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Unveiling the impact of carbon sources on phosphorus release from sediment: Investigation of microbial interactions and metabolic pathways for anaerobic phosphorus recovery

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ABSTRACT

The aim of this study was recovery of phosphorus (P) from marine sediment, and our results revealed the influence of P release from the sediment stimulated with different types and concentrations of carbon sources. During the 15-day anaerobic operation, the sediments stimulated with 1 g/L propionic acid and glucose exhibited more prominent effects compared to other trials, with 5.98 mg/L and 6.44 mg/L of P released, respectively, with a total solid content of 4 %. Notably, the excessive addition of carbon sources was shown to can partially inhibit P release. As microbial activity intensified, P was utilized for microbial synthesis, resulting in a decreased P in the supernatant. For example, in glucose-fed systems with concentrations of 5 g/L and 10 g/L, the P concentration decreased from 5 mg/L on Day 3 to approximately 3 mg/L on Day 15. The sequencing results indicated distinct evolutions within different carbon source-fed systems over the 15-day operations. Feeding high concentrations of glucose resulted in rapid enrichment of fermentative bacteria under anaerobic conditions, while sulfate-reducing bacteria promoted P release in volatile fatty acids-fed systems. Metabolic analysis revealed that carbon sources not only influence gene expression in different systems, but also impact the metabolic pathways involved in nutrient cycling, which can be interrelated. For example, a significant positive correlation was observed between the abundance of P and sulfur cycling functional genes (phoD, cysD).

1. Introduction

The importance of phosphorus (P) as a limited and nonrenewable mineral resource has received widespread attention. Due to the global depletion of this mineral and highly European dependence on imports of this mineral, phosphate rock and P were listed as the most critical raw material in the EU in 2014 and 2017, respectively [1,2]. Considering the limited availability of P in Europe, sustainable solutions for easing the burden of P mineral shortages should be developed.

In terms of the P flow in modern society highlights that, in addition to waste streams, a significant portion of P is lost when it flows into surface water and oceans [3], thereby triggering the problem of eutrophication. For instance, an estimated 31,000–37,000 tons of potentially mobile P have been found in the sediment surface of certain archipelago areas of the northern Baltic Sea [4]. Currently, the estimated annual P input to the entire Baltic Sea is 28,000 tons [5]. Therefore, to tackle the dual challenges of P supply shortages and P-induced marine eutrophication, the P buried in the eutrophic marine environment could be a promising alternative resource; mitigating the P in aquatic ecosystems and implementing P recovery technologies are imperative approaches. Although reclaiming P from waste streams has received growing attention in recent years, there has been little focus on P recovery from

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eutrophicated aquatic environments. One of the major contributing factors to this is technological bottlenecks that hinder the recovery of naturally diluted P [3].

Biological P recovery strategies, with their cost-effectiveness and straightforward operations, offer promising solutions to concentrate dilute P [6]. Biological-based P recovery focuses on microbial enhancement; for instance, enhanced biological phosphorous removal (EBPR) aims to remove P from wastewater through the enrichment of polyphosphate-accumulating organisms (PAOs) [7]. The growth and metabolism of microbial organisms are influenced by the type of carbon (C) sources. Ni et al. [8] observed that the growth of PAOs (*Acinetobacter lwoffii*) in a biofilm sequencing batch reactor was primarily regulated by C source under anaerobic conditions. This can be attributed to the fact that C sources provide electron donors, facilitating polyhydroxyalkanoate synthesis for energy storage, and enabling the subsequent aerobic stage to utilize the stored energy for *A. lwoffii* growth. Dai et al. [9] investigated the stimulating effects of P release by feeding three different C sources (sodium acetate, sewage, and effluent of treated wastewater) into the sludge P release process. The results indicated that the type and concentration of C had significant effects on the P release performance. Within the sodium acetate feeding system, 44.40 mg/L of P could be released in the first 4 h, while when sewage and effluent were fed as a general and lower C source, only 21.45 mg/L and 0.83 mg/L of P could be released in 5 h, respectively. Another observations from Chen et al. [10] showed that, compared with acetic acid, the propionic acid could improve EBPR performance for P removal ability after long-term cultivation. Hence, there is tangible evidence that strategically chosen C sources and doses will determine P release.

However, currently, the limited research on P recovery from the eutrophic environment has resulted in a knowledge gap regarding the bioavailability potential of P naturally present in the environment and the metabolic process of microbial organisms involved in P release. Further exploration of the development of P recovery technologies for the feasibility of P reclamation from the natural environment is also needed. Therefore, in our study, anaerobic batch reactors were applied to achieve P recycling from the Baltic Sea sediment, and for expanding research efforts in revealing different C feeding sources on the P release process. Four different types of C sources (acetic acid, propionic acid, butyric acid, and glucose) were compared under different concentrations (0–10 g/L), and the potential mechanism of P release induced by microbial activity under the influence of C has been deeply explored. Our results can provide technical support and theoretical analysis which can aid understanding of nutrient cycling, optimization of P recovery from marine sediment, and remediation strategies in marine environments.

2. Materials and methods

2.1. Anaerobic batch experiments

The sediments were collected from the Baltic Sea area on 30th January 2022, Stockholm, as our study focuses on surface sediments where P cycling is most active. The characteristics of the sediments and pore water were described by Zhu et al. [11]. Four different typical C sources − acetic acid, propionic acid, butyric acid, and glucose − were tested to compare their performance in stimulating P release with the marine sediment under anaerobic conditions. These C sources are commonly used in microbial studies due to their diverse metabolic researches and widespread use in anaerobic processes [12–14].

Briefly, each anaerobic fermenter was filled with 16 g of fresh sediment into a 120-mL serum bottle in triplicate, then the pure solutions of sodium acetate, sodium propionate, sodium butyrate, and glucose were respectively added to corresponding C-fed systems, respectively, with each total working volume of 80 mL, resulting in a final loading of 0 (control), 0.5, 1, 2.5, 5 and 10 g/L target C sources (electron donors). The control group was established to provide a baseline for comparison with the experimental groups where external C sources were added. The control was maintained under the same anaerobic conditions as the experimental groups but without any additional carbon input. The anaerobic fermenters were set up at an initial pH of approximately 7 and were sealed with septa and aluminum caps. Each bottle was flushed with nitrogen gas for more than 5 min to provide an anaerobic environment. The bioreactors were run for 0, 1, 2, 3, 6, 9, 12, and 15 days with daily manual mixing at room temperature, since each group was set up with 3 parallel reactors, a total of 144 bottles were required for each C-fed system individually for destructive sampling. On the sampling day, the flasks of each group were analyzed (18 reactors on each sampling day), and throughout the operation, the analysis of the PO_4-P, pH, C source consumption and transformation (electron/donor) in the fermenters, as well as the sediment microbial community were conducted to investigate the P-releasing capability and explore the potential microbial activities driven by different C doses.

2.2. Analytical methods

The concentration of PO_4-P was determined according to the Murphy-Riley method [15], and the pH was monitored by using the pH meter (Mettler Toledo FiveEasyTM pH bench meter, FE20). The gas chromatography (GC) (Agilent Intuvo 9000), equipped with CP-Sil 5 CB column (25 m × 0.32 mm × 5 μm, Agilent) and a flame ionization detector, was utilized to analyze the concentrations and compositions of three volatile fatty acids (VFAs, acetic acid, propionic acid, butyric acid) [11]. The glucose content in the supernatant was determined by the dinitrosalicylic acid (DNS) method described by McKee [16]. The chemical speciation of P in sediments by sequential extraction was also conducted for clarifying the variations of P speciation during the anaerobic operation following the methodology detailed in the study of Jiang et al. [17]. The sulfate concentration in the supernatant was determined using an Ion Chromatography (IC)(850 Professional IC Anion, Metrohm, 2.850.2030) equipped with a Metrosep A Supp 5–150/4.0 column (Metrohm, 6.1006.520) and a 863 Compact IC Autosampler (Metrohm, 2.863.0010). The concentrations of metal ions were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES; Thermo Scientific iCAP 7000 Plus).

