



Enhancing phosphorus mobilization from sediments toward recovery via carbon-stimulated sulfate reduction under anaerobic conditions

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ABSTRACT

Mobilization of sedimentary phosphorus (P) for subsequent recovery is a promising strategy to mitigate long-term eutrophication and alleviate global P resource shortages, yet the coupled biogeochemical mechanisms controlling this process remain poorly understood. In this study, anaerobic batch reactors were used to examine the individual and combined effects of glucose (1 g/L) and sulfate (up to 8 mM) addition on P release from the Baltic Sea sediments. Combined glucose and sulfate addition markedly enhanced dissolved P release compared with single-factor treatments and controls. Early-stage enhancement (Day 12 of a 36-day incubation) was dominated by inorganic P (IP) release (~10%), likely driven by sulfate reduction and sulfide-mediated Fe-P dissolution. In the later stage (Day 36), IP removal in the 8 mM sulfate treatment decreased to 1.4%, suggesting P re-retention in the sediments, whereas organic P (OP) mobilization increased to 18%, indicating a shift towards OP mineralization as the main release pathway. Microbial community analysis revealed that sulfate addition under glucose-rich conditions had limited effects on overall taxonomic composition, but induced functional shifts associated with P cycling, particularly genes related to P mineralization during glucose depletion and increasingly reducing conditions. Sulfate may appear to promote the conversion of butyrate to acetate/propionate, potentially enhancing energy availability for microbial OP mineralization. Overall, this study provides mechanistic insights into carbon-sulfur-P coupling in brackish sediments, offering a scientific basis for designing strategies to enhance sediment P mobilization toward downstream recovery and internal P loading control.

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1. Introduction

Phosphorus (P) is an essential element for agricultural fertilizers and a critical raw material for numerous industrial products (i.e., detergents, feed additives) (Zhu et al., 2023). However, P is a non-renewable resource that is primarily obtained through phosphate rock mining (Azizi, 2018; March et al., 2016). Production is similarly dominated by limited regions, with China, Morocco, and the United States accounting for 45.8%, 12.5%, and 8.3%, respectively, of the world's total phosphate output (USGS, 2025). Meanwhile, the European Union (EU) faces a severe shortage of P resources and depends on imports for 84% of its phosphate rock demand and nearly 100% of total P consumption (Zhu et al., 2023), leaving it vulnerable to geopolitical and supply chain disruptions (Nedelciu et al., 2020; Yang et al., 2019).

Meanwhile, natural P reserves are being rapidly depleted (Reijnders, 2014), while excessive discharge of P through wastewater and soil erosion has led to severe eutrophication of aquatic ecosystems (Comber et al., 2013; Ngatia et al., 2019; Withers and Haygarth, 2007). In Europe, the Baltic Sea is one of the most affected regions (Andersen et al., 2017; Stigebrandt and Andersson, 2020). The report of HELCOM (2023) identified eutrophication as one of its most pressing environmental challenges. Most mitigation strategies to date have targeted external nutrient loads, yet "legacy P" accumulated in sediments, often referred to as internal P sources, continues to drive eutrophication for decades (Müller-Karulis et al., 2024). In fact, internal P sources have been recognized as the primary driver of Baltic Sea eutrophication since the late 1990s (Stigebrandt and Andersson, 2020). Addressing internal P pollution through in situ sediment treatment or nutrient recovery therefore holds significant potential for both water quality improvement and circular nutrient use.

Under anoxic or anaerobic conditions, the release of sediment-bound P is closely linked to carbon-sulfur-iron-P interactions (Wu et al., 2019; Zhao et al., 2021). Labile carbon (i.e., glucose) inputs can stimulate microbial fermentation and generate volatile fatty acids (VFAs), which serve as electron donors for anaerobic respiratory processes (Zhu et al., 2024). Sulfate, when present, can function as an electron acceptor, driving sulfate reduction and sulfide formation. The produced sulfide can react with Fe minerals to form FeS, releasing Fe-bound inorganic P (IP), and may also promote the mineralization of organic P (OP). Such processes are often facilitated by sulfate-reducing bacteria (SRB) but can also proceed via chemical sulfide-iron reactions or other microbial pathways (Han et al., 2023; Wu et al., 2019; Zhao et al., 2021). Despite growing recognition of these coupled processes, the specific effects of co-supplementing readily degradable carbon and sulfate, particularly on IP/OP mineralization, P fraction transformations, and associated functional gene dynamics, remain insufficiently understood. Clarifying these effects is essential for designing strategies that deliberately stimulate sedimentary P mobilization as a first step toward its controlled recovery and recycling.

Previous studies have investigated sulfate- and carbon-related P mobilization in freshwater lake sediments, often under scenarios driven by natural oxic-anoxic interfaces or temperature fluctuations (Chen et al., 2021, 2016; Han et al., 2023). These studies generally highlight that Fe redox cycling, and organic matter decomposition jointly regulate sedimentary P mobility, but most evidence is derived from freshwater systems with relatively low background sulfate. In contrast, brackish coastal sediments such as those in the Baltic Sea naturally contain millimolar sulfate in porewater, where sulfate reduction may represent a more persistent and competitive terminal electron-accepting process under anoxic conditions. Notably, our previous work also showed a high baseline abundance of SRB in the native Baltic Sea sediments (Zhu et al., 2024).

However, the dose-dependent effects of sulfate addition under defined electron-donor enrichment (e.g., glucose) remain poorly constrained in brackish sediments. In particular, it is still unclear how increasing sulfate availability regulates the balance between P mineralization, and how these transformations are temporally coupled to microbial functional shifts (e.g., fermentation, sulfate reduction, and P cycling genes). To address this knowledge gap, we conducted a series of anaerobic batch reactor experiments using Baltic Sea sediments with a sulfate concentration gradient (1–8 mM), with and without glucose addition. By integrating dissolved geochemical indicators, sequential P fractionation, and microbial community and functional gene prediction analyses, this study systematically elucidates the coupled carbon-sulfur-P mechanisms controlling IP and OP mobilization. This integrated approach provides a systematic basis for optimizing electron donor/acceptor manipulation to enhance controlled P mobilization, thereby supporting the development of in situ or engineered strategies for sustainable P recovery from sediments. In this context, P release refers to the transformation and transfer of sediment-bound P (IP/OP) into dissolved phosphate in the aqueous phase under anaerobic incubation, whereas P recovery refers to the subsequent engineered capture or separation of dissolved P from water (e.g., via adsorption, precipitation, or membrane concentration) (Zhu et al., 2023). Therefore, the present work focuses on the mobilization step as a prerequisite for controlled P recovery.

