The effect of natural materials using as sediment remediation on phosphorus and nitrogen control in a mesocosm

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Abstract

Background: Nitrogen (N) and phosphorus (P) control in sediment remediation through adding natural material has been paid more and more attention. In this study, different natural material including iron-rich clay mineral, calcite, kaoline, sugarcane bagasse and Phragmites detritus was applied to test the effect on N and P control in a mesocosm experiment.

Results: Iron-rich clay mineral and Phragmites detritus had an obvious advantage on P control in terms of almost undetectable soluble reactive phosphorus (SRP) in water column throughout the experiment, which could be explained by strong P adsorption ability. The high available organic carbon in sediment and water column after sugarcane bagasse addition provided enough electron donors for denitrification and dissimilatory nitrate reduction to ammonium (DNRA), which was responsible for nitrate (NO$_3^-$-N) deficiency and ammonium (NH$_4^+$-N) accumulation in water column. Also this resulted in anaerobic status, further fuelling P release from iron-bound P induced by anoxia. Thus, sugarcane bagasse application should be considered its dosage in order to balance the denitrification and DNRA as well as P release. Calcite and Phragmites detritus should be recommended as remediation material for N removal according to the significant promotion of denitrification and limitation of DNRA, finally resulting in low NO$_3^-$-N and NH$_4^+$-N. These results could be explained by the shift of functional microbial community composition and abundance after natural material addition.

Conclusions: Iron-rich clay mineral and Phragmites detritus should be regarded as the promising sediment remediation material for P immobilization due to the increase of P adsorption ability. Taken together, the selection of sediment remediation material should combine the N and P coupling relationship, avoiding that NO$_3^-$-N removal
caused the P leaching or NH$_4^+$-N accumulation.

Key words: Sediment remediation; Natural material; Organic carbon; Denitrification; DNRA

1. Background

Eutrophication, one of the most serious environmental problem, has caused harmful algae to multiply, and led to severe degeneration in water quality of lakes worldwide (Heisler et al., 2008; Schindler et al., 2016). Pollution of nutrients such as N and P is a prominent reason which has caused the problem (Wu et al., 2008). Furthermore, sediments that play a role in gathering pollutants can transport the nutrients into water column owing to biochemical and physical reactions (Chon et al., 2012). Thus, when the external N and P loading has been controlled effectively, the release of internal nutrients dedicates to eutrophication increasingly (Song et al., 2017).

The movement of endogenous nutrients is mainly caused by the relative environmental condition (Lee et al., 2019). For instance, several reasons that drive P release generally contain pH, dissolved oxygen, redox potential, temperature, flow rate as well as biological activity (Jiang et al., 2008; Li et al., 2013). N released into the water body usually as NH$_4^+$-N, NO$_3^-$-N and nitrite (NO$_2^-$-N). The crucial factors, which can cause the exchange of N at the sediment-water interface, mainly involve the capacity of sediments to retain nutrients (e.g. sediment accumulation rates), the conditions of the overlying water (e.g. dissolved oxygen, pH, redox potential, temperature) and the biota (e.g. bioturbation or bioadvection or rates of microbial N transformation) (Medina et al., 2003; Cornwell & Owens, 2011).

So far, numerous methods have been developed to control the release of
endogenous nutrients. To reduce internal P release in sediments, there are some ideas including artificial aeration, the chemical applications such as flocculation precipitation or adding Fe, Al or Ca-based agents, and sediment capping (Freeman et al., 2018; Hansen et al., 2003). The application of CaO$_2$ could effectively control the release of N and P from the sediment and the concentrations of N and P in the overlying water were obviously reduced because of the enhancement of the microbiological action (Wang et al., 2019). Illite is useful as an agent to deactivate P in control techniques for lake eutrophication while it has showed high efficiency (>130%) as the capping material for both SRP and total phosphorus (TP) (Gu et al., 2019). Similarly, several efforts have been proposed to mitigate N release. These strategies contain capping (Zhu et al., 2019), chemical injection (Liu et al., 2014) and microorganism strengthening (Wu et al., 2016). Among capping technique, the biochar was applied and indicated that the fluxes of NH$_4^+$-N, NO$_3^-$-N and total nitrogen (TN) from sediments to water were significantly reduced or reversed (Zhu et al., 2019). In addition, the removal efficiency of TN and NH$_4^+$-N increased obviously after the bioaugmentation of specialized bacteria (Jiao et al., 2011).