2.3. DNA extraction, PCR amplification and sequencing

High-throughput sequencing was adopted to characterize the microbial community structure in the batch reactors. Microbial genomic DNA was isolated using DNeasy PowerSoil Pro Kit (QIAGEN, Germany) by following the instructions of the manual protocol.

Library preparation was performed according to the Illumina 16S Metagenomic Sequencing Library Preparation (Illumina, San Diego, CA, USA). The V3-V4 hypervariable region of the 16S rRNA gene was amplified via polymerase chain reaction (PCR) in a final volume of 25 μL with 25 ng microbial DNA, 2X KAPA HiFi HotStart ReadyMix (Roche, Basel, Switzerland), and 200 nmol/L of 341F and 785R primers with added Illumina adapter overhang sequences [18]. The PCR thermocycle consisted of 3 min at 95 °C, 25 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C, and a final 5-min step at 72 °C [19]. PCR products were purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) and indexed libraries were prepared by limited-cycle PCR with Nextera technology (Illumina). Indexed libraries were subsequently cleaned-up as described above and then quantified using the Qubit 3.0 fluorimeter (Invitrogen, Waltham, MA, USA), normalized to 4 nM and pooled. The sample pool was denatured with 0.2 N NaOH and diluted to a final concentration of 4.5 pM with a 20 % PhiX control. Sequencing was performed on an Illumina MiSeq platform using a 2 × 250 bp paired-end protocol, according to the manufacturer's instructions (Illumina).

Raw sequences were processed using a pipeline combining PANDAseq [20] and QIIME 2 [21]. High-quality reads were retained using

the "fastq filter" function of the Usearch11 algorithm [22], then clustered into amplicon sequence variants (ASVs) using DADA2 [23]. Taxonomy was assigned using the VSEARCH classifier [24] and the SILVA database (December 2017 release) as a reference [25]. Overall, an average sequencing depth of 4.3 ± 2.4 thousand high-quality reads per sample was obtained, resulting in a total of 16,116 ASVs. PICRUSt2 with default parameters was used to predict metagenome functions (KEGG orthology database) based on the ASVs identified in our dataset [26]. Our study focuses on microbial activities specifically related to P cycling under anaerobic conditions, we also analyzed pathways involved in the cycling of other elements related to P release, such as sulfur (S), C, and nitrogen (N) metabolism. The functional annotation of these pathways was mapped to the KEGG orthology database to identify relevant metabolic functions. Processed reads for 16S rRNA gene sequencing are openly available in European Nucleotide Archive (ENA), reference number PRJEB74909.

2.4. Statistical analysis

The analysis of variance (ANOVA) was conducted to assess the significance of P release results ($p < 0.05$). The differences in microbial diversity between different C dosing groups were analyzed using nonmetric multidimensional scaling (NMDS). The Anosim (analysis of similarities) analysis was used to investigate differences in microbial composition. At the family level, redundancy analysis (RDA) and Pearson's correlation were used to ascertain the interaction between multiple factors and microbial abundance. The OmicShare tool was used to analyze the KEGG enrichment pathways. To minimize the impact of confounding variables, consistent experimental conditions were maintained across all treatment groups, with the only variation being the type and concentration of C sources. Additionally, three parallel replicates were included for each treatment to ensure the reliability of the results.

3. Results and discussion

3.1. Effects of different carbon sources on phosphorus release

The key objective of our experiment was to evaluate P decomposition stimulated by different C loading in four C-fed systems. The dissolved PO_4-P released from the sediment are depicted in Fig. 1. The introduction of additional C sources has enhanced the release of P under anaerobic conditions. The highest concentrations of released P were observed in the system fed with acetic acid, propionic acid, butyric acid, and glucose, which were 4.99 mg/L, 5.98 mg/L, 5.76 mg/L, and 6.44 mg/L, respectively. In comparison with the corresponding control groups, the enhancement was 26.9 %, 32.0 %, 23.1 %, and 44.1 %, respectively. P experienced rapid release during the initial 3 days, with 67.0 %, 57.2 %, 69.1 %, and 77.7 % of the maximum final P solubilization being observed in the system of acetic acid-, propionic acid-, butyric acid- and glucose-fed, respectively. By Day 6, for the anaerobic reactors fed with three VFAs, this value exceeded 80 %, therefore, although the increasing trends of P release were observed in all systems

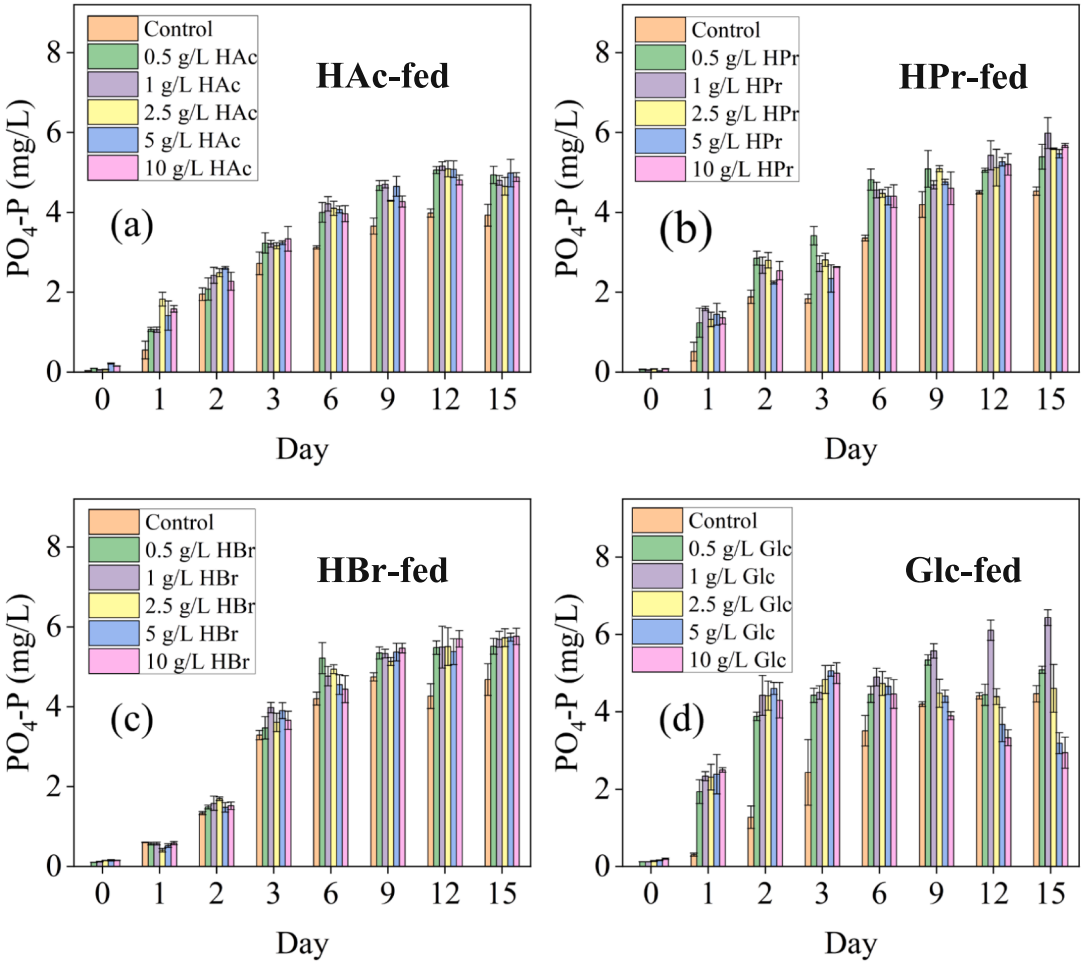

Fig. 1. PO_4-P release performance of sediment under different concentrations with four types of carbons-fed systems: (a) acetic acid-, (b) propionic acid-, (c) butyric acid- and (d) glucose fed system. (HAc: acetic acid, HPr: propionic acid, HBr: butyric acid, Glc: glucose).

with the extension of operation time to 15 days, the final P release only increased slightly.