2. Materials and methods

2.1. Set up of anaerobic bioreactors

Sediment samples were collected on March 31, 2023, from the Baltic Sea region (N 58°46.5769', E 17°26.1202'), with basic sediment properties detailed in Table S1. Each reactor contained 16 g of fresh sediment, which was placed in a 120 mL serum bottle with a working volume of 80 mL. The working volume was achieved by adding synthetic Baltic seawater (6.5 psu), prepared using deionized water and NaCl to match the average salinity of the Baltic Sea. The total solids (TS) content of the sediment in the reactors was 3.6%. In our previous tests, the TS of the original sediment after removing overlying water typically ranged from approximately 18–20% (w/w), representing a consolidated sediment matrix with limited supernatant after long-term settling. However, such a high-solid system is not suitable for batch incubation aiming to quantify P release into the liquid phase, because it provides insufficient supernatant for repeated sampling and limits homogeneous mixing during incubation. Therefore, to allow reliable measurement of

dissolved P in the supernatant and to ensure well-mixed and reproducible conditions across treatments, we adopted a slurry incubation with TS around 4%-5%.

All treatments were set up in triplicate (Table 1), with the following groups: control (no carbon or sulfate added); experimental groups, including 1 g/L glucose (S0), 1 g/L glucose + 1 mM sulfate (S1), 1 g/L glucose + 2 mM sulfate (S2), 1 g/L glucose + 4 mM sulfate (S4), and 1 g/L glucose + 8 mM sulfate (S8); and a comparison group, with 8 mM sulfate only, without glucose (no Glc + S8). The glucose concentration was set at 1 g/L according to our previous study, which demonstrated that this dosage provides sufficient labile carbon to effectively stimulate microbial activity and P release, while avoiding excess organic substrate that could otherwise lead to uncontrolled fermentation or inhibitory effects (Zhu et al., 2024). Sulfate concentrations are reported in millimolar (mM), with equivalent values in mg/L (1 mM sulfate = 96.06 mg/L) provided in the Results and discussion section for ease of comparison. The background sulfate concentration in the sediment porewater prior to amendments was 4.48 mM (Table S1). The sulfate amendment range (1–8 mM) was selected to establish a dose-response concentration gradient relative to the porewater baseline and to represent enhanced sulfate availability conditions for mechanistic evaluation of sulfate-driven redox processes and associated P mobilization.

All reactors were sealed with septa and aluminum caps, and flushed with nitrogen gas for over 5 min to provide an anaerobic environment. The bioreactors were operated at room temperature, with sampling occurring at 0, 1, 2, 3, 6, 9, 12, 18, and 36 days, and mixing at 150 rpm. Each group had 3 parallel reactors, resulting in a total of 189 reactors for destructive sampling. On each sampling day, a total of 21 reactors were analyzed (7 treatments $\times$ 3 replicates). Independent reactors were destructively sacrificed at each sampling time (i.e., each bottle was sampled only once). The sediment slurry was immediately centrifuged at 4 °C (4500 rpm, 10 min), and the supernatant was collected and filtered through a 0.45 μ m filter for dissolved-phase analyses (PO₄-P, NH₄-N, SO₄²⁻ and dissolved metals). The remaining sediment pellet was collected for sequential P fractionation and microbial community analysis. To minimize oxidation, reactors were kept sealed until sampling and all sample handling steps were performed rapidly. Sediment samples were stored at –20 °C prior to DNA extraction and P fractionation analysis.

2.2. Analytical methods

The concentration of PO₄-P in the supernatant was determined using the Murphy and Riley colorimetric method (Cho and Nielsen, 2024). NH₄-N was measured with commercial cuvette test kits (LCK 303, Hach Lange, Germany) using a DR 3900 spectrophotometer (Hach Lange). Sulfate concentrations were analyzed via ion chromatography (850 Professional IC Anion, Metrohm, 2.850.2030). pH levels were monitored with a benchtop pH meter (FiveEasy™, FE20, Mettler Toledo). VFAs produced during glucose metabolism, were identified and quantified by gas chromatography (GC) using an Agilent Intuvo 9000 equipped with a CP-Sil 5 CB column (25 m $\times$ 0.32 mm $\times$ 5 μ m, Agilent) and a flame ionization detector. The chemical speciation of P in the sediment was analyzed through a sequential extraction procedure, as described by Jiang et al. (2011) to assess the transformations of P forms under anaerobic conditions. Concentrations of Fe and Mn ions in the supernatant were quantified using inductively coupled plasma optical emission spectrometry (ICP-OES; Thermo Scientific iCAP 7000 Plus).

2.3. Microbial community analysis and functional annotation

Microbial genomic DNA was extracted from samples using the DNeasy PowerSoil Pro Kit (Qiagen, Germany) following the manufacturer's instructions. DNA extracts were stored at –20 °C and subsequently sent to Novogene Europe for 16S rRNA gene amplicon sequencing and bioinformatics analysis.

Briefly, the V3-V4 region of the 16S rRNA gene was amplified using primers 341 F and 806 R, each containing a unique barcode. PCR reactions were performed in 15 μ L volumes using Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μ M of each primer, and ~10 ng of template DNA. Thermal cycling included an initial denaturation at 98°C for 1 min; 30 cycles of 98°C for 10 s, 50°C for 30 s, and 72°C for 30 s; followed by a final extension at 72°C for 5 min. PCR products were purified with magnetic beads and pooled in equimolar concentrations. Sequencing libraries were constructed with appropriate adapters and quality-checked using Qubit, qPCR, and a Bioanalyzer. Libraries were sequenced on an Illumina platform. Paired-end reads were demultiplexed, quality-filtered with Fastp (v0.23.1), and merged using FLASH (v1.2.11). Chimeric sequences were removed using Vsearch (v2.16.0) with the SILVA database (v138.1). Amplicon sequence variants (ASVs) were generated using DADA2 in QIIME2 (v2022.2). Taxonomic classification was performed using QIIME2 with the SILVA database. For unclassified reads, additional annotation was conducted using the Micro_NT sub-database from NCBI, with the least common ancestor (LCA) algorithm (Bokulich et al., 2013; Edgar et al., 2011; Magoč and Salzberg, 2011; Wang et al., 2021).