However, even though these proposals had effects on the remediation of sediment in eutrophic lakes, some drawback appeared. For instance, several ecological risks are related to the use of chemical products. In addition, biological methods are susceptible to environmental factors (Guo et al., 2017). Consequently, looking for a material which is environmentally friendly, economic and efficient for the control of endogenous nutrients pollution in the eutrophic lake is a crucial issue.

In this study, five different natural materials including iron-rich clay mineral, calcite, kaoline, sugarcane bagasse and Phragmites detritus were added in eutrophic lake sediments to test the effect on N and P control in a mesocosm experiment. N and P
species, dissolved organic carbon (DOC) and chlorophyll $a$ (Chl $a$) in water samples were analyzed. Organic carbon composition, activities of leucine aminopeptidase (LAP), $\beta$-glucosidase (GLU) and alkaline phosphatase (AP), P fractionation and sorption, N transformation rates as well as the abundance and composition of the functional microbial communities in sediment samples were determined. The purpose of this study is to 1) test the effect of different natural material on N and P control; 2) illuminate the chemical and microbial mechanisms; 3) underline the mutual effect of N and P control based on their coupling relationship; 4) propose some promising natural material for N and P control in sediment remediation.

2. Materials and methods

2.1. Experimental design and sampling

The 4-month experiment was conducted in twelve lab-scale columns which were constructed by PVC pipe. The inner diameter of column is 300mm and the height is 1200mm. Sediment and overlying water were collected from Lake Chaohu, which is seriously eutrophic. Sediment mixed with different natural material was put into each column with 50cm height, followed by overlying water, which was infused slowly to avoid the sediment suspension. The twelve columns were divided into six treatments of duplicates. The six groups are control group (Treatment CT), iron-rich clay mineral (Treatment IR), calcite (Treatment CA), $Phragmites$ detritus (Treatment PH), sugarcane bagasse (Treatment SU), and kaoline (Treatment KA) respectively. Iron-rich clay mineral, calcite and kaoline were chosen because of their high phosphorus sorption ability, while $Phragmites$ detritus and sugarcane bagasse were used to provide organic carbon as electron donor for denitrification.

Water samples of all treatments were collected and tested weekly, equivalent
water from Lake Chaohu were replenished to the columns after sampling. Sediment
was sampled by a Peterson grab sampler twice at the beginning of experiment (9
March) and the end (4 July). The water samples were processed the same day and the
sediments were stored at 4 °C in the dark within 1 week for analysis.

2.2. Nutrients analysis

Water samples were tested for TN, dissolved total nitrogen (DTN), NH$_4^+$-N, NO$_2^-$
-N, NO$_3^-$-N, TP, dissolved total phosphorus (DTP), SRP, DOC and Chl a. Among the
analysis of N and P species, water samples were filtered through 0.45 μm cellulose
acetate membrane for soluble nutrients preparation. All the methods followed national
standards (APHA, 2012). Dissolved organic nitrogen (DON) was calculated as:
DON=DTN-DIN, while DIN was calculated as the sum of NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$
N. Particulate nitrogen (PN) was followed: PN=TN-DTN. Dissolved organic
phosphorus (DOP) was calculated as: DOP=DTP-SRP. Particulate phosphorus (PP)
was followed: PP=TP-DTP. For the analysis of DOC, water samples were filtered
through GF/C filters (Whatman, USA) and measured by Multi N/C 2100 TOC
Analyzer. Chlorophyll a (Chl a) was extracted from GF/C filters (Whatman, USA)
with 90% acetone and measured by fluorometric method according to Yentsch and
Menzel (1963).