Among the three VFA-fed systems, the stimulations of P release by both propionic acid- ($p < 0.01$) and butyric acid- ($p < 0.05$) were significantly higher than that by the acetic acid-fed system. The propionic acid-fed system exhibited the highest P release at 5.98 mg/L, achieved from an initial dosing of 1 g/L. While the stimulatory effect of 1 g/L glucose-fed on P release was more pronounced compared to the VFA-fed groups, reaching an optimal release of 6.44 mg/L on Day 15, despite that the ANOVA analysis revealed there was no significant difference between 1 g/L of propionic acid and glucose feeding treatments ($p = 0.055$). Interestingly, although there was no statistically significant difference in the detected dissolved PO_4-P content, further analysis of the release ratios of inorganic phosphorus (IP) and organic phosphorus (OP) revealed distinct patterns. Under 1 g/L propionic acid-fed conditions, the release ratios for IP and OP were 11.6 % and 12.0 %, respectively. In contrast, under 1 g/L glucose-fed conditions, the ratios were 1.6 % and 23.9 %. This could suggest that microbial activity predominantly drives the release of OP in the glucose-fed system. Furthermore, the addition of varying concentrations of C led to a diverse influence on P release from the sediment. Excessive C input did not result in an amplified stimulatory effect on P release; instead, excessively high C concentrations inhibited P release. This observation has also been substantiated by statistical analysis. The two-way ANOVA analysis revealed a highly significant inter-group difference ($p < 0.001$) regarding the impact of different C concentrations on P release on day 15. For instance, in the glucose-fed systems, the optimal concentration for P release was 1 g/L, while no significant difference was observed between 0.5 g/L and 2.5 g/L ($p > 0.1$), as well as between 5 g/L and 10 g/L ($p > 0.1$).

It is noteworthy that the stimulation of P decomposition under high-concentration glucose feeding is particularly strong during the initial days. For instance, the concentration of PO_4-P can reach 5.1 mg/L and 5.0 mg/L when fed with 5 g/L and 10 g/L glucose on Day 3, respectively. However, the dissolved PO_4-P gradually decreased over time, with only 3.2 mg/L and 2.9 mg/L of PO_4-P detected in supernatants on Day 15; this value is even significantly lower than that of the control group (4.47 mg/L, $p < 0.05$). This process of declining P concentration could be potentially triggered by the glucose fermentation process. The addition of high-concentration glucose generated a higher yield of organic acids (e.g., VFAs), which led to a drop in the pH. Simultaneously, during the fermentation, the metal ions can be released from the sediment under the acidic condition, given that these metal ions can complex with P adsorption, leading to its reduction in the supernatant [27].

Therefore, the variations in P content in the sediment were examined, to validate the reason for P concentration reduction in the supernatant. As shown in Fig. S1, the high dose of glucose did not lead to a decrease in the Fe-P or Al-P content of the sediments after 15 days ($p > 0.05$) compared to the initial content on Day 0. Simultaneously, we also observed the changes in metal ion concentrations in the supernatant, where the concentration of Al(III) was extremely low, detected only at 0.9 mg/L and 3.2 mg/L in the 5 g/L and 10 g/L glucose-fed groups on Day 15, respectively, while concentrations in other groups were below 0.1 mg/L. Furthermore, the release of Mn(II) and Fe(II)/Fe(III) in anaerobic cultivation was more apparent, as illustrated in Fig. S2 and Fig. S3, especially in the glucose-fed reactors. On Day 3, under 10 g/L glucose feeding, 1.8 mg/L of Mn and 6.5 mg/L of Fe were detected. Although subsequent PO_4-P concentration decreased since Day 3 (Fig. 1 (d)), the concentrations of released Mn and Fe on Day 15 significantly increased ($p < 0.01$), reaching 7.8 mg/L and 85.9 mg/L, respectively. Overall, the released P from the sediments did not form complexes with the released metal ions (i.e., Fe(II), Al(III), Mn(II)), leading to a reduction in P concentration in the supernatant. The variations in concentration may be attributed to microbially-mediated physiological responses. Nonetheless, further analysis associating with microbial activities and metabolism need to be conducted.

3.2. Variations of carbon consumption in different carbon-fed systems

Tracking the consumption of C sources is crucial for exploring the microbial metabolic processes. As depicted in Fig. S4, the concentration variations of the four different C sources indicate that three VFAs in the VFAs-fed systems only had gentle consumption in all groups (<30 %) after 15 days, and this consumption was mainly detected in the low-concentration C supply groups. For example, the bioreactors fed with 0.5 g/L and 1 g/L propionic acid showed 28.1 % and 19.7 % consumption, respectively; and those fed with 0.5 g/L and 1 g/L butyric acid consumed 15.0 % and 17.8 % of butyric acid. This result is inconsistent with previous studies involving sewage sludge. In anaerobic conditions, the PAO can rapidly take organic carbons (e.g., VFAs) and hydrolyze the stored poly-P; and subsequently, PO_4-P is released to the environment [11]. In a batch experiment conducted by Gerber et al. [28], it was exhibited that under anoxic-anaerobic conditions, the 200 mg/L of equivalent COD (chemical oxygen demand) short-chain C compounds (including acetic acid, propionic acid, butyric acid) could effectively induce PO_4-P release, resulting in above 65 mg/L phosphate released from around 4 g/L MLSS (mixed liquor suspended solids) sludge system. Meanwhile, the VFAs compounds experienced rapid consumption, reaching 100 % depletion within 2–4 h. However, in our anaerobic sediment system, only glucose exhibited substantial consumption. Over 99 % of the glucose in the 1 g/L-fed system was consumed within 3 days, whereas for systems initially dosed with 5 g/L, this timeframe was extended to 12 days. This phenomenon is potentially attributed to variations in microbial communities between sewage sludge and marine sediment.

3.3. Anaerobic carbon sources transformation

As depicted in Fig. 2, under anaerobic conditions, the addition of different C sources led to a fermentation process and organic acid production. The dominant VFAs newly generated are mainly acetic acid and butyric acid. Compared to propionic acid- and butyric acid-fed systems, it is evident that, when provided with a similar amount of C source, glucose yielded a higher production of newly generated VFAs.

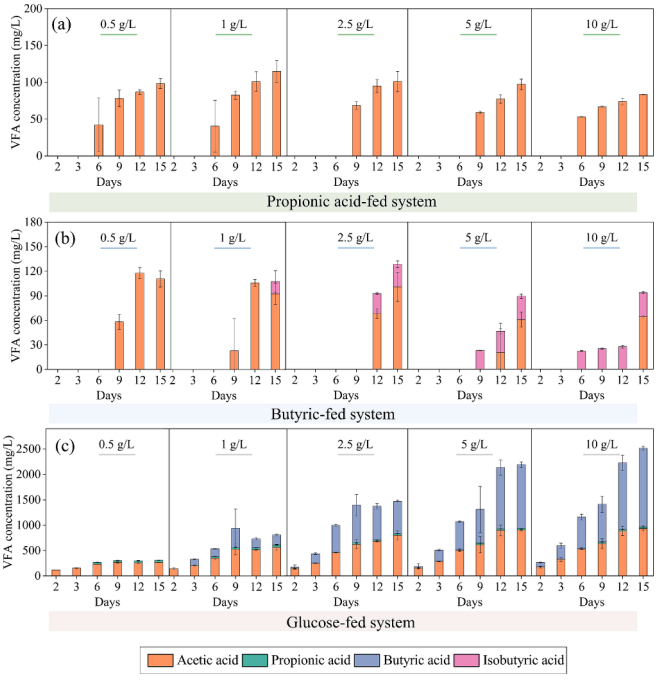

Fig. 2. Species and amount of VFAs transformation/production during fermentation of sediment under different concentrations within (a) propionic acid-, (b) butyric acid-, and (c) glucose-fed system.