Table 1

Experimental setup for different reactors.

Group Name	Glucose (g/L)	Sulfate (mM)	Description
Control	0	0	No external carbon source or sulfate
S0	1	0	Glucose only
S1	1	1	Glucose + low sulfate
S2	1	2	Glucose + moderate-low sulfate
S4	1	4	Glucose + moderate sulfate
S8	1	8	Glucose + high sulfate
no Glc + S8	0	8	Sulfate only (no glucose added)

PICRUSt2 (v2) was utilized by Novogene with default parameters to predict microbial functional profiles based on the ASVs identified from 16S rRNA amplicon data. The predicted functions were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology (KO) database. This analysis focused on pathways associated with P cycling under anaerobic conditions, along with functions related to the transformation of sulfur, carbon, and iron, which are closely linked to P release mechanisms.

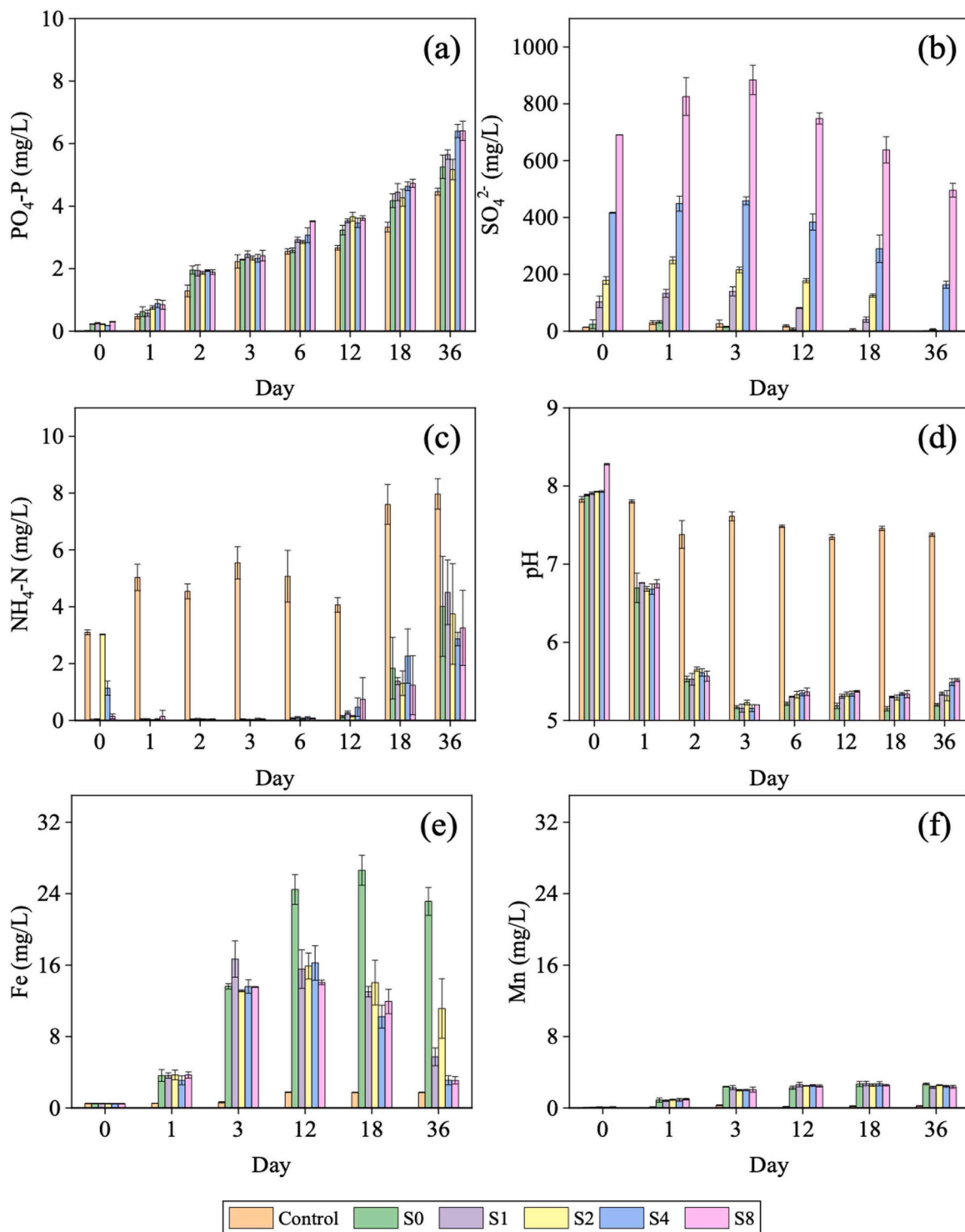


Fig. 1. Variations in (a) $\text{PO}_4\text{-P}$ concentration; (b) sulfate concentration; (c) $\text{NH}_4\text{-N}$ concentration; (d) pH; (e) dissolved Fe concentration; and (f) dissolved Mn concentration over the incubation period.

The raw 16S rRNA sequence data can be found online in the NCBI database with the BioProject ID number: PRJNA1294851.

2.4. Statistical analysis

All statistical analyses were performed using R version 4.3.1. Microbial community structure was analyzed via non-metric multidimensional scaling (NMDS) visualization and permutational multivariate analysis of variance (PERMANOVA). Predicted functional gene expression profiles were derived from KEGG KO tables generated by PICRUSt2. KO abundances associated with P metabolism, sulfur cycling, and carbon transformation were extracted and summarized by treatment group (mean of triplicates). Values were transformed using a strict $\log_2(x)$ approach. Differential expression relative to the initial condition was evaluated using Welch's two-sided *t*-test on $\log_2$ -transformed replicate values. Significant changes (FDR-adjusted $p < 0.05$) were annotated in heatmaps using directional arrows ($\uparrow/\downarrow$), with double arrows ($\uparrow/\downarrow$) indicating stronger shifts (FDR-adjusted $p < 0.01$ combined with fold-change thresholds). Microbial-environment associations were further tested using: (1) Spearman rank correlations for linear relationships, and (2) Mantel tests for distance matrix correlations. Significant links (FDR-adjusted $p < 0.05$) were visualized through integrated network-heatmap plots.

