranged from 5°C to 35°C) were selected for molecular analysis. The genomic DNA was extracted using the Mobio PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc, Carlsbad, CA) following the manufacturer's protocol. All DNA extracts were stored at -80 °C until analyzed. The V3-V4 region of 16S rRNA gene was amplified using the 515F/806R primer set. The forward primer (515F) was GTGCCAGCMGCCGCGGTAA and the reverse permit (806R) was GGACTACHVGGGTWTCTAAT. Amplification conditions were as follows: an initial denaturation step (98°C for 3 min), which was followed by 30 cycles of denaturation at 98°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 45 s. The final extension was for 7 min at 72°C. 16S rRNA gene tag-encoded ultra-high-throughput sequencing was carried out using Illumina MiSeq platform at the Genomics Institute (Beijing, China). The reads were deposited into the NCBI short reads archive database (SRP074060).

2.5. Statistical analysis

Data analysis for chemical indexes was carried out using Excel 2007 and SPSS19.0 (SPSS Corporation, USA). The least significant different (LSD) for the comparison of means value was verified using Duncan's test at a significance level of $p=0.05$, while correlation was determined by Pearson's coefficients at $p=0.05$ level. Sigmaplot (10.0) was used for drawing. For DNA sequencing data analysis, after sequencing, the original data of DNA were processed to obtain the Clean Data and the high quality paired-end reads were combined to tags using the software FLASH (Fast Length Adjustment of Short reads, v1.2.11). In this work, 201968 tags were obtained in total with 28852 tags per sample on average, and the average length of each tags is 252 bp. Filtered tags are clustered into OTU (Operational Taxonomic Units) at 97% similarity by scripts of software USEARCH(v7.0.1090). Rarefaction curve tended to approach the saturation plateau (shown in Fig. S2). OTU

representative sequences were taxonomically classified using Ribosomal Database Project (RDP) Classifier v.2.2 trained on the Greengenes database, using 0.8 confidence values as cutoff if the length of tags is equal or greater than 250 bp, otherwise, 0.5 confidence values as cutoff. Then the tags number of each taxonomic rank (Phylum and Genus) or OTU in different samples were summarized in a profiling table or histogram, and the histogram(heatmap) was drawn with the software R(v3.1.1). Moreover, the diversity of samples including Alpha diversity analysis, Beta diversity analysis, PCA (Principal coordinates analysis, PCA) and cluster analysis was analyzed. Figures were drawn by software R(v3.1.1). RDA analysis was conducted with software Cannoco for window 4.5 and R(v3.1.1) with vegan package. Correlation analysis was carried out by SPSS 19.0 with Pearson method.

3. Results

3.1. Nitrate reduction and sulfide/ferrous oxidation

Fig. 1 Variation of nitrate(a), nitrite(b), AVS(c), sulfate(d), ferrous(e) and ferric iron(f) in treated sediments with the increase of temperature.

Fig.1a showed that after addition of calcium nitrate, the concentration of nitrate in interstitial water increased from 1.8 mg/L (table 1) to 978.4 mg/L immediately. And three days later (at the end of 5°C), nitrate concentration increased to 1093.1 mg/L then slightly fluctuated when temperature ranged from 5°C to 15°C. Next, it began to decline at 25°C with a 14.9% reduction compared with the value at the end of 15°C. Sharp decline was found at the end of 35°C, which decreased by 45.3% compared with the value at 25°C. The result suggested that nitrate reduction reaction was limited when temperature was below 15°C, while was promoted obviously at temperatures of 25°C and 35°C.

Accumulation of nitrite was found in Fig.1b. Nitrite accumulation was usually reported as an intermediate product of nitrate reduction or ammonium oxidation (Burgin and Hamilton, 2007). In this work, the nitrite production was quite slight at low temperature stages (5°C and 10°C), indicating the reaction was weak. Obvious nitrite accumulation was observed when temperature rose higher than 15°C. Concentration of nitrite at temperature stage of 15 °C, 25 °C, 35 °C were 17.1mg/L, 13.6mg/L and 27.8mg/L, respectively, which further confirmed that high nitrate reduction reaction occurred at 25°C and 35°C.