Furthermore, as the initial dosing concentration increased, the C conversion from glucose to VFAs also enhanced (Fig. 2(c)). On day 15, 932.5 mg/L of acetic acid, 33.2 mg/L of propionic acid, and 1549.5 mg/L of butyric acid were detected in the system under 10 g/L glucose treatment. However, the conversion of C in the systems fed with propionic acid and butyric acid did not show noticeable increasing trends as the C feeding concentrations increased. For instance, as shown in Fig. 2 (a), the addition of propionic acid partially conversed into acetic acid. Two-way ANOVA analysis indicated that the new acetic acid production (114.9 mg/L) in the dosage of 1 g/L significantly exceeded other concentration fed groups ($p < 0.01$). In the low concentrations of butyric acid-fed groups, the C transformation came solely from butyric acid to acetic acid; whereas at higher dosing concentrations, the isobutyric acid could be converted (Fig. 2(b)). Interestingly, during the beginning operation in the system of 10 g/L butyric acid-fed, the transformation of acetic acid did not occur in the initial 12 days.

Previous studies indicate a trend of long-chain organic acids (including VFAs) being oxidized or degraded into smaller organic acids; VFAs with more than three C atoms can undergo biodegradation to produce acetic acid during anaerobic fermentation, which can generate energy through the β-oxidation pathway [29]. Thus, part of the propionic acid was transformed into acetic acid in anaerobic bioreactors, and butyric acid can be converted into acetic and propionic acids. However, as observed in Fig. 2(b), when provided with high concentrations of butyric acid (5 g/L and 10 g/L), the reaction underwent isomerization to produce isobutyric acid first, rather than the breakdown of butyric acid into smaller molecules. In contrast, under low concentration feeding, the C transformation related to acetic acid formation. Hence, high concentrations of butyric acid feeding limited the efficiency of its conversion into shorter-chain C2 and C3 compounds. Furthermore, the transformation of butyric acid into short-chain VFAs occurred relatively late (on Day 12). Newly produced VFAs (acetic acids) in the bioreactors were only detected from Day 9, except in the 10 g/L feeding group.

Microorganisms in the sediments can also transform glucose into smaller organic molecular compounds under anaerobic conditions, and this process yields energy that can be utilized for cellular energy supply [30]. In the absence of oxygen, a portion of the newly produced VFAs can be further converted into CH_4, often facilitated by methanogenic archaea, and biomass prefoliation [29]. Therefore, when the external supply of C source is limited, the accumulation of VFAs in the bioreactors will cease. Due to the lack of additional external C sources available for VFAs conversion, utilizing internal sediment C for VFAs transformission is more challenging [31]. Conversely, the generated VFAs may be further consumed by organisms. As shown in Fig. 2(c), the bioreactors with initial glucose feeding concentrations of 0.5, 1, and 2.5 g/L exhibited a slight decrease in accumulated VFA from day 9; for instance, the total amount of VFAs production within the 1 g/L glucose-fed groups decreased from 939.53 mg/L to 809 mg/L.

3.4. Anaerobic microbial conversion of carbon sources

The energy generated from C transformation serves to support microbial catabolic and anabolic activities, such as the production of CH_4 and the growth of biomass. According to our calculation, considering the equilibrium of C transformation, a significant portion of added C sources were utilized by the bioprocess. The results indicate that with an increase in the concentration of C sources, the total amount of C utilized by biological processes also rises. For example, in the glucose-fed systems, at glucose concentrations of 0.5 g/L and 1 g/L, the C utilized by biological processes is 519.33 mg/L and 466.32 mg/L (calculation based on COD, Fig. S5(d)), respectively. While at higher concentrations of 5 g/L and 10 g/L, the corresponding number increased to 3618.36 mg/L and 2600.93 mg/L (calculation based on COD), respectively. The relevant calculation of C mass balance transformation can be seen in Fig. S5.

When excessive C was dosed into the system, the proliferation of biomass may become predominant, as previously observed by Low and Chase [32]. This dominance of biomass growth can be counterproductive for synthesizing target products or supporting microbial reactions, such as the release of orthophosphate from sediment. The Pearson correlation analysis results indicated that in the propionic acid-fed system (Table S1), there was a significant positive correlation between the concentration of released PO_4-P and the concentration of newly generated acetic acid ($p < 0.01$, $r = 0.816$). Similarly, in the butyric acid-fed system, a significant positive correlation ($p < 0.01$, $r = 0.748$) was observed between the concentration of newly transformed VFAs and the PO_4-P concentration. In the glucose-fed system, a significant positive correlation ($p < 0.05$, $r = 0.926$) between the concentration of newly transformed VFAs and the released PO_4-P was detected within the first 3 days. However, over the entire 15-day period, a significant negative correlation was found with a Pearson coefficient of $r = -0.702$ ($p < 0.01$).

Considering the results from Fig. S4 and Fig. 2, it can be concluded that the higher amount of newly generated VFAs observed in the 1 g/L propionic acid- and butyric acid-fed systems surpassed those of other C concentration dosage groups. This suggests greater energy production through the electron transport chain, which may support higher P release from the sediment. The increased VFA production enhances microbial respiration and redox reactions, likely altering the sediment's chemical environment to favor P release. Previous studies have confirmed that changes in redox conditions significantly influence P release in sediments under anaerobic conditions [33,34]. In contrast, although high-concentration glucose feeding initially stimulated rapid P release, it likely promoted biomass synthesis due to the excess C supply. Since biomass synthesis consumes P, this led to a decrease in P concentration after Day 3.

Redox processes play a crucial role in the release of P from sediment, as they directly influence the chemical environment and microbial activity within the system. The significant impact of these redox processes is evident from the observed C transformations, which demonstrate how the availability and metabolism of different C sources can drive P release. This finding is further supported by the notable changes in other electron acceptors, such as sulfate, within the system. The reduction of sulfate, for instance, indicates active microbial respiration and electron transfer processes, which alter the redox state of the sediment and contribute to the mobilization of P. As observed in Fig. S6, sulfate concentration considerably decreased after 15 days of anaerobic operation, particularly in the 0.5 g/L and 1 g/L C-fed groups, where the concentration approached to 0. Correlation analysis revealed significant positive correlations ($p < 0.05$) between the PO_4-P release and sulfate consumption within all C sources addition systems. The correlation coefficients in acetic acid-, propionic acid-, butyric acid-, and glucose-fed systems are 0.77, 0.69, 0.67, and 0.79, respectively. Zhuo et al. [35] found that the addition of electron acceptors (i.e., sulfate), significantly enhanced VFAs production and promoted the release of total P from iron-containing sludge. Similarly, the study from Yang et al. [36] showed that the enhancement of sulfate reduction processes led to the strengthening of microbial functions associated with P release in sediment.

Anaerobic fermentation in different C-fed systems involves the generation and consumption of H^+, which consequently causes variations of pH. Therefore, the variations of pH were also monitored in all reactors during 15-day operation. As depicted in Fig. S7, the initial pH in all systems was primarily 7.2 ± 0.2. Due to the relatively low C conversion in VFAs-fed systems, there were minor pH fluctuations over the 15-day operation, while the acetic acid-fed system showed a slight pH increase from 7.25 to 7.45, propionic acid- and butyric acid-fed systems remained relatively stable around 7.2 during the 15 days. The production of VFAs during glucose fermentation brought a rapid drop in the pH. By Day 3, the C feeding groups with a dosage exceeding 1 g/L all experienced a pH decrease to around 5.5, and as more VFAs accumulation was observed, the high concentration of glucose added gradually drove the pH below 4.5. According to the glucose consumption in Fig. S4 (d), it is evident that the 10 g/L glucose was not entirely depleted within

the 15 days of operation, only around 44 % of glucose was consumed. Consequently, if the experimental duration is further extended, more VFAs could be produced, which is expected to result in a lower pH. Actually, the decline in pH is unfavorable for glucose acting as an electron donor.