Total organic carbon (TOC) in sediment was measured by loss-on-ignition in a
muffle furnace (Auer and Yingst, 1980). Protein (PRT) analysis was conducted
according to Hartree (1972) and expressed as bovine serum albumin (BSA)
equivalents. Carbohydrate (CHO) was determined according to Gerchakov & Hatcher
(1972) and expressed as glucose equivalents. Lipid (LIP) was extracted by direct
elution with chloroform-methanol on the basis of Bligh & Dyer (1959) and Marsh &
Weinstein (1966).
Sediment P fractionation was carried out according to Golterman (1996). The method groups sediment P into iron-bound P (Fe(OOH)~P), calcium-bound P (CaCO$_3$~P), acid-soluble organic P (ASOP) and hot NaOH-extractable organic P (P$_{alk}$). Different forms of P were extracted sequentially and SRP was detected.

The method for sediment P sorption was in the light of James (1992). 5mL of sediment suspension were mixed with 10mL of 0, 0.1, 0.2, 1, 2, 5, 8, 10, 15, 20, 25, 30, 40, 50 mg / L KH$_2$PO$_4$ solution (containing 0.01 mol / L KCl) respectively. They were mixed and shaken thoroughly for 24h at 25℃. After centrifugation, the supernatant was filtered through 0.45 μm cellulose acetate membrane and detected for SRP. Reduced phosphate from solution was considered adsorbed by sediment. The sorption parameters at equilibrium were calculated as (1).

\[
S_e = (C_0 - C_e)V/m \quad (1)
\]

Where $S_e$ is the P sorption capacity at equilibrium (mg P/kg), V is the sample volume (mL), $C_0$ is the initial P concentration (mg P/L), $C_e$ is the aqueous P concentration at equilibrium (mg P/L), and m is the adsorbent amount (kg).

Data obtained were fitted to following Langmuir adsorption isotherm equation (2) (Sakadevan & Bavor, 1998).

\[
\frac{C_e}{S_e} = \frac{1}{S_{max}K_L} + \frac{1}{S_{max}}C_e \quad (2)
\]

where $S_{max}$ is maximum adsorbed concentration (mg P/kg), $K_L$ is the Langmuir equilibrium constant (L/mg P).

### 2.3. Microbial activity analysis

The activities of leucine aminopeptidase (LAP), β-glucosidase (GLU) and alkaline phosphatase (AP) were analyzed fluorometrically according to Hoppe (1983), using the methylumbelliferone (MUF) labelled substrates L-leucine-4-methylcoumarinylamid, MUG-Beta-D-glucopyranoside and 4-methylumbelliferyl.
phosphate. In brief, slurries were made at the ratio of 1:10 by weight, triplicates of
3mL subsamples as initial samples were added to 100 μM concentration of each
specific fluorogenic substrate and stopped the reaction immediately at 90°C
electrically-heated thermostatic water bath, while other triplicates of 3mL slurries as
final samples were incubated separately for 1 hour with a same concentration of each
specific fluorogenic substrate. The fluorescence released by substrate hydrolysis was
measured with a Sanco 960 spectrofluorometer as the increase between initial time and
1 hour incubation.