Change of AVS content in treated sediments at 5°C (Fig.1c) was insignificant. However, at temperature stage of 10°C and 15°C, 22.2% and 17.4% of AVS in sediments were removed with no nitrate decrease (Fig.1a). Moreover, when temperature increased from 15°C to 35°C, AVS content fell to 355.3mg/kg from 3253.2 mg/kg. The AVS removal rate at the end of 25°C and 35°C increased by 40.8% and 78.9%, respectively. AVS is an operationally -defined concept which is a complex and variable component of dissolved S^{2-} species, iron sulfide and pyrite, etc. (Rickard, 2005). Thus, AVS content was rarely used for stoichiometric analysis. According to relevant research (Park, 2009), sulfide could be oxidized to sulfur, sulfate and other sulfur forms depending on the oxidizer, bacteria and reaction condition, but sulfate was the main end product when nitrate served as electron acceptor (Jiang, 2009; Shao, 2010). Therefore, nitrate consumption and sulfate production were usually detected and used as indexes for sulfide oxidation (Shao, 2009; Hayakawa, 2013; Zhang, 2015). As shown in Fig.1d, there was no significant accumulation of sulfate at 5°C and 10°C. Sharp sulfate increase was observed at 25°C and 35°C, with sulfate production rate of 110%, 331%, respectively. The result suggested that high AVS oxidation driven by nitrate reduction could be achieved at high temperature rather than at low temperature.

Fig.1e and Fig.1f illustrated the effect of temperature on iron cycle during nitrate addition. As temperature rising up from 5°C to 10°C, there was a small Fe (III) content decline and Fe(II) content increase. Though the change was insignificant, it hinted a possibility of iron reduction at these two temperature stages. In contrast, at 25°C and 35°C, Fe(II) showed a clearly downward trend, its content reduced by 12.6% and 34.8% compared with those in previous temperature stages. At the same time, the content of Fe (III) increased significantly by 475% and 82.0% at 25°C and 35°C. The results suggested that at high temperature stages (25°C and

35°C) Fe(II) could be oxidized coupling with sulfur-driven nitrate reduction, resulting in accumulation of Fe (III).

Table 2 Correlation between temperature and other parameters

	Nitrate	Nitrite	AVS	Sulfate	Ferrous	Ferric
Temperature	-0.814*	0.923**	-0.997**	0.876*	-0.814*	0.829*
Nitrate		-0.730	0.808	-0.967**	0.973**	-0.837*
Nitrite			-0.921**	0.842*	-0.695	0.647
AVS				-0.869*	0.812*	-0.803
Sulfate					-0.964**	0.771
Ferrous						-0.775

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

According to the correlation analysis shown in Table 2, temperature displayed a significant correlation with other parameters. Seen from the high negative correlation coefficient, it could be inferred that the higher the temperature, the higher nitrate reduction rate, AVS oxidation rate and Fe(II) oxidation rate. Moreover, nitrate was significantly positively related to Fe(II), and negatively related to Fe(III) and sulfate, while both ferrous and sulfate were high positively and negatively correlation with AVS, indicating the oxidation of AVS(formation of sulfate) and Fe(II) (formation of ferric iron) were strongly linked with the process of nitrate reduction. Furthermore, nitrite was found highly connected with AVS and sulfate, meaning its accumulation was closely related to sulfide oxidation. [Though the change of organic compound \(TOC\) was not detected in this work, according to our relevant experiment\(data not shown\), there was no significant reduction of TOC was observed in such short study period.](#) These results were consistent with previous reports that autotrophic sulfur-driven nitrate reduction and nitrate dependant-Fe(II) oxidation were the major process in sulfide-rich, ferrous-rich sediments enhanced by calcium nitrate addition (Weber, 2006; Zhang, 2009; Yang, 2012; Robertson, 2016). Possible pathways could be expressed by using the equation S1 to S14 summarized by Schippers (2002), Cardoso (2006), Bosch (2012) and Robertson (2016), as represented in table S1.