3. Results and discussion

3.1. The addition of sulfate on phosphorus release

Under anaerobic conditions, P release was significantly enhanced by the co-addition of external carbon and sulfate (Fig. 1(a)). The final dissolved P concentrations in S4 and S8 treatments (6.41 and 6.40 mg/L, respectively) were approximately 40% higher than the control (4.46 mg/L) after 36 days of incubation. The comparable P release between S4 and S8 treatments may be explained by the presence of residual sulfate that was not completely consumed, as evidenced by residual sulfate concentrations of 163 mg/L (60.8% consumption) and 496 mg/L (51.3% consumption) on Day 36, respectively. The stimulation of P release by sulfate addition may arise from multiple mechanisms. Although the sediment TS content in this study was relatively low (3.6%), this slurry-based design enabled homogeneous mixing and reliable quantification of dissolved P in the liquid phase during batch incubation. We acknowledge that such a well-mixed, high-moisture system differs from intact natural sediments with higher solid contents and stronger diffusion constraints; therefore, the P release mechanisms observed here should be interpreted within the context of slurry incubation conditions.

From a geochemical perspective, sulfate reduction under anaerobic conditions can promote the reductive dissolution of P-containing minerals, particularly iron-bound P (Fe-P), thereby liberating previously bound P into the porewater. Alternatively, sulfate may also influence microbial processes that accelerate P mobilization, an aspect that needs further validation through microbial community analyses. Previous studies have shown that sulfate reduction could promote the release of OP and Fe-P from sediments (Han et al., 2023), and elevated sulfate levels promote endogenous P release and soluble P fluxes across the sediment-water interface via sulfate-driven redox shifts (Zhang et al., 2024). In contrast, the sulfate-only treatment (no Glc + S8), which lacked an external carbon source, showed a final P concentration of 3.7 mg/L, which was slightly lower than the control (4.46 mg/L), and only $8 \pm 2\%$ of the supplied sulfate was consumed (Fig. S1(b)). These results indicate that the stimulatory effect of sulfate on P release depends strongly on the availability of external electron donors. Without additional labile carbon, the redox conditions remained relatively stable and sulfate reduction was limited, resulting in minimal sulfide generation and consequently little enhancement of P mobilization (Hao et al., 2014). Although sulfate addition promoted P mobilization in the present study, sulfate reduction may also generate H_2S , which could potentially cause environmental toxicity if released to the overlying water (Vaquer-Sunyer and Duarte, 2010). Moreover, sulfide-metal interactions may influence the mobility of trace metals through precipitation/dissolution and complexation processes (Sundelin and Eriksson, 2001). Notably, the maximum sulfate amendment applied here (8 mM) was within the millimolar range and only moderately higher than the measured porewater background level (4.48 mM; Table S1). Therefore, any potential application of sulfate amendment for P recovery should consider appropriate dosage control and monitoring/management of sulfide production and metal release.

The variation of NH_4 -N concentrations also differed markedly among treatments (Fig. 1(c)). In the control group, NH_4 -N concentrations increased continuously from Day 0 (from 3.1 to 8.0 mg/L), likely due to the mineralization of organic nitrogen in sediments (Ding et al., 2022). While in all glucose-supplemented groups (S0-S8), NH_4 -N concentrations followed a "decrease-increase" pattern: the initial decline may have resulted from microbial assimilation of NH_4 -N facilitated by external carbon, while the subsequent rise was likely due to ongoing organic nitrogen mineralization similar to the control group. pH values remained around 7.0 in the control group, whereas all glucose-supplemented treatments stabilized at approximately 5.2–5.5, likely due to acid production (e.g., VFAs) during glucose fermentation. Notably, sulfate-amended treatments exhibited slightly higher pH than the glucose-only treatment (S0) (Fig. 1(d)), which is consistent with the consumption of H^+ during reductive processes such as sulfate reduction (Braissant et al., 2007). However, the bulk pH in our reactors was dominated by fermentation-derived acidification, and therefore reflects the net balance between acid production and H^+ consumption.

For Fe/Mn release (Figs. 1(e), 1(f)), in the S0 group (glucose only), Fe concentrations peaked at 26 mg/L on Day 18, indicating active iron reduction. However, Fe concentrations subsequently declined in all glucose-fed groups (S0-S8). This trend suggests that sulfate addition may have dual effects on iron reduction. In the early stage, sulfide generated from sulfate reduction can chemically reduce Fe(III) minerals, thereby temporarily enhancing Fe(II) release (Poulton et al., 2004). At this stage, the concurrent increase in dissolved P concentrations more likely indicates enhanced dissolution of Fe-bound P (Fe-P) and subsequent P mobilization into the porewater/supernatant, rather than re-precipitation. The later decline in dissolved Fe suggests immobilization of Fe(II), potentially via

precipitation as FeS and/or other reduced Fe minerals, which may subsequently favor re-association of phosphate with the solid phase and reduce net IP release. Mn(II) concentrations, in contrast, remained at approximately 2 mg/L in all treatments except the control, possibly because Mn(II) has a weaker affinity for sulfide and thus precipitates less readily. In the control group, Fe and Mn concentrations showed negligible increase, suggesting that in the absence of external carbon and sulfate, metal reduction/dissolution processes were limited.

As shown in Fig. S2, sequential extraction of P fractions revealed that the enhancement of TP release in sulfate-addition treatments primarily originated from OP mobilization. The temporal shift from IP-dominated release at Day 12 to OP-dominated mobilization at Day 36 is clearly illustrated by comparing Fig. S2(b) and Fig. S2(c). Taking the S8 treatment on Day 36 as an example, TP removal reached 9.9%, with contributions of 1.4% from IP and 18.4% from OP. Interestingly, at the early stage of incubation (Day 12), the removal of IP exceeded that of OP (8.8% vs. 6.4% in S8), whereas the trend reversed in the later stages. In the glucose-only treatment (S0), TP removal on Day 36 was dominated by IP loss (3.3% IP vs. 0.6% OP), consistent with early-stage data (Day 12: 10.5% IP vs. 3.7% OP). Overall, these results suggest a temporal shift during our 36-day batch incubation, with early-stage IP release followed by enhanced OP mobilization in the later stage.