Potential rates of denitrification and ANAMMOX were tested via isotope tracing
method with a membrane inlet mass spectrometer (MIMS) (Risgaard-Petersen et al.,
2003; Hou et al., 2012) and DNRA rates were determined by OX/MIMS (Yin et al.,
2014). The specific processes had been described by peer researchers (Deng et al.,
2015). In short, at the ratio of 1:7 by weight, sediments and water were made into
slurries and purged by helium for about 30 min, then were transferred into 20
respective 12mL gas-tight vials (Labco Exetainers) under a helium atmosphere. To
eliminate residual nitrite, nitrate, and oxygen, the vials were preincubated in a shaker at
150rpm for 24h. Then $^{15}\text{NO}_3^-$ were added into the vials (final concentration
approximately 100 μmol $^{15}\text{N} \text{L}^{-1}$) with a syringe and 100 μL of saturated HgCl$_2$ were
added into 10 of the replicates for preservation, 5 of which are DNRA initial samples,
another 5 vials are initial samples of ANAMMOX and denitrification. The remaining
10 samples were shaken (200 rpm) and incubated for about 8 h and then the sample
were preserved with HgCl$_2$ as the final samples, half of which were measured
dissolved nitrogen gas ($^{29}\text{N}_2$ and $^{30}\text{N}_2$) produced during the incubations for
ANAMMOX and denitrification rate calculation(Nielsen, 1992; Risgaard-Petersen et
al., 2003), and the other half were oxidized with hypobromite iodine solution to
transfer the $^{15}\text{NH}_4^+$ produced by DNRA into nitrogen gas ($^{29}\text{N}_2$ and $^{30}\text{N}_2$) and DNRA rates were calculated from concentration changes in $^{15}\text{NH}_4^+$ during the incubations.

2.4. Metagenome DNA extract and sequencing

To determine the abundance and composition of the functional microbial communities in sediment samples, metagenomic analysis was conducted. Genomic DNA was extracted with commercial kits according to the manufacturer’s instructions. DNA integrity and purity were monitored on 1% agarose gels. DNA concentration and purity were measured using Qubit 2.0 (Thermo Fisher Scientific, Waltham, USA) and Nanodrop One (Thermo Fisher Scientific, Waltham, USA) at the same time. Sequencing libraries were generated using NEB Next Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA) following manufacturer’s recommendations and index codes were added. The library quality was assessed on the Qubit 3.0 Fluorometer (Life Technologies, Grand Island, NY) and Agilent 4200 (Agilent, Santa Clara, CA) system. At last, the library was sequenced on an Illumina Hiseq X-ten platform.

2.5. Statistical analysis

In this study, triplicate samples were analyzed, and the data were presented as the mean ± SD. Average value and standard deviation were analyzed by IBM SPSS Statistics 23. A statistical significance level was within 0.05 that employed for all analyses. Non-linear regression estimation for the model parameters was conducted by Sigma-Plot 10.0 and dynamic fit wizard function. The data were drawn using OriginPro 9.0, the concentrations of chemical parameters in water and sediments were displayed. Among different treatments, clusterings based on the relative abundance of functional genes were performed using hierarchical clustering method and visualized through heatmaps using Primer 5 and Adobe Illustrator CS6.

3. Results
3.1. Nutrients in water column

The effect of different natural materials for eutrophic sediment remediation on nutrients level in water column and algal growth and species was not exactly the same. There had been a sharp increase of SRP concentration on the second sampling day (18 Mar.) in Treatment SU, which was maintained until 29 April. In the other treatments, SRP kept a low level until the end of experiment (3 June), in which day SRP began to increase in Treatment CT, KA and CA, especially Treatment CT, indicating the P release from the sediment. DOP and PP maintained the low level in almost all treatments except in Treatment SU, which peaked on 8 April and 1 April, respectively. Also, in Treatment PH, PP value was relatively high (Fig. 1).

$\text{NH}_4^+ - \text{N}$ showed high value at the beginning of experiment, and then began to decrease and remained the low level until the end of experiment in all treatments except Treatment SU, which always exhibited high value throughout the experiment. The low $\text{NO}_2^- - \text{N}$ value was found in all treatments. There was a common pattern with a peak from 1 to 8 April for $\text{NO}_3^- - \text{N}$ concentration in all treatments, which showed the significantly low level in Treatment PH and SU. DON and PN did not exhibited the significant different in all treatments except Treatment SU with a peak on 6 May and 13 May, respectively (Fig. 2).

The DOC content in Treatment SU was an order of magnitude higher than that of the other treatments, showing a peak on 15 April. In almost all treatments, the Chl $a$ content was considerably low except for Treatment SU with a peak on 6 May. At the end of experiment, relatively low Chl $a$ content was found in Treatment IR (Fig. 3).