3.2. Shift of microbial community structure

3.2.1. Diversity

Alpha diversity indexes including the OTU numbers, Chao, Ace, Shannon, Simpson and Peilou based on OTU analysis were shown in Table S2. Compared with indexes in the initial sediments, the OTUs, Chao, Ace and Shannon indexes in treated sediments at low temperature stages (5°C Beginning and 5°C End) all increased, while the Simpson index decreased. It meant that microbial diversity was enhanced by calcium nitrate addition. However, with the temperature increase, a total tendency of decrease of microbial diversity was observed. Diversity value in sediment samples at the end of 25°C and 35°C were even lower than that in initial sediments. Analyzing from the OTUs and Peilou index, the reason for diversity increase in 5°C sediments could be considered as the increase of richness and evenness of microbial species. On the contrary, for 25°C and 35°C sediments, not only the OTUs but also the Peilou value were apparently decreased. It meant that the enrichment of species occurred, leading to the decline of abundance, evenness and finally the diversity.

3.2.2. Microbial community structure

Fig. 2. The taxonomic composition distribution at phylum level in treated sediments samples under different temperature stage

There were about forty phylum identified in this work based on OTU taxonomic analysis and

the main phylum with the maximum relative abundance larger than 0.05% were shown in Fig.2. Venn diagram (Fig. S3) showed that the set of OTU commonly presented in each temperature stage occupied about 50%, and the unique OTU in various temperature stage was lower than 10%. It seemed that the change of microbial composition in treated sediments with temperature increase was not significant. Instead, a great variation on percentage and proportion of OTU -representing species, as shown in Fig.2, might be the main reason for the change of microbial diversity.

As illustrated in Fig.2, *Proteobacteria* were the highest abundant phylum with average relative abundance of 54.70%, followed by *Bacteroidetes* (15.18%), *Chloroflexi* (13.65%), *Firmicutes* (8.47%), *Caldiserica* (4.54%) and *Synergistetes* (1.04%), all of which occupied 97.6% of total sequencings. In addition, as reported in relevant reference (Chen,2013) *Proteobacteria* also exhibited the most obvious change in this work. It accounted for 49.19% bacterial sequences in initial sediments, then declined to its lowest proportion of 38.54% at 5°C Beginning when calcium nitrate was injected. However, a significant increase began at 15°C and finally reached its maximum value (77.16%) at 35°C. With the sharp increase of *Proteobacteria* at 25°C and 35°C, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Caldiserica* and *Synergistetes* showed a decreasing trend as the temperature rising. It confirmed that the enrichment of *Proteobacteria* resulted in the microbial diversity decline (table S2) in treated sediments.

According to the reviews by Shao (2010) and Hedrich (2011), most common-reported bacteria with capacity of nitrate-driven sulfur oxidation and ferrous iron oxidation were presented in the α -, β -, δ -, γ - and ϵ -*Proteobacteria*. The district change of class within *Proteobacteria* was shown in Fig. S4, which suggested a high correlation between microbial community and nitrate reduction process. This was similar with the previous research (Chen,2013).

Fig. 3. Percentage heat map of the distribution of dominant genus.

Fig.3 and Fig.S5 showed the classified genus with relative abundance $> 0.05\%$, in which *Pseudomonas* (with average relative abundance of 29.3%) and *Acinetobacter* (7.91%) were the two dominant microbial genus in initial sediments. It seemed that species within these two genus were suitable for growing up at low temperatures. In fact, Psychrotrophic *Pseudomonas* and psychrotrophic *Acinetobacter* had been widely reported that could grow at low temperatures (Zachariah et al., 1973; King and Nedwell, 1984; Suzuki, 2001). As the temperature rose, the relative abundance of *Pseudomonas* in treated sediments displayed a decline tendency, but was still dominant at 5°C, 10°C and 15°C. However, when the temperature was raised to 25°C, *Pseudomonas* was significantly decreased to 14.4%. In contrast, *Sulfurimonas* became the dominant genus (26.81%). Furthermore, at 35°C, *Pseudomonas* only accounted for 7.57%, while *Thiobacillus* increased from 0.1% (initial sediments) to 37.99%(the maximum value) and became the dominant species.