This shift suggests that net IP release decreased during the later phase, likely due to phosphate re-retention in the solid phase. The decline in dissolved Fe concentrations (Fig. 1(e)) indicates Fe removal from the aqueous phase, which could be associated with Fe-related phosphate re-retention; however, this cannot be confirmed based on dissolved Fe trends alone, since Fe may also be immobilized via FeS precipitation under sulfate-amended sulfidic conditions. Therefore, the late-stage decrease in net IP release is more plausibly associated with phosphate re-retention processes (e.g., re-adsorption and/or retention onto mineral surfaces) (Gomez et al.,

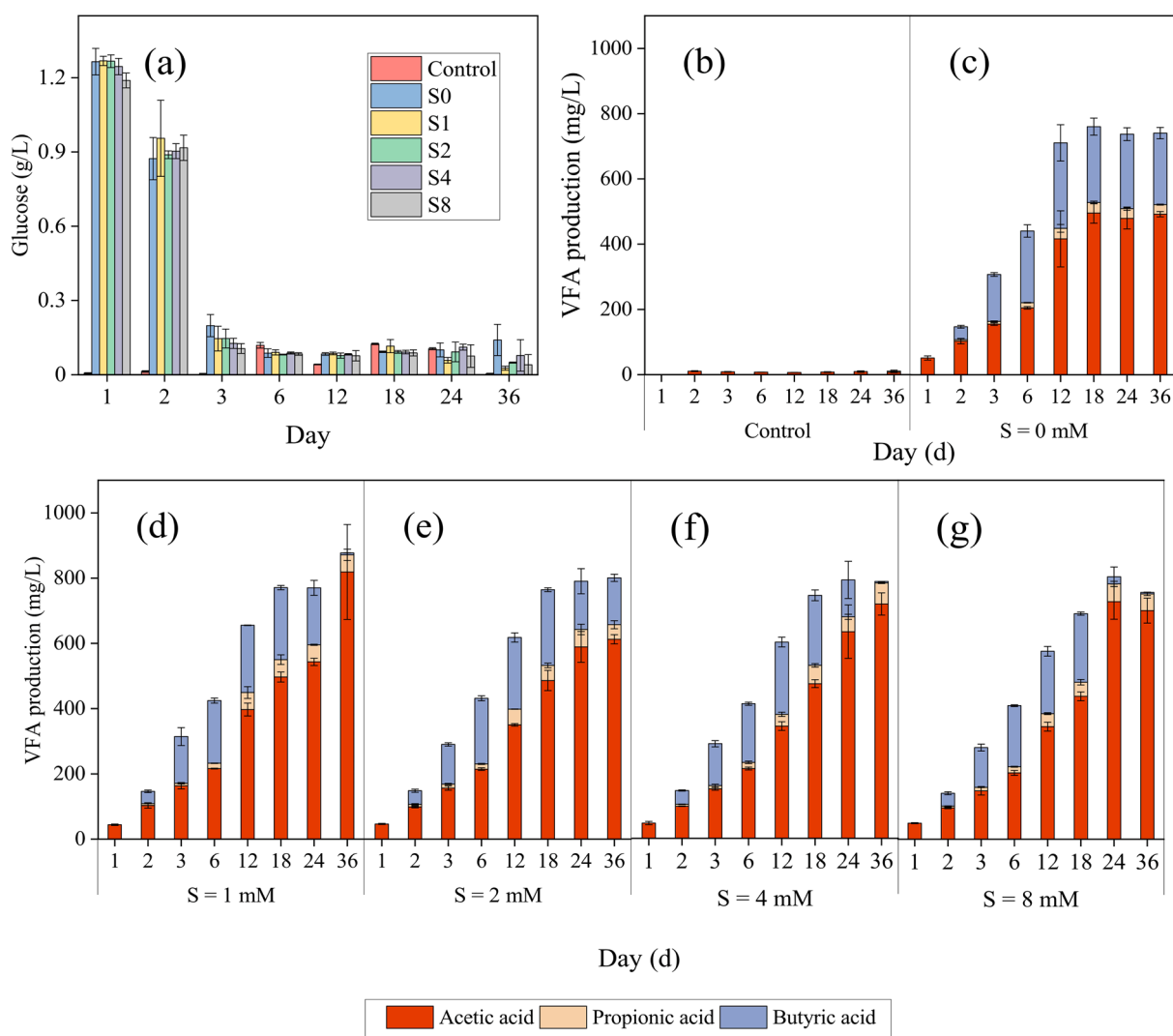


Fig. 2. (a) Glucose consumption dynamics and VFAs production profiles across experimental groups: (b) Control, (c) S0 (glucose only), (d) S1, (e) S2, (f) S4, and (g) S8. VFA speciation (acetate, propionate, butyrate) and concentrations are shown in the Figure (b)-(g).

1999), potentially occurring in parallel with FeS formation. Meanwhile, the “no-Glc S8” treatment showed limited Fe mobilization and no obvious late-stage decline in net IP release (Fig. S3). It is worth noting that direct identification of secondary Fe minerals was not conducted in this study, and newly formed phases may be poorly crystalline and difficult to detect in complex sediments. Therefore, the specific mineral forms remain uncertain and require further verification using techniques such as X-ray absorption spectroscopy and scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy.

We observed that the pronounced OP release occurred only when both sulfate and an external carbon source (glucose) were supplied, whereas no comparable trend was found in treatments with either carbon or sulfate alone. An explanation is that the co-presence of an electron donor and an efficient electron acceptor enables sediment microorganisms to exploit additional metabolic pathways, thereby accelerating OP hydrolysis and mineralization. This synergistic effect may involve the disruption of OP complexes by sulfide produced via sulfate reduction, releasing OP for microbial decomposition and ultimately increasing dissolved P concentrations. This interpretation is consistent with Zhang et al. (2024), who reported that sulfate reduction in the presence of lactate not only mobilized Fe-P but also markedly stimulated OP mineralization, with OP depletion accounting for more than 80% of the total TP loss in surface sediments of eutrophic lakes. It should be noted that the above temporal dynamics were observed under well-mixed laboratory batch incubation conditions over 36 days. P cycling in natural sediments typically occurs over longer time scales and is further affected by sediment stratification, and natural changes in redox conditions (Katsev and Dittrich, 2013; Sun et al., 2022). Therefore, the temporal pattern reported here should be interpreted as mechanistic insights under controlled incubation conditions rather than being directly extrapolated to long-term in situ sediment processes.