Additionally, pH, as a crucial environmental factor, significantly influences the release of P, particularly IP. Jin et al. [37] studied P fractions and the effect of pH on P release in sediments and found that when IP is predominantly in the form of NaOH-P (e.g., Fe-P, Al-P), an increase in pH above 7 led to a rapid increase in the rate of P release. Conversely, when IP is primarily HCl-P (e.g., Ca-P) and the pH is below 7, a rise in pH results in a marked decrease in the rate of P release. Similar experimental observations were reported by Peng et al. in a wastewater stabilization pond [38]. In our experiment, the pH in the VFAs-fed systems remained relatively stable at around 7.3, which suggests that this pH level had less impact on IP release. However, in the high-concentration glucose-fed groups (>2.5 g/L), the pH dropped below 5, which likely promoted the release of HCl-P (Ca-P). After 15 days, the Ca-P release in high glucose-fed groups exceeded 21 %, compared to only ~ 6 % in the other groups (Fig. S1). On the other hand, the release of other IP fractions in the high-concentration glucose-fed groups was much lower than in groups fed with lower concentrations of glucose.

Overall, the P release in anaerobic systems under different C-fed bioreactors is intricately connected to organic C degradation, electrons transfer, and microbial activities. Lower C concentrations struggle to generate sufficient energy, while an excess of C supply is more likely to be utilized for biomass accumulation. It is essential to further infer on these aspects with the analysis of microbial structure. This will be addressed in the following section, where more detailed microbial activities will be explored.

3.5. Microbial community responses under varied carbon stimulation

The evolutions of microbial structures of sediments were analyzed. Through NMDS analysis, it was observed that microbial communities exhibited distinct changes when exposed to different C sources, with notable variations in the bacterial community structure observed among VFAs-fed and glucose-fed systems (Fig. 3(a)). To further investigate these differences, we conducted Anosim by comparing the sediment organisms provided with 1 g/L of C, revealing significant distinctions among the various C-fed systems. Also, microbial communities at the phylum and family levels on each sampling day are provided in Fig. S8 and Fig. 4.

At the phylum level, Actinobacteriota, Proteobacteria, Chloroflexi, Desulfobacterota and Planctomycetota initially dominated the microorganisms (Fig. S8), accounting for more than 68 % of the total phyla. These bacteria are involved in C, N, S cycling, as well as organic matter (OM) biodegradation and metabolism [39–41]. However, significant changes were observed in the microbial community, especially in the glucose-fed system starting from day 3. For example, there was a rapid increase in the abundance of Firmicutes: on day 3, the abundance of Firmicutes with glucose dosages of 0.5, 1, 2.5, 5, and 10 g/L reached 18.3 %, 28.4 %, 39.4 %, 26.3 %, and 49.3 %, respectively, while the control group had an abundance of only 0.4 %. Moreover, the richness of Firmicutes exceeded 66 % in the high-concentration (5 g/L and 10 g/L) glucose-fed groups by Day 15.

Similar changes were also evident at the family level, especially in the glucose-fed systems. As shown in Fig. 4(d), initially, *Desulfosarcinaceae* and *Ilumatobacteraceae* were the dominant families, and gradually, *Clostridiaceae*, *Fusobacteriaceae*, and *Psychromonadaceae* dominated the communities, with *Clostridiaceae* being particularly notable. These three families belong to the Firmicutes and Proteobacteria phyla, and their significant increase is consistent with the phylum level. The abundance of the family *Clostridiaceae* increased from nearly 0 % at day 0 to 17.2 %, 28.1 %, 38.8 %, 25.8 %, and 46.0 % with glucose dosages of 0.5, 1, 2.5, 5, and 10 g/L on Day 3, respectively. Although different concentrations and types of C sources can induce significant microbial evolutions, a further study with Anosim revealed that varying concentrations of the same type of C may not necessarily lead to microbial community changes. As shown in Fig. S9, microbial community structures exhibited no significant differences under the addition of different concentrations of acetic acid and butyric acid ($p > 0.05$), while significant differences were observed with the addition of different propionic acid and glucose dosages ($p < 0.05$). This indicates that the concentration of the added C source does not have a direct correlation with the stimulation effect on sediment microorganisms. Under anaerobic reactor cultivation, the sediments selectively uptake and transform C sources, consequently influencing the P release and internal cycling.

Redundancy analysis (RDA) and correlation tests were conducted

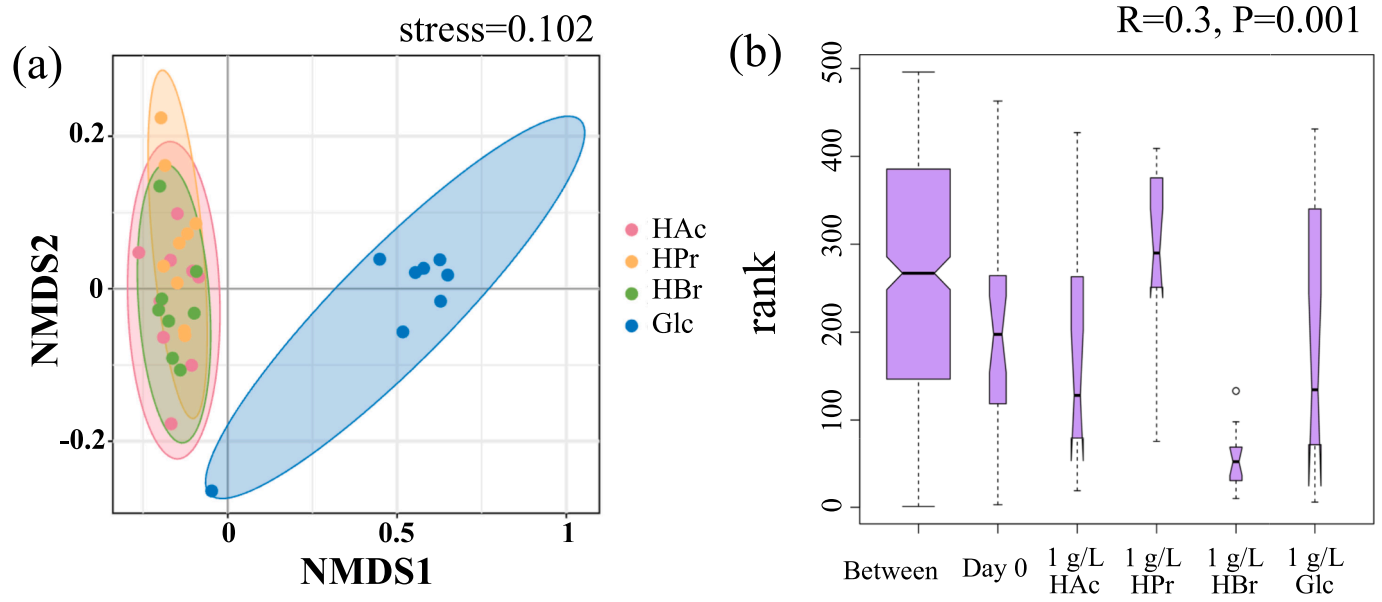

Fig. 3. The comparison of microbial structure at family level: (a) within different carbon-fed systems on day 15 using Anosim analysis; (b) within 1 g/L carbon dosing in four systems, for this analyses variable the day 0, 9, 12 and 15 were considered (HAc: acetic acid, HPr: propionic acid, HBr: butyric acid, Glc: glucose).