Seen from the CV (coefficient of variation) index, *Sulfurimonas*, *Thermomonas*, *Lysobacter*, *Thiobacillus*, *Microvirgula* and *Agrobacterium* had a significant change ($CV > 1.5$) in the sediment compared with others. However, taking the average and standard deviation(σ) index into account, the σ value of *Thiobacillus*, *Sulfurimonas*, *Pseudomonas*, *Acinetobacter*, *Thermomonas*, *Fusibacter*, *Longilinea*, etc. were obvious higher than others, which meant

that these genus exhibited a remarkable change during the variation of temperature. These genus severally became dominant at different temperature stage as well. Compared with table S2 and Fig.3, it could be found that the variation of those genus which simultaneously had high average, σ and CV values, such as *Sulfurimonas* (member of *Epsilonproteobacteria*), *Thermomonas* (member of *Gammaproteobacteria*), *Thiobacillus* (member of *Betaproteobacteria*) and *Pseudomonas* (member of *Gammaproteobacteria*), contributed to the shift of microbial community structure and diversity. It also indicated that the significant change of these genus was closely related to the process of nitrate reduction and temperature variation.

4. Discussion

4.1. Effect of temperature variation on S and Fe cycle and associated genus

Fig.4 Cluster analysis of sediments parameters during the variation of temperature

Table 2 revealed that temperature had a significant impact on other sediments parameters. Cluster analysis based on all parameters (AVS, Fe^{2+} , Fe^{3+} , nitrate, nitrite and sulfate) at different temperature stage was shown in Fig.4. The variance could be obviously divided into three distinct temperature categories, below 15°C (including 15°C), 25°C and 35°C. Actually, previous studies had confirmed that in sulfur-rich and ferrous-rich sediments at a suitable temperature, nitrate reduction processes (e.g. heterotrophic denitrification, sulfide-driven autotrophic denitrification, nitrate-dependent ferrous oxidation, sulfide and ferrous driven dissimilatory nitrate reduction to ammonium (DNRA), etc.) were the main observed biochemical reaction after the addition of calcium nitrate (Hutchins et al, 1998; Burgin, 2007;

Robertson, 2016). Therefore, the effect of temperature on the sediments parameters could be reflected by the process of nitrate reduction. As shown in Fig.1, only when temperature rose above 25°C, nitrate concentration displayed a clear sign of downward, and other indexes were obviously in line with the reported characteristic of nitrate-driven sulfide and ferrous oxidation. It represented that nitrate reduction reaction was just activated at 25°C and reached a significant level at 35°C in this work. In brief, nitrate reduction was promoted with the increase of temperature, which was similarly found in relevant studies, including in lab researches and in the effect of seasonal changes in summer on surface-water body (King, D., & Nedwell, D. B., 1984; Piña-Ochoa and Álvarez-Cobelas, 2006; Silvennoinen et al., 2008). And for nitrate-driven sulfide oxidation, results from former lab researches conducted under medium temperature (20-30°C) were very similar to what were found at 25°C and 35°C in this work. For example, in Shao's research (2009), approximately 60% AVS was oxidized after the first week of treatment at temperature of 28°C with *Sulfurimonas* as dominant bacteria, which was very close to the AVS oxidation rate (59.1%) and dominant bacteria observed in this study at 25°C.