3.2. Anaerobic carbon sources transformation

As shown in Fig. 2(a), glucose was rapidly consumed in all treatments, with less than 0.2 g/L remaining after 3 days and a consumption rate exceeding 92% within 6 days. Further calculation revealed that the COD of VFAs produced from glucose first increased with VFA accumulation and then declined; by Day 36, the COD in the S8 treatment (833.4 mg/L) was significantly lower than that in other treatments, particularly the S0 treatment (968.0 mg/L) (Fig. S4). However, COD values showed no significant correlation with P release ($p > 0.05$). Notably, Fig. 3 shows a distinct difference in the major VFA products between treatments. In the absence of sulfate (S0), glucose was more likely converted into butyric acid, whereas in the presence of sulfate (S8), conversion was directed toward acetic acid. For example, on Day 36, the concentrations of acetic, propionic, and butyric acids in S0 were 492.1, 29.2, and 219.3 mg/L, respectively, whereas in S8 they were 700.5, 51.4, and 4.5 mg/L (Fig. 2(c)).

Under anaerobic conditions, marine sediments typically contain abundant SRB capable of oxidizing various organic acids and hydrocarbons, including VFAs and short-chain alkanes (Kniemeyer et al., 2007; Laanbroek and Pfennig, 1981). In sulfate-amended systems, fermentation-derived intermediates (e.g., propionate and butyrate) can be further oxidized/turned over, thereby shifting the VFA compositions and reducing net VFA accumulation. This phenomenon has been observed in marine systems, where increased sulfate availability promotes the oxidation of propionate and butyrate by SRB (Visser et al., 1993). Meanwhile, enhanced secondary oxidation under sulfate-amended conditions may promote further mineralization of fermentation intermediates (e.g., acetate) to gaseous products such as CO₂, which were not captured in the liquid-phase VFA analysis, thereby contributing to the observed decrease

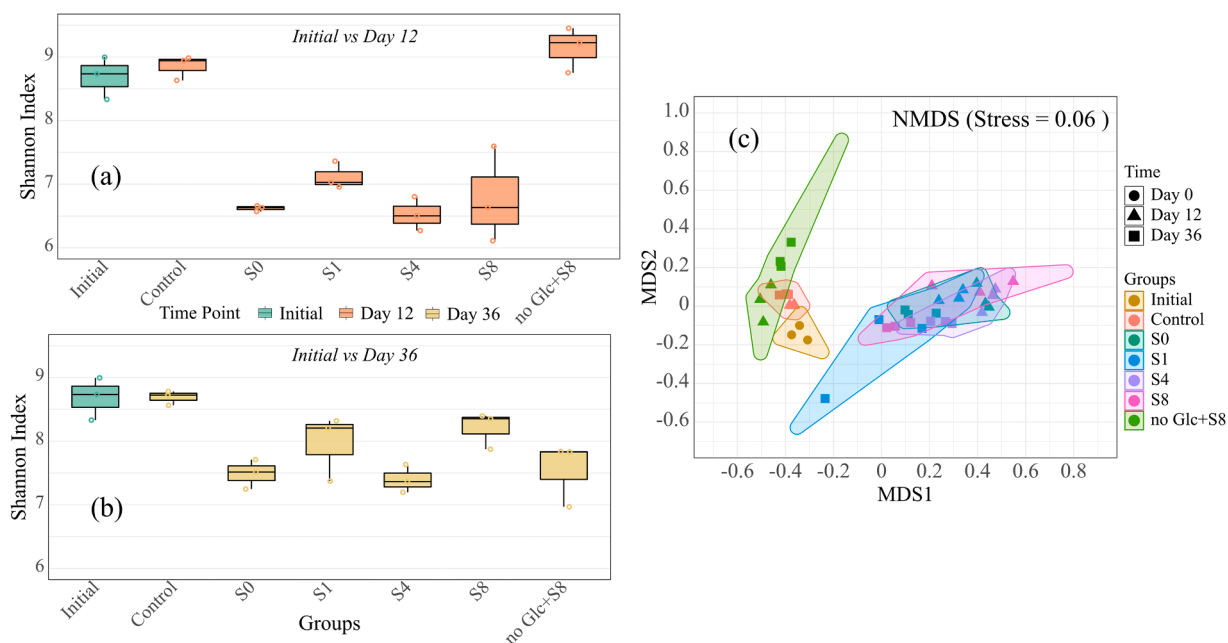


Fig. 3. Alpha diversity at (a) Day 12 and (b) Day 36, and (c) NMDS ordination of microbial community composition.

in VFA-COD.

In this study, sulfate consumption showed a significant positive correlation with the production of acetic and propionic acids (Spearman, $p < 0.05$), and P release was likewise positively correlated with these short-chain VFAs, indicating that enhanced VFA generation favors P mobilization. The energy released during carbon transformation, along with the oxidation of long-chain to short-chain organic acids, likely alters sediment redox conditions and facilitates P release (Zhu et al., 2024). Furthermore, the sulfide generated during sulfate reduction can react with oxides such as iron, leading to the dissolution of Fe-bound P compounds and the concomitant release of IP into the supernatant (Wilfert et al., 2020).

3.3. Microbial community responses

According to Fig. 3(a) and 3(b), the alpha diversity in the control and no Glc + S8 groups showed no significant change compared with the initial (Day 0) samples, remaining relatively stable throughout the incubation. In contrast, the S0-S8 treatments exhibited a marked decline in diversity during the early stage, followed by an increase in the later stage. This “decline-recovery” pattern may reflect an initial disturbance caused by rapid substrate consumption under high-carbon conditions, leading to a temporary decrease in the relative abundance of certain dominant taxa, followed by a gradual restoration of community structure as anaerobic metabolic products accumulated and functional adaptation occurred.