3.2. Nutrients fractionation and sorption in sediment

The addition of sugarcane bagasse and *Phragmites* detritus (Treatment SU and PH) at the beginning of experiment could significantly stimulate the accumulation of
TOC, PRT, CHO and LIP, and the addition of kaoline (Treatment KA) at the beginning of experiment increased PRT content, compared to control. At the end of experiment, the PRT, LIP and CHO in Treatment PH, LIP in Treatment SU increased significantly, and CHO in Treatment SU decreased significantly, compared to the beginning of experiment. The carbon fractionation in most treatments did not change greatly during the whole experiment (Fig. 4).

The calcium and iron bound phosphors content was significantly enhanced after the addition of calcite and kaoline (Treatment CA and KA), respectively. Surprisingly, in Treatment PH and SU with organic carbon as main component, the obviously increased iron bound P were observed at the beginning of experiment, which declined considerably in Treatment SU at the end of experiment (Fig. 5). In addition, compared to control, relatively low P sorption parameter EPC₀ value in Treatment PH and SU was found, coupled with high Q_max value. The higher EPC₀ value was recorded in Treatment IR and CA. Through comparison between the beginning and end of experiment, Q_max value significantly increased in all treatments. The Q_max showed the significantly higher value in Treatment SU, compared to the others (Fig. 6).

3.3. Microbial activities, functional genes and microbial community composition in sediment

The addition of sugarcane bagasse (Treatment SU) significantly inhibited the LAP, GLU and AP activities in sediments at the beginning of experiment, the latter two of which sharply increased at the end of experiment. The LAP activity did not vary greatly after the addition of natural materials except Treatment SU, and then dropped down a very low level at the end of experiment in all treatments. The addition of Phragmites detritus markedly stimulated the GLU activity in Treatment PH throughout the experiment. The AP activity was inhibited at the beginning of experiment in all
treatments (Fig. 7). The denitrification process dominated in all N transformation process. The denitrification rate was significantly promoted in Treatment CA, PH, SU and KA, compared to control. The difference was that in Treatment PH, SU and KA, the DNRA rate was also greatly raised, while limited in Treatment CA. The Anammox process was negligible in all treatments (Fig. 8).

The functional genes mediating C, N and P cycling in all treatments showed the completely different level, with the highest and lowest level in Treatment KA and SU, respectively. The functional genes mediating denitrification, DNRA, ammonification and phosphatase in Treatment IR, CA, PH and SU were significantly lower than those of Treatment CT. The functional genes mediating nitrification and carbon decomposition in Treatment CA were significantly higher than those of the others except for Treatment KA (Fig. 9).

The dominant microbial community composition was completely different in all treatments. The dominant community was Cytophagales and Nitrosomonadales in Treatment CT and IR, respectively. In Treatment CA, the Dehalococcoidia, Planctomycetales and Nitrospirales was dominated. In Treatment PH, the dominant community included Aeromonadales, Rhizobiales, Opitutales, Delta-proteobacteria, Syntrophobacterales and Chlorobiales. In Treatment KA, the proteobacteria, Chlamydiales and Bacillales was dominated. Compared to the other treatments, the microbial community composition diversity in Treatment SU was abundant, which was dominated by Methanomicrobiales, Methanocellales, Methanobacteriales, Methanosarcinales, Anaerolineales, Sulfuricurvum, Bacteroidales, Erysipelotrichales, Pseudomonadales, Clostridiales, Desulfovibrionales, Flavobacteriales and Desulfuromonadales (Fig. 10).
4 Discussion