Fig.5 Pearson correlation between bacteria at genus level and sediments environmental parameters

The Pearson correlation analysis (Fig.5) further confirmed that most bacteria were significantly correlated with the variation of temperature, AVS, Fe(II) and nitrate, which reached to a significant level at $p < 0.01$ and $p < 0.05$. It revealed that these bacteria might play an important role on nitrogen, sulfur and iron cycle during the sediment treatment. Genus related to S and Fe cycles found in this work and commonly reported species were represented in Table S3. At high temperature, genus *Thiobacillus* and *Thermomonas* displayed significant connection with AVS oxidation, ferrous oxidation and nitrate reduction. *Thiobacillus denitrificans* and *Thiobacillus thiophilus* were well known nitrate-driven sulfide oxidation bacteria. Moreover, *Thiobacillus denitrificans* has been reported able to oxidize ferrous, FeS and FeS₂ (Straub et al., 1996; Jørgensen, 2009; Vaclavkova, 2014). *Thermomonas BrG3*, *Dechloromonas agitata CKB*, *Dechloromonas RCB* were other reported NRFeOB within genus of *Thermomonas* and *Dechloromonas* (weber, 2006; Chakraborty,

2013), which also reached their highest richness at 35°C. All these genus were considered to be highly connected with the oxidation of ferrous. Besides *Thiobacillus*, *Sulfurimonas* (eg. *Sulfurimonas denitrificans* and *Sulfurimonas autotrophica*), there was another famous nitrate reduction sulfur oxidation bacteria (Zhang, 2009), which became dominant at 25°C in this work. Otherwise, *Sulfuricurvum*, in which *Sulfuricurvum kujiense* was identified as facultative sulfur oxidation bacteria (Kodama, 2004), was discovered in initial sediments with a relative abundance of 1.9%. Yet its richness decreased when calcium nitrate was added and with the increase of temperature.

The presence of *Sulfuricurvum* and Fe(III) in initial sediments revealed that the sediment samples, which was taken from surface sediments in a tidal rivers, was not exactly anaerobic. It also suggested that during the low tide period, sulfide and ferrous in the surface sediments would be oxidized by oxygen when sediments were exposed to the air. Furthermore, it could be inferred that during the high tide period, when sediments were covered with water and under an anoxic condition, Fe(III) reduction occurred and the iron redox cycle proceeded. However, the high content of AVS and ferrous in initial sediments (Table 2) indicated that the AVS oxidation by oxygen was weak due to the limited oxygen concentration. Instead, nitrate was a more effective oxidant for sulfide oxidation since 91% of AVS was removed from 5°C to 35°C in this work. However, it should be pointed out that high efficiency was achieved only when temperature > 15°C due to the high activity and richness of NRSOB and NRFeOB at high temperature stage.

4.2. Effect of temperature on microbial community structure and diversity

Fig. 6 Bray–Curtis cluster tree (a) and PCA analysis based on OTU abundance (b)

Cluster analysis of species composition (based on representative OTU sequence) among different temperatures samples was shown in Fig.6a. It demonstrated that the similarity in species composition among sediment samples at 5°C, 10°C and 15°C were high compared to initial sediments, while significantly low with samples of 25°C and 35°C. PCA analysis (Fig.6b) based on OTU abundance showed a similar result, that four sediment samples with temperature below 15°C crowded together to represent a high similarity, while samples at 25°C and 35°C were far away from others to reveal a high dissimilarity. Referring back to the change of diversity indexes (Table S2) and bacterial richness at genus level (Fig.3), it seemed that enrichment of species at different temperature gradient was the reason for the great influence of temperature on the shift of microbial community structure and diversity.

What deserved to be mentioned was that the cluster result of microbial structure (Fig.6a) was highly similar to that of sediments parameters (Fig.4), suggesting a close relationship between one and another. Redundancy analysis between bacteria at genus level and sediments parameters was shown in Fig.7.