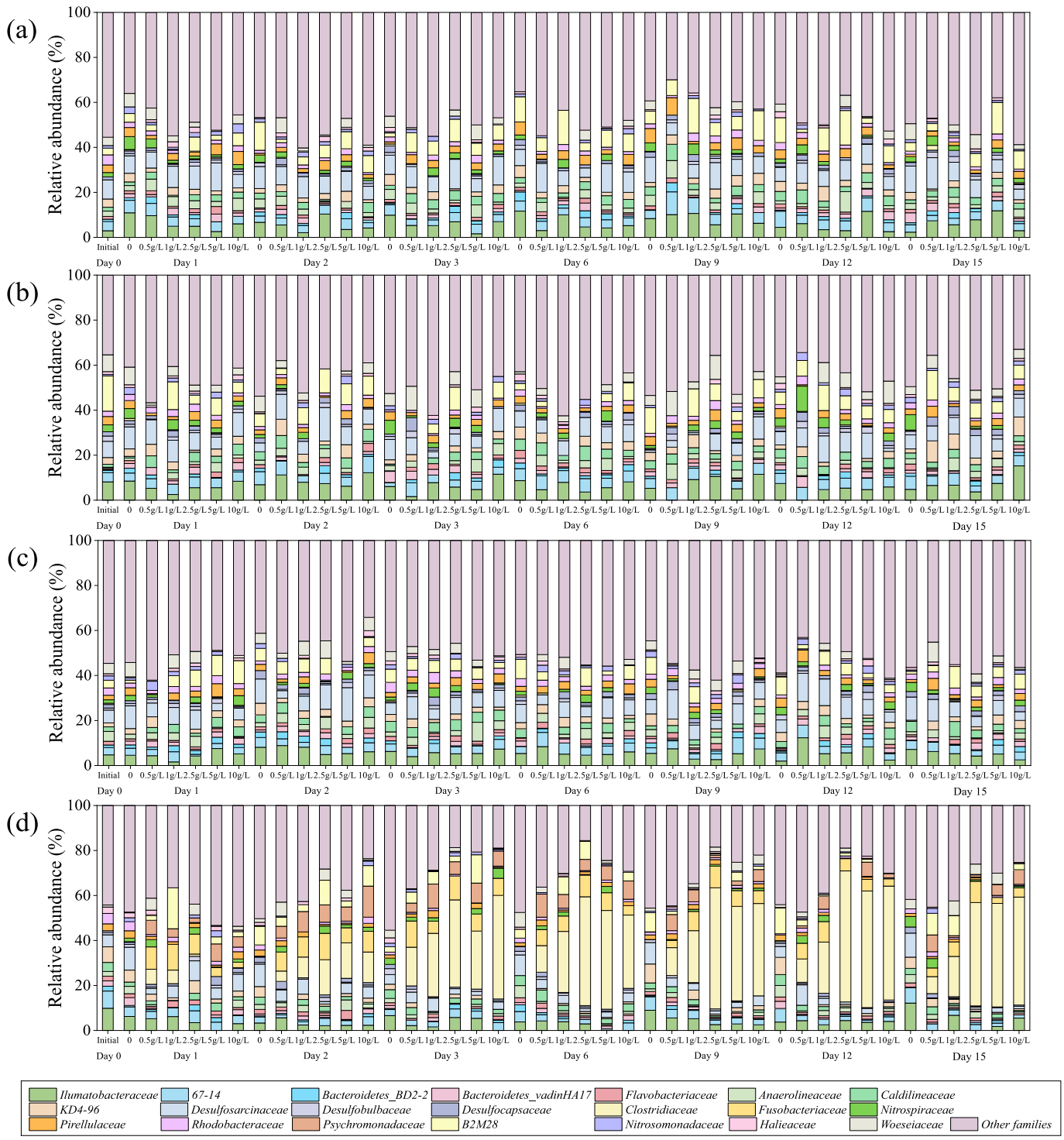

Fig. 4. Compositional structure of microbial communities at family level under different concentrations with four types of carbons-fed systems: (a) acetic acid-, (b) propionic acid-, (c) butyric acid- and (d) glucose fed system.

(Fig. 5). RDA interpreted the variances of the first and second axes of 56.2 % and 30.0 % in the acetic acid-fed system, 52.5 % and 21.9 % in the propionic acid-fed system, 53.9 % and 24.7 % in the butyric acid-fed system, and 94.2 % and 3.2 % in the glucose-fed system, respectively. The results showed that the first-axis of bacterial microbial communities separated most of environmental variables and different C dosages, especially for the glucose-fed bioreactors. *Clostridiaceae* plays a significant role in the decomposition and fermentation of OM, contributing to the production of organic acids, including VFAs. Although its initial abundance was low (almost 0 %), the addition of C sources, especially glucose, rapidly accelerated its enrichment in the anaerobic reactors. This transformation led to an overall microbial community structure that favors the rapid utilization of C sources and processes conducive to decomposition and fermentation. As the correlation analysis illustrated in Fig. 5(h), the abundance of *Clostridiaceae* is notably influenced by the dosage of glucose ($cr = 0.64$, $p < 0.05$). Meanwhile, the dominance of *Clostridiaceae* in the fermentation reactions significantly dropped the pH in anaerobic systems.

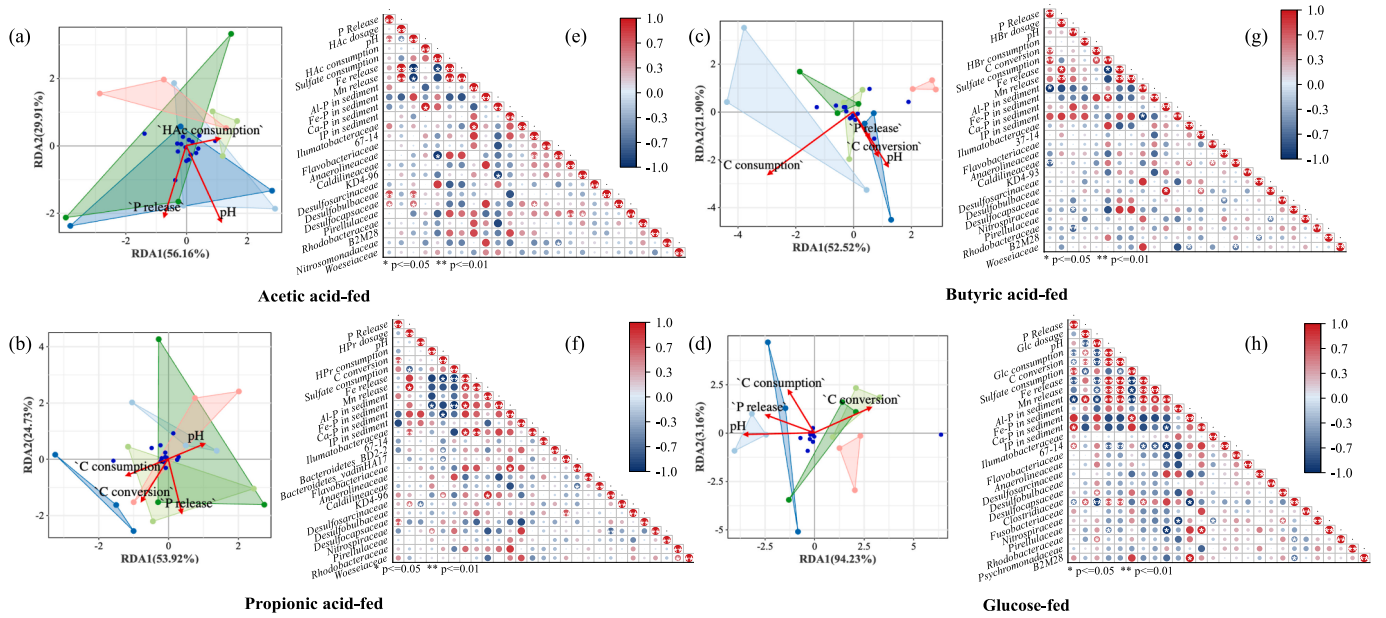

Fig. 5. Redundancy analysis for the relationships between environmental factors and microbial community for (a) acetic acid-, (b) propionic acid-, (c) butyric acid- and (d) glucose fed system; Pearson correlation analysis between the relative abundance of bacteria at the family level and different factors (e) acetic acid-, (f) propionic acid-, (g) butyric acid- and (h) glucose-fed system. For these analyses variables on day 3, 9 and 15 were considered.