4.1. Effect of different natural materials addition on P control

At the beginning of experiment, in Treatment SU, all forms phosphorus were accumulated (Fig. 1), indicating that the addition of sugarcane bagasse could cause P leaching at the beginning phase. Sugarcane biomass was significantly increased with P fertilizer application, suggesting that abundant of phosphorus was stored inside sugarcane cells (Alvarado et al., 2019). Also, the addition sugarcane-derived biochar could effectively stimulate phosphorus release (Motaghian et al., 2019). Thus, the enzymatic hydrolysis of organic P in sugarcane bagasse should be responsible for the P leaching to some extent, in accordance with the sharp increase of AP (Fig. 7). In addition, the significant increase of iron bound P in Treatment PH and SU (Fig. 5) should be explained that the addition of sugarcane bagasse and Phragmites detritus resulted in inorganic P immobilization with ferric iron in part (Zhang et al., 2015; Yu et al., 2018). The immobilized iron bound P in Treatment SU was subsequently released in terms of the decline of iron bound P at the end of experiment (Fig. 5). The most possible reason was the formation of anaerobic conditions, which facilitated desorption of iron bound P (Hupfer and Lewandowski, 2008). Sustained hypoxia results in an initial decrease in sediment P content due to dissolution of P-bearing iron oxyhydroxides, resulting in a pulse of phosphate to overlying waters (Reed et al., 2011). Furthermore, in Treatment SU, a great deal of DOC was produced in sugarcane bagasse decomposition process (Fig. 3), in which dissolved oxygen was consumed and anaerobic conditions was formed. In a word, the addition of sugarcane bagasse caused the P leaching, which should be attributed to the hydrolysis of organic P in plant cells and the release of iron bound P due to serious anoxia.

However, it was worth noting that at the end of experiment, all released P in
Treatment SU was re-immobilized (Fig. 1), which was associated with strong P sorption ability and potential expressed by the relatively low EPC₀ value and high Qₘₐₓ value (Fig. 6). In others word, even though sugarcane bagasse addition resulted in P leaching at the beginning, in the meantime it promoted the P sorption ability, which finally adsorbed all released P. At the end of experiment, in Treatment CT, KA and CA, SRP was released gradually from sediments (Fig. 1). This fact indicated that, the addition of calcite and kaolinite could not effectively inhibit P release in the long term. Taken together, Phragmites could be considered as an effective natural material for P control in sediments due to high sorption ability, low P leaching and long-term stability, although it might increase the iron bound P content. Iron mineral could maintain low P level in water column throughout the experiment, while the P sorption ability was weak. Sugarcane bagasse should be applied prudently due to the P leaching at the beginning, although it could distinctly enhance the P sorption ability and re-adsorb the released P. Calcite and kaolinite could not maintain the P fixation for long period (Fig. 1, 5 and 6).

4.2. Effect of different natural materials addition on N control

NH₄⁺-N accumulation and NO₃⁻-N deficiency in Treatment SU (Fig. 2) manifested weak nitrification and/or strong NH₄⁺-N production ability. As mentioned above, in Treatment SU, high DOC content and quite possibly anaerobic status (Fig. 3) indicated the blocked nitrification. On the other hand, the increase of all carbon fractions after the addition of sugarcane bagasse, especially CHO which was greatly consumed at the end of experiment (Fig. 4), illustrated that sugarcane bagasse could provide abundant electron donor for NO₃⁻-N reduction. Elevated DOC including organic-rich sugar has been detected in groundwater beneath irrigated sugarcane, which may be positive to denitrification (Thayalakumaran et al., 2015). Sugarcane straw increased the DOC by
more than 50% in the topsoil layer and was positively correlated with N$_2$O emissions (Vargas et al., 2019). Also, the organic carbon content of sugarcane bagasse facilitated denitrification in the vertical flow wetlands (Saeed and Sun, 2013). That is the reason of NO$_3^-$-N deficiency due to strong denitrification in Treatment SU. Furthermore, the significantly high denitrification and DNRA rate in Treatment SU (Fig. 8) proved the above conclusion. As a result, NH$_4^+$-N and N$_2$ was largely produced (Fig. 2). In addition, the obvious low LAP activity suggesting low ammonification in Treatment SU (Fig. 7) further illustrated that DNRA process should be major responsible for NH$_4^+$-N accumulation. The organic carbon was the primary factor controlling the rates of dissimilatory nitrate reduction, accounting for 28.3% and 37.9% of the variance in denitrification and DNRA rates, respectively (Jiang et al., 2019). High organic carbon and reducing conditions shifted NO$_3^-$-N reduction toward more pronounced DNRA (Nizzoli et al., 2010). So, in Treatment SU, high organic carbon fuelled the DNRA process. In a word, weak nitrification, strong denitrification and DNRA after sugarcane bagasse addition jointly caused the NH$_4^+$-N accumulation and NO$_3^-$-N deficiency. Coupled with P release, algal growth was greatly promoted in Treatment SU (Fig. 3).