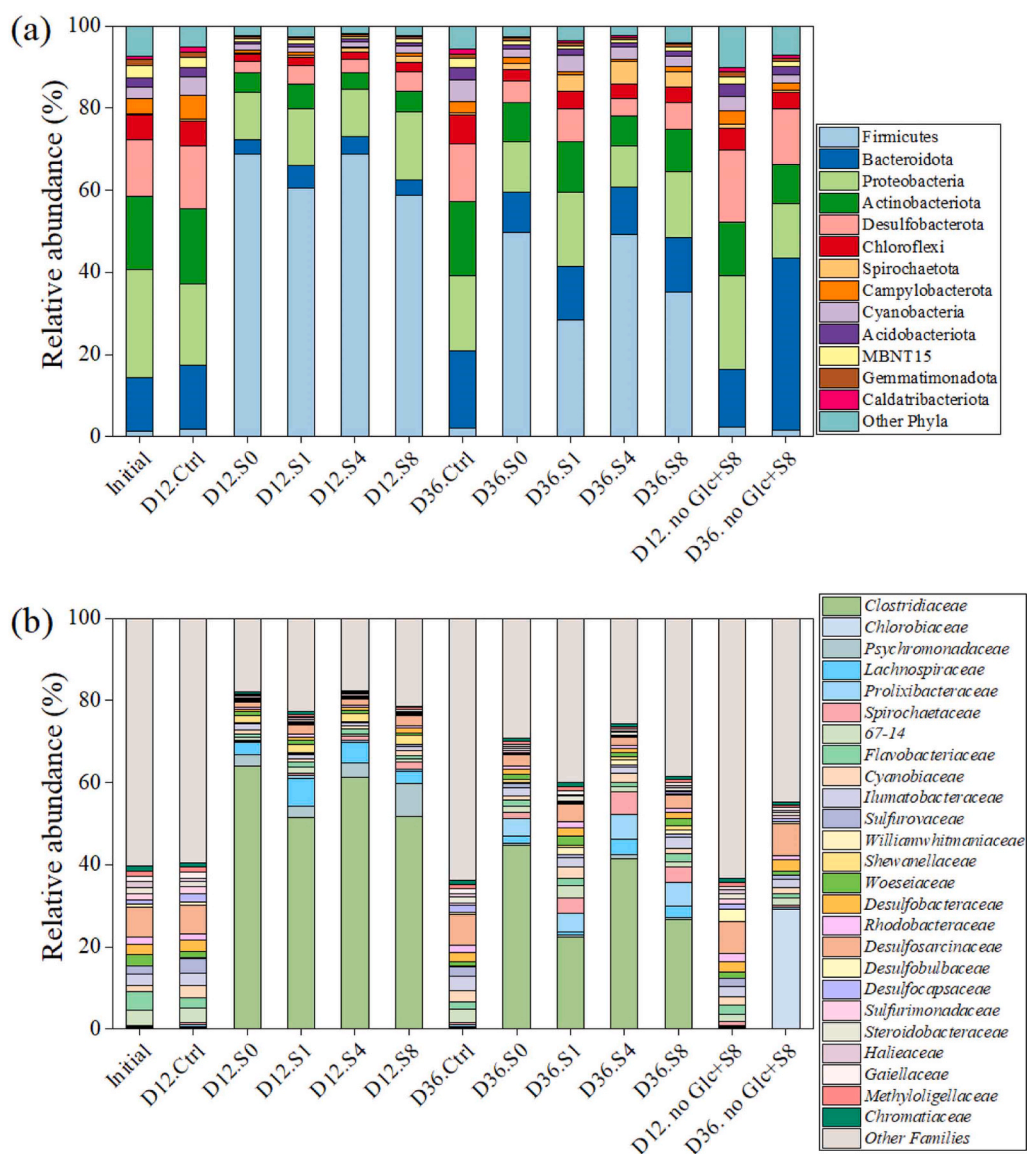


Fig. 4. Temporal microbial community structure at (a) phylum and (b) family levels.

NMDS analysis further revealed that the initial, control, and “no Glc + S8” samples clustered closely (Fig. 3(c)), whereas the remaining S0-S8 samples were grouped together. PERMANOVA results showed that pairwise differences among the S0-S8 treatments were not significant ($\text{adj } p > 0.05$), indicating that, under glucose addition, variations in sulfate concentration did not cause substantial shifts in overall microbial community composition. Although the control and “no Glc + S8” treatments clustered relatively closely in the NMDS plot, PERMANOVA analysis still indicated a significant difference between these two groups ($p < 0.05$). This suggests that sulfate addition alone, even without external carbon input and with limited net sulfate reduction, can reshape microbial community structure under anaerobic conditions. Such shifts may partly explain why the final dissolved P concentration in the “no Glc + S8” treatment was slightly lower than that in the control, potentially due to the reshaped microbial community promoting P immobilization processes (e.g., microbial uptake and/or enhanced $\text{PO}_4\text{-P}$ retention in sediments), as sulfate enrichment has previously been shown to decrease dissolved P and alter microbial nutrient-related processes (Seifert-Monson et al., 2014).

Taken together with the P release, these results suggest that the additional P release observed in sulfate-fed treatments is unlikely to be primarily driven by large-scale shifts in community structure. Rather, it is more likely linked to chemical processes (e.g., Fe-S interactions) or to changes in microbial metabolic functions that are not readily captured at the taxonomic level. Nevertheless, examining taxonomic changes remains informative, as functional shifts, often driven by relatively small variations in the abundance of key taxa, may play a more critical role in P mobilization than wholesale community restructuring. In this context, we further investigated the variations at the phylum and family levels to identify potential functional contributors to P release.

At the phylum level (Fig. 4(a)), glucose addition led to a pronounced enrichment of typical anaerobic fermentative bacteria. In the initial sediment, the dominant phyla were Proteobacteria (26.2%), Actinobacteriota (17.9%), and Bacteroidota (13.2%). By Day 12, the community shifted to be dominated by Firmicutes in glucose-addition groups, reaching 68.9% in the S0 group and 58.8% in the S8 group. Subsequently, Firmicutes abundance declined to 49.8% (S0) and 35.3% (S8) by Day 36, while Bacteroidota, Proteobacteria, and Actinobacteriota remained at approximately 10%. In the sulfate-only group, Bacteroidota became enriched during the later stage, reaching 41.9% at Day 36. Functionally, fermentative Firmicutes play a key role in organic matter hydrolysis and hydrogen/acid production during anaerobic digestion and possess potential electron transfer capacity (Zhou et al., 2020). Actinobacteriota, Bacteroidota, and Proteobacteria share similar roles in organic matter degradation and mineralization, and they are also important participants in nutrient cycling involving nitrogen, iron, and sulfur (Hedrich et al., 2011; Wasmund et al., 2017; Yang et al., 2023).