The role of *Clostridiaceae* in sediment involves the utilization of sulfate, thiosulfate, S, and nitrate as electron acceptors, which has been extensively reported [42]. Additionally, as a representative fermentative anaerobic bacterium, it also actively participates in the decomposition of OM and electro-fermentation reactions [43], yielding products such as hydrogen and methane [44,45]. Previous studies have indicated that *Clostridiaceae* can get enriched in the presence of electron sinks [46]. The experimental results from Toledo-Alarcón et al. [43] exhibited a positive correlation between hydrogen yield and the abundance of *Clostridiaceae*, as well as butyrate accumulation, which is indeed with our results. The Pearson correlation analysis demonstrated a significant positive correlation between the abundance of *Clostridiaceae* and butyric acid production ($r = 0.892$, $p < 0.001$) in our experiment. Moreover, we observed a significant negative correlation between pH and the abundance of *Clostridiaceae*, suggesting that as pH decreased, the proliferation of *Clostridiaceae* was likely enhanced, further contributing to the formation of VFAs.

In three VFAs-fed systems, the abundance of the above-mentioned families remained consistently low during the anaerobic operation, with a total abundance of less than 1 %. The correlation analysis indicated a positive correlation between C conversion and P release in VFAs-fed systems, in contrast to a negative correlation in the glucose-fed system (Fig. 5). Therefore, it can be concluded that the addition of different C sources led to variations in the metabolic pathways for C utilization in sediment. It is here speculated that feeding with high concentrations of C, especially those conducive to electron transfer, which are easily utilized by microorganisms, may result in the rapid proliferation of fermentative bacteria under anaerobic conditions. If this process predominates, the process of OM decomposition and the release of P may be inhibited, and in some cases, P might be depleted due to biomass production, or even complexed with metal ions during the fermentation. Similar results have been observed in previous studies. Lin et al. [47] investigated the organic resources recovery from sewage sludge through alkali pretreatment and acidogenic fermentation, where no PO_4-P was detected in Al-$NaHCO_3$ and Al-Na_2CO_3 pretreated reactors after the fermentation period. Additionally, it is noteworthy that the enrichment of *Clostridiaceae* inhibited PAO, showing a significant negative correlation with the family *Rhodobacteraceae* ($p < 0.05$, as presented in Fig. 5(h)). The function of PAO involves utilizing C under anaerobic conditions, releasing P from poly-P [48]. This may indirectly confirm the inhibitory effect of fermentative bacteria proliferation on P release.

Furthermore, it is also important to highlight the significant contribution of sulfate-reducing bacteria (SRB) in sediment P cycling, as they are involved in the decomposition of OM and the reduction of sulfate [49–51]. The data demonstrated that the abundance of phylum Desulfobacterota or the sum of three SRB families *Desulfosarcinaceae*, *Desulfobulbaceae*, and *Desulfocapsaceae* consistently represented around 10 % of the total microbial abundance (Fig. S8 & Fig. 4). SRB primarily facilitate P release in sediment through anaerobic respiration, OM decomposing and reducing sulfate to generate S^{2-} and H_2S. This promotes the formation of FeS, the reduction of Fe(III), and indirectly induces the release of Fe-P in sediment [50]. In our study, in the low C-fed groups (0.5, and 1 g/L), more than 95 % of sulfate was consumed (Fig. S6), while in groups with no external C addition or high C addition, the average consumption was below 60 %. Correlation analysis indicated that SRB families play a significantly positive role in the release of P in the acetic acid and propionic acid-fed systems ($p < 0.05$).

Although SRB can also participate in the oxidation of acetate and the utilization of formate to produce CO_2 [51]; in our experiment, we observed significant negative correlations ($p < 0.05$) between the concentration of added glucose and the abundance of phylum Desulfobacterota, as well as the combined abundance of three SRB families, with correlation coefficients of −0.36 and −0.315, respectively. Due to the inhibition of SRB at high glucose concentrations, the corresponding reduction of sulfate has consistently been restricted. This leads to the suppression of P release, partly explaining why high concentrations of C addition do not promote P release.

3.6. Potential metabolic activities induced by varying carbon stimulation

The potential functional characteristics of microbial metabolism were examined through metagenomic analysis, revealing functional genes were mainly distributed in carbohydrates, amino acids, energy, and metabolism of cofactors and vitamin (Fig. S10). The study focused on genes involved in P cycling, particularly those related to IP and OP solubilization, P uptake and transport, and the regulation of P-starvation responses (Fig. 6(a)). For example, under anaerobic conditions, P release

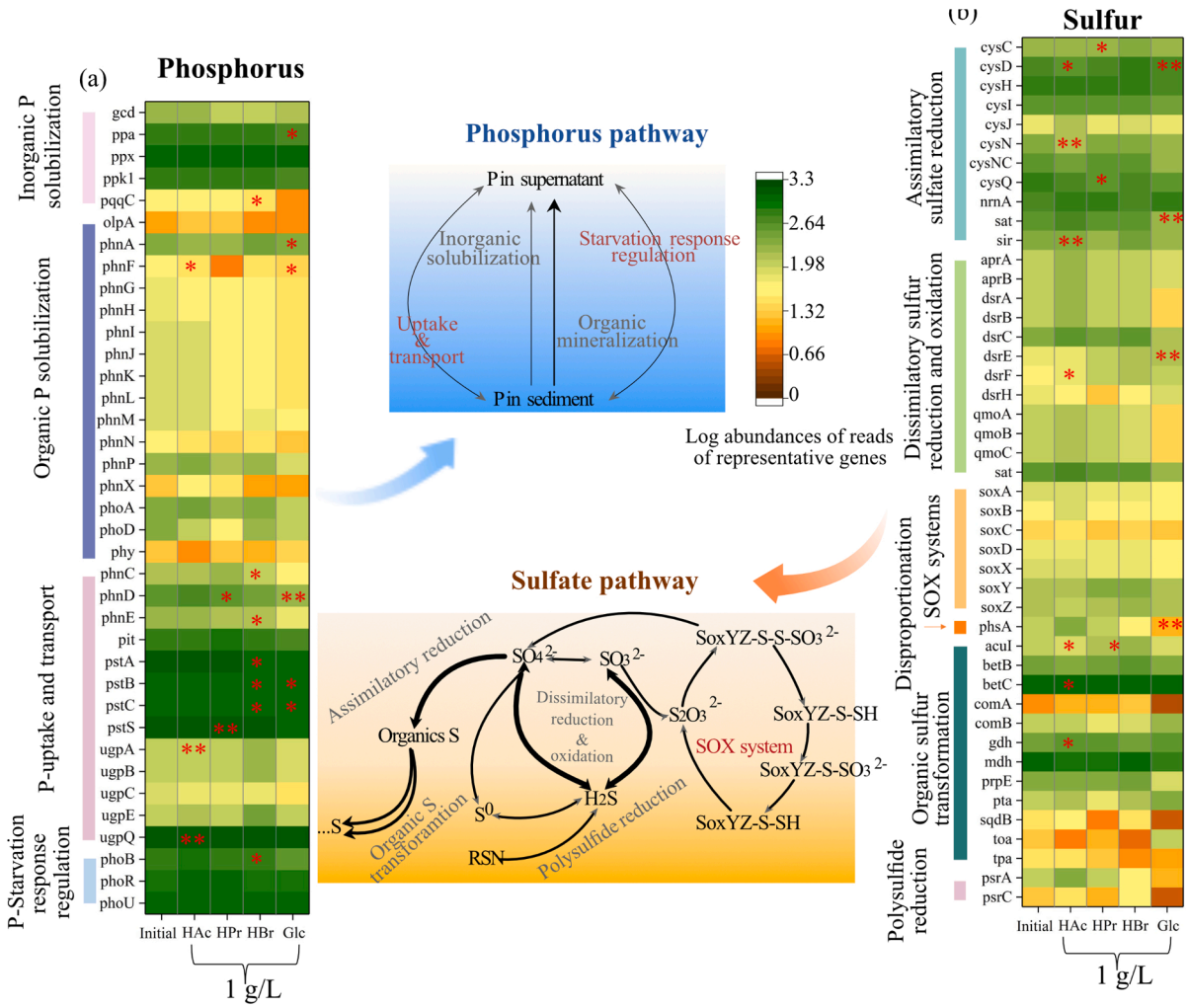

Fig. 6. The abundance key genetic genes in the anaerobic reactors for initial, 1 g/L acetic acid- (HAc), propionic acid- (HPr), butyric acid- (HBr), and glucose- (Glc) fed systems involved in (a) P and (b) sulfur-cycling; and corresponding genetic potential for several steps of the P and sulfur cycling pathway (Using the paired sample *t*-test to test differences for genes between initial and Day 15, *: $p < 0.1$ regarded as a trend, and **: $p < 0.05$ regarded as significant).

was predominantly regulated by genes associated with P uptake and transport, such as phnD, pstS, ugpA, and ugpQ, which showed significant alterations in their abundance during the 15-day period ($p < 0.05$, Fig. 6a). These shifts indicate that different C sources not only influence the microbial community composition but also the metabolic pathways activated over time, particularly those related to nutrient cycling.