Fig.7 RDA(Redundancy Analysis) for correlation analysis between microbial community structure at genus level and sediments environmental parameters

RDA Analysis (Fig.7) illustrated that all parameters except for nitrite were significant factors for structuring the bacterial community, and temperature, Fe(III) and AVS showed a significance at $p < 0.01$ levels. Furthermore, all the genus were clearly divided into two parts that clustered together at the left and right side respectively along the temperature arrow, exhibiting the phenomenon of "temperature dependency", which meant that the rising temperature would lead to the shift of community from psychrophilic to mesophilic, and resulted in the variance of microbial community diversity. Moreover, Pearson analysis (Fig.5) showed that those genera(eg. *Thiobacillus*) which were highly negative with nitrate, AVS and Fe(II), were highly positive with temperature. In contrast, those genera(eg. *Geobacter*) that highly negative with Fe(III) (corresponding to ferric iron reduction) were negative with temperature. It confirmed that the temperature variation could restructure the microbial community and then influence the process of sulfur and iron cycle. Canion et al. (2014) reported a similar temperature response of denitrification and ammonia oxidation in the Arctic fjord sediments. They found that the optimum ammonia oxidation was observed at 12-17°C. However, when temperature increased above 25°C, ammonia oxidation almost disappeared and the optimum denitrification occurred at 25-27°C. More earlier, King and Nedwell(1984) and Ogilvie et al. (1997) indicated that the variation of temperatures would cause the enrichment of different denitrifier, which would affect the process of nitrate

reduction. Here, based on the results of sulfur, iron and nitrogen variation (Fig.1), change of microbial community structure (Fig.3) and redundancy analysis (Fig.6), one significant implication proposed here was that with the temperature increased from 5°C to 35 °C, microbial community shifted from psychrophilic to mesophilic, meanwhile, the main microbial process changed from ferric iron reduction to AVS and ferrous oxidation.

4.3. Effect of temperature on sediments odor control in south China areas

As mentioned above, the promotion of sulfide/ferrous oxidation (odor control) was the main purpose of sediments treatment by calcium nitrate addition. In this work, sulfide and ferrous oxidations were proportionally promoted only when temperature increased above 15°C. Pearson correlation (Fig.5) and RDA analysis (Fig.7) further demonstrated that the variation of temperature had a prominent influence on microorganisms, thereby affecting the AVS/ferrous oxidation. *Sulfurimonas* and *Thiobacillus* were found to be two dominant bacteria appearing at 25°C and 35°C, which would display different feature on sulfide and ferrous oxidation. As shown in Fig. 1f, when temperature was near 25°C, ferrous oxidation was limited with *Sulfurimonas* as dominant bacteria. On the contrary, species in *Thiobacillus* (eg. *Thiobacillu denitrificant*) could select many forms of sulfur and iron (S^{2-} , S^0 , HS^- , Fe^{2+} , FeS , FeS_2) as electron donor coupled with nitrate reduction, therefore, it could oxidize sulfide and ferrous simultaneously. As a result, sediments odor and black color problems would be solved simultaneously at a faster speed.

Therefore, for sediment in-situ treatment in cities at south China areas, the efficiency of sulfide removal by calcium nitrate addition might be inhibited in winter and spring when temperature was below 15°C. Moreover, nitrate releasing into overlying water would be accelerated due to the weak nitrate positive consumption(eg. sulfide oxidation) in these seasons. Thus, more calcium nitrate was required and the potential ecological risk induced by the promoted nitrate release should deserve greater attention(Liu, 2015). On the contrary, as shown in Fig.S1, the temperature (25°C-35°C) suitable for sulfide oxidation appeared from May to September. Thus, when adding calcium nitrate into sediment for odor control, selecting the appropriate season (temperature) was one of the key factors.

5. Conclusions

The results in this work revealed that microbial community in sediments treated with calcium nitrate would be remarkably restructured by temperature variation. Meanwhile, the process of sulfide/ferrous oxidation were significantly influenced due to the change of dominant bacteria determined by temperature variation. Thus, nitrate-driven sulfide and ferrous oxidation were proportionally promoted only when temperature increased above 15 °C , in which nitrate-driven sulfide and ferrous oxidizing associated bacteria (e.g. *Thiobacillus*, *Sulfurimonas* and *Thermomonas*) became dominant. As a result, a higher treatment efficiency by calcium nitrate addition for odor control would be achieved in summer than any other seasons in south China areas.

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