At the family level, the initial sediment contained no highly dominant families, with the highest abundance being *Flavobacteriaceae* (4.5%). In glucose-added groups, *Clostridiaceae*, originating from the phylum Firmicutes and recognized as major contributors to fermentative metabolism (Toledo-Alarcón et al., 2021), rapidly became dominant, reaching 64.1% in S0 and 51.8% in S8 by Day 12, before declining to 44.8% (S0) and 26.7% (S8) at Day 36. In contrast, *Chlorobiaceae* was only detected in the no Glc + S8 group, with a relative abundance of 28.9% at Day 36; this family represents typical anaerobic photoautotrophic green sulfur bacteria, which use sulfide as an electron donor for photosynthesis to fix carbon into organic matter (Imhoff, 2014).

Spirochaetaceae and *Prolixibacteraceae* showed moderate enrichment at the later stage (Day 36) in the S0-S8 treatments, with the

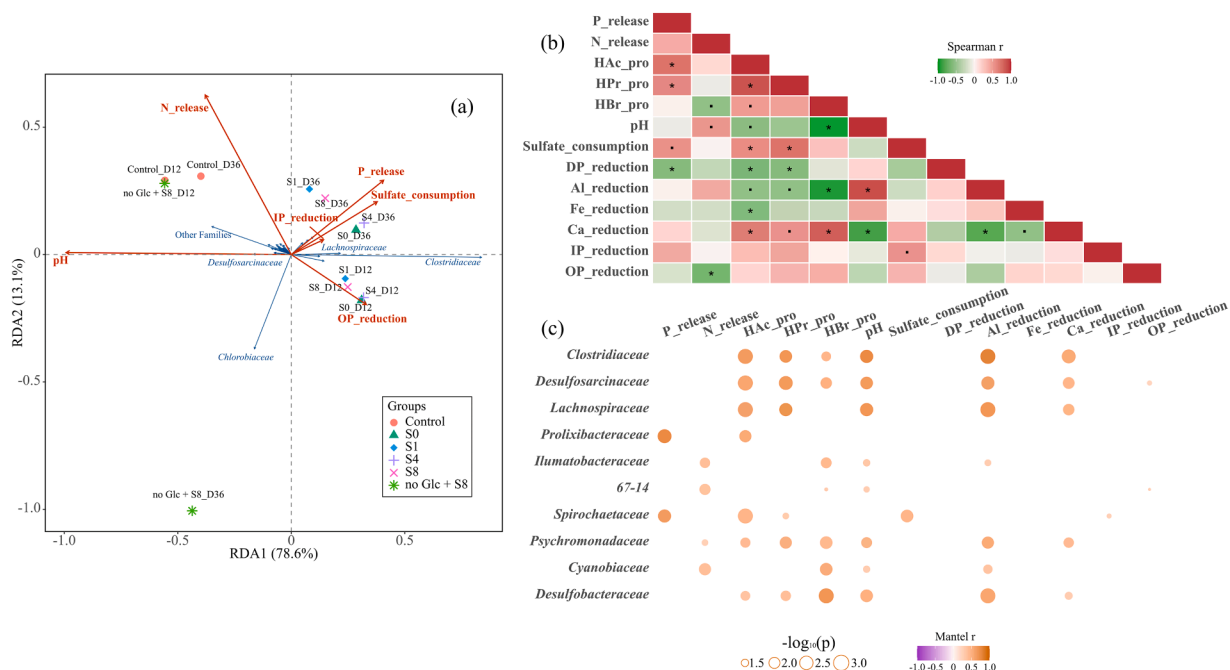


Fig. 5. (a) Redundancy analysis (RDA) of microbial families constrained by environmental factors (red arrows; time as covariate); (b) Spearman correlations among environmental factors (* $p < 0.05$, $\cdot p < 0.1$); (c) Mantel tests showing significant microbe-environment associations ($p < 0.05$); bubble size indicates $-\log_{10}(p)$ and color represents Mantel r . (pro: production; DP: dissolved P; IP: inorganic P; OP: organic P).

latter exceeding 5.5% in S4 and S8 groups. Both taxa are known to degrade complex organic compounds under anaerobic conditions (Cao et al., 2025; Chan et al., 2025; Orellana et al., 2025), and their increased abundance after glucose depletion suggests a reliance on intermediate fermentation products such as VFAs. Spearman correlation analysis showed that both families were significantly associated with P release ($p < 0.05$, Fig. S5) and positively correlated with acetate and propionate production, suggesting a possible role in the conversion of butyrate to acetic and propionic acid. Similar syntrophic butyrate- and acetate-oxidizing potentials have been reported for *Spirochaetaceae* (Cao et al., 2025; Yi et al., 2020).

Notably, although our initial hypothesis emphasized the potential role of SRB in promoting P release, the microbial data revealed a different pattern. Typical SRB families (*Desulfobacteraceae*, *Desulfosarcinaceae*, *Desulfobulbaceae*, and *Desulfocapsaceae*) decreased from ~12% in glucose-free treatments to below 5% following glucose addition and showed no significant correlation with P release (Fig. S5, $p > 0.05$). However, sulfate reduction is a defined biogeochemical process that involves sulfate-reducing microorganisms (including SRB) (Muyzer and Stams, 2008), and SRB functional contributions cannot be excluded solely based on relative abundance correlations. Although microbial abundance has been shown to influence functional roles (Rivett and Bell, 2018), taxonomic abundance does not necessarily reflect in situ metabolic activity or ecological function. Direct abiotic sulfate reduction is unlikely under the mild incubation conditions of this study (typically requiring high-temperature hydrothermal settings) (Machel, 2001); thus, the observed sulfate consumption was most likely microbially mediated. Therefore, taxonomic patterns alone may not fully reflect sulfate-reducing activity, which should be interpreted together with geochemical evidence and functional potential.

Fermentative taxa such as *Spirochaetaceae* and *Prolixibacteraceae* were significantly positively correlated with the production of acetic and propionic acids as well as sulfate consumption (Fig. S5, $p < 0.05$), suggesting that fermentative metabolism could play an important upstream role by supplying labile electron donors (e.g., VFAs/ H_2) that support sulfate reduction (Finke and Jørgensen, 2008; Finke et al., 2007). This interpretation is consistent with previous studies showing that fermentation-derived VFAs represent key