Similarly, in Treatment PH, all carbon fractions were accumulation in sediments, especially PRT (Fig. 4). However, no high DOC production (Fig. 3) indicated the low availability of introduced organic carbon (Phragmites detritus) and inactive organic carbon decomposition process, compared to sugarcane bagasse. The low decomposition rate of Phragmites has been proved due to its component properties (Zhang et al., 2014), resulting in the low organic carbon availability and DOC leaching rate (Duke et al., 2015). Thus, it could be deduced that dissolved oxygen in sediment-water interface should be sufficient and organic carbon for electron donor should be
enough but not excessive. Hence, the expected strong nitrification and denitrification as well as weak DNRA expressed as denitrification and DNRA rate (Fig. 8) jointly led to the low NH$_4^+$-N and NO$_3^-$-N concentration in Treatment PH (Fig. 2), even if there might exist strong ammonification process (Fig. 7). Simply speaking, *Phragmites* detritus should be considered as a promising natural material introduction for N removal in sediment remediation project.

NO$_3^-$-N accumulation in Treatment CT, IR, CA and KA at the beginning of experiment should be ascribed to different reason. In Treatment CA and KA, it should be believed that at the beginning of experiment weak denitrification occurred due to electron donor lack. On the other hand, high LAP activity indicated the strong ammonification process (Fig. 7), coupled with predicted strong nitrification and weak denitrification, jointly resulting in NO$_3^-$-N accumulation at the beginning of experiment. However, during the experiment, organic detritus and some reductive metal inside calcite and kaoline were dissociated and served as electron donor for denitrification (Fig. 4), which could be explained by high denitrification rate and low NO$_3^-$-N concentration at the end of experiment (Fig. 2 and 8). There is evidence that electron donor exists on the surface of calcite (Ryzantseva and Bunin, 2015). During the formation of calcite, electron donors were needed for bacterial sulfate reduction (Han et al., 2016). The low denitrification rate (Fig. 8) due to electron donor deficiency (Fig. 4) in Treatment CT and IR directly resulted in the sustained maintenance at the high level for NO$_3^-$-N concentration (Fig. 2).

**4.3. Effect of different natural materials addition on microbial functional genes and community composition**

The lowest level of functional genes mediating C, N and P cycling (Fig. 9), coupled with different microbial community composition and rich microbial diversity
in Treatment SU (Fig. 10), indicated that sugarcane bagasse addition shaped microbial community and reduced the abundance of functional microorganisms, which responded with lower EEAs at the beginning of experiment (Fig. 7). On the other hand, the raised denitrification and DNRA rate (Fig. 8) suggested that excess organic carbon in Treatment SU (Fig. 3 and 4) fuelled for a minority of functional microorganisms to perform the greater function (depending on organic carbon). Also, in Treatment SU, the dominant microbial community species (Fig. 10) all grew in the strict anaerobic environments, most capable to decompose organic carbon and produce methane as well as sulfate reduction, which was consistent with the anaerobic status and abundant of organic carbon as well as high denitrification and DNRA rate (Fig. 3, 4 and 8). Even though similar functions (organic carbon decomposition and methane production) were found between Treatment SU and PH, the dominant microbial community composition was different (Fig. 10), indicating the difference of organic carbon components during sugarcane and Phragmites detritus decomposition.

In Treatment KA, the high functional genes abundance and rate mediating denitrification and DNRA (Fig. 8 and 9) suggested that kaoline triggered functional promotion through increasing greatly the quantity of functional microorganisms. Also, the significantly high functional genes mediating nitrification and carbon decomposition in Treatment CA and KA (Fig. 9) were corroborated by the similar dominant microbial community composition, such as Dehalogenimonas and Nitrospira (Fig. 10), which could function as nitrification and organic matter decomposition. All these results illustrated that natural material addition could both shape community composition and change the quantity of functional microorganisms, further determining the ecological functions during the C, N and P cycling to a great extent.
5. Conclusions

The high available organic carbon in sediment and water after sugarcane bagasse addition provided a great deal of electron donors for denitrification and DNRA, also resulted in anaerobic status. The first consequence was that NO$_3^-$-N deficiency and NH$_4^+$-N accumulation due to strong denitrification and DNRA as well as weak nitrification. The second consequence was that a large amount of P leaching due to iron-bound P release induced by anaerobic status. The third consequence was serious algal growth due to high P and NH$_4^+$-N. Thus, sugarcane bagasse application as sediment remediation should control its dosage to balance the denitrification and DNRA, and also to reduce P leaching. Calcite and *Phragmites* detritus should be recommended as remediation material for N removal, because their addition significantly accelerated the denitrification rate and restricted the DNRA rate, finally resulting in low NO$_3^-$-N and NH$_4^+$-N. These results mentioned above could be explained by the change of functional microbial community composition and abundance after natural material addition. Iron-rich clay mineral and *Phragmites* detritus should be regarded as the promising sediment remediation material for P immobilization due to the increase of P adsorption ability. Taken together, the selection of sediment remediation material for N and P joint control should combine the N and P coupling relationship, avoiding that NO$_3^-$-N removal caused the P leaching or NH$_4^+$-N accumulation.

Abbreviations

SRP: soluble reactive phosphorus; DNRA: dissimilatory nitrate reduction to ammonium; NO$_3^-$-N: nitrate; NH$_4^+$-N: ammonium; NO$_2^-$-N: nitrite; TP: total phosphorus; TN: total nitrogen; DOC: dissolved organic carbon; Chl a: chlorophyll a;
LAP: leucine aminopeptidase; GLU: β-glucosidase; AP: alkaline phosphatase; CT: control; IR: iron-rich clay mineral; CA: calcite; PH: *Phragmites* detritus; SU: sugarcane bagasse; KA: kaoline; DTN: dissolved total nitrogen; DTP: dissolved total phosphorus; DON: Dissolved organic nitrogen; PN: Particulate nitrogen; DOP: Dissolved organic phosphorus; PP: Particulate phosphorus; TOC: Total organic carbon; PRT: Protein; BSA: bovine serum albumin; CHO: Carbohydrate; LIP: Lipid; CaCO$_3$~P: calcium-bound P; ASOP: acid-soluble organic P; $P_{\text{alk}}$: hot NaOH-extractable organic P.

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Authors’ contributions

MT, QD and XL were involved in the experiments and manuscript writing, XC, ZZ, YZ and QS were responsible for the data analysis. CS contributed to the study design and manuscript correction. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets obtained and analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Fig. 1 Concentrations of SRP, DOP, and PP in water column among different treatments
Fig. 2 Concentrations of NH$_4^+$-N, NO$_2^-$-N, NO$_3^-$-N, DON and PN in water column among different treatments
Fig. 3 Concentrations of DOC and Chl a in water column among different treatments
Fig. 4 Comparison of TOC, PRT, CHO and LIP in sediments among different treatments
Fig. 5 Different P in sediments among different treatments
Fig. 6 The parameters of P sorption in sediments among different treatments
Fig. 7 The activities of leucine aminopeptidase, β-glucosidase and alkaline phosphatase in sediments among different treatments.
Fig. 8 Potential rates of denitrification, Anammox and DNRA in sediments among different treatments
Fig. 9 Relative abundance of key functional genes mediating C, N and P cycling among different treatments
Fig. 10 The functional microbial community composition and abundance in sediments among different treatments.