Sulfate levels, a crucial electron acceptor promoting P release from sediment under anaerobic conditions, are mainly regulated by key genes associated with assimilatory sulfate reduction [52]. As depicted in Fig. 6 (b), genes such as cysD, cysNC, sat, and sir showed significant changes compared to initial values ($p < 0.05$). Previous studies have indicated a close connection between microbial communities involved in P and S cycles, supported by significant positive correlations between P and S concentrations and functional gene abundances (e.g., phoD, cysD) [35]. Our study similarly revealed a significant correlation between S and P-related genes, as illustrated in Fig. S11. Specifically, in each C-fed system, notable correlations were observed within the phn, pst, and cys groups of genes, with consistent significant positive correlations ($p < 0.05$) between sat and phnD.

Environmental factors significantly impact gene expression, particularly evident in the glucose-fed system (Fig. S11(d)). With increasing sulfate consumption, more P was released; however, this was accompanied by a downregulation of regulatory genes for P release. This further underscores the interconnection of S and P functional genes. Additionally, key genes involved in N and C cycling, such as narI, nirB, and porB, are regulated by those associated with dissimilatory nitrate reduction and anaerobic carbon fixation, as shown in Fig. S12.

Incorporating C metabolism, during the initial phases, easily degradable C sources such as glucose rapidly stimulate microbial activity. This leads to a quick increase in VFAs production as microbes predominantly engage in glycolysis and fermentation pathways, converting into VFAs. As easily degradable C sources are consumed, the microbial community shifts towards the metabolism of more complex organic matter, which is slower to break down. Over time, the microbial community adapts to the prevailing environmental conditions and available substrates. For instance, in the glucose-fed systems, the initial high production of VFAs may decrease as the microbial community shifts towards pathways associated with biomass synthesis, consuming available P and C. This shift reduces the net production of VFAs as the microbes divert energy towards growth rather than fermentation, which naturally influences P release dynamics. As observed in Fig. S12, the abundance of anaerobic C fixation genes decreased after 15 days only in the 1 g/L glucose system, suggesting a shift in microbial metabolic activities towards pathways that may facilitate P release. This indicates that the choice of C source can steer microbial metabolic pathways towards either enhancing or inhibiting P release, depending on the community structure and the metabolic capabilities of the dominant organisms. It is important to note that these findings, based on PiCRUST

predictions, are hypothetical. More direct methods, such as meta transcriptomics or proteomics, would be needed to confirm these proposed mechanisms.

Overall, when considering the evolution of the microbial community, these findings emphasize the importance of time-dependent shifts in metabolic pathways when assessing the impact of different C sources on P release. For example, the enrichment of families such as *Clostridiaceae*, while promoting OM decomposition, also led to increased utilization of released P, thus hindering its recovery. The critical role of SRB in VFAs-fed systems was validated, as different C sources modulated key genes involved in P uptake and sulfate reduction, thereby influencing the overall efficiency of P release under anaerobic conditions. Therefore, understanding these shifts is crucial for optimizing the conditions needed to maximize P recovery. This knowledge can inform the design of strategies that target specific stages of microbial succession to enhance P release efficiency.

3.7. Implications of the study

Our research aims to enhance the recovery of P from marine sediments. Inspired by resource recovery technologies from waste streams, we conducted experiments using batch reactors to investigate the effects of different types and concentrations of C sources on P release from sediments. The results confirmed that the addition of extraction C sources can indeed increase P release from marine sediments under anaerobic conditions.

While our current study has limitations, particularly in terms of direct application to full scale engineering, such as the fact that the PO_4-P concentration in the solution obtained after 15 days of operation and filtration was less than 10 mg/L, making it challenging to efficiently recover P through precipitation, the limitations might be related to the TS content of our reactors being only 4 %. To achieve a higher concentration of P in the solution, increasing the TS content of the sediments and scaling up the reactor could be potential solutions. Additionally, the Baltic Sea's unique sediment composition, particularly its brackish conditions, differs from fully saline oceans. While findings may vary by location, the observed trends in P release are likely relevant to other marine sediments. Future work should account for site-specific differences when applying these results.

The primary focus of this study was to elucidate the biological mechanisms behind the promotion of OP release by adding C sources. Previous research has extensively reported the release of IP through the addition of acids, bases, or chelating agents. However, in our experiment, the maximum OP release detected in the filtered supernatant was only about 20 % after 15 days of anaerobic reaction. Microbial analysis also indicated that the natural abundance of functional bacteria capable of promoting P release, including PAOs, was relatively low in the native sediment. This could explain the relatively low OP release rate observed. Therefore, future research should consider using reactors with longer operation that can enrich functional organisms, such as sequencing batch reactors (SBRs). The findings from this study could provide valuable insights into how organisms adapt to C external feeding during the anaerobic phase of SBRs, potentially improving OP release efficiency. Additionally, while the release of IP has been demonstrated in previous studies, combining strategies for releasing both OP and IP could maximize P recovery. Such enhanced strategies could be broadly applied to the practical recovery of P from sediments in the future.

4. Conclusion

C addition stimulated the P release from the sediments under anaerobic condition. For instance, the addition of 1 g/L propionic acid and glucose resulted in the release of 5.98 and 6.44 mg/L P, respectively, representing a 32 % and 44 % increase compared to the control group. Under anaerobic conditions, there is an optimal concentration of extra C sources addition in stimulating P release. We observed that, when an excess of C is added, microbial utilization of P intensifies, resulting in a decrease in P concentration in the supernatant. Notably, in glucose-fed systems at feeding concentrations of 5 g/L and 10 g/L, the P concentration in the supernatant decreased from 5 mg/L on Day 3 to around 3 mg/L on Day 15. Microbial analysis revealed that microbial communities within different C sources-fed systems underwent distinct evolution over 15 days. The addition of different C sources led to variations in the metabolic pathways of C utilization in the sediment. For instance, feeding high concentrations of glucose resulted in rapid enrichment of fermentative bacteria under anaerobic conditions, while SRB contributed to improving P release in VFA-fed systems. The addition of different C sources influenced metabolic pathways under anaerobic conditions. These pathways, especially those involved in nutrient cycling, are interconnected and play a crucial role in P release. For example, a strong positive correlation was observed between the abundance of P and S cycling genes, such as phoD and cysD. Our findings emphasize the importance of selecting appropriate C sources and concentrations, and understanding microbial succession to optimize conditions for maximizing P recovery in bioremediation processes.

CRediT authorship contribution statement

Fengyi Zhu: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elena Radaelli:** Software, Formal analysis. **Giorgia Palladino:** Writing – review & editing, Software, Formal analysis. **Chen Chen:** Writing – review & editing, Formal analysis. **Andressa Mazur:** Formal analysis. **Frederico Marques Penha:** Writing – review & editing, Supervision, Project administration, Data curation, Conceptualization. **Maria Cuartero:** Writing – review & editing, Project administration, Data curation. **Silvia Turroni:** Writing – review & editing, Project administration, Funding acquisition, Data curation, Conceptualization. **Zeynep Cetecioglu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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 data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2024.157058.

Data availability

Data will be made available on request.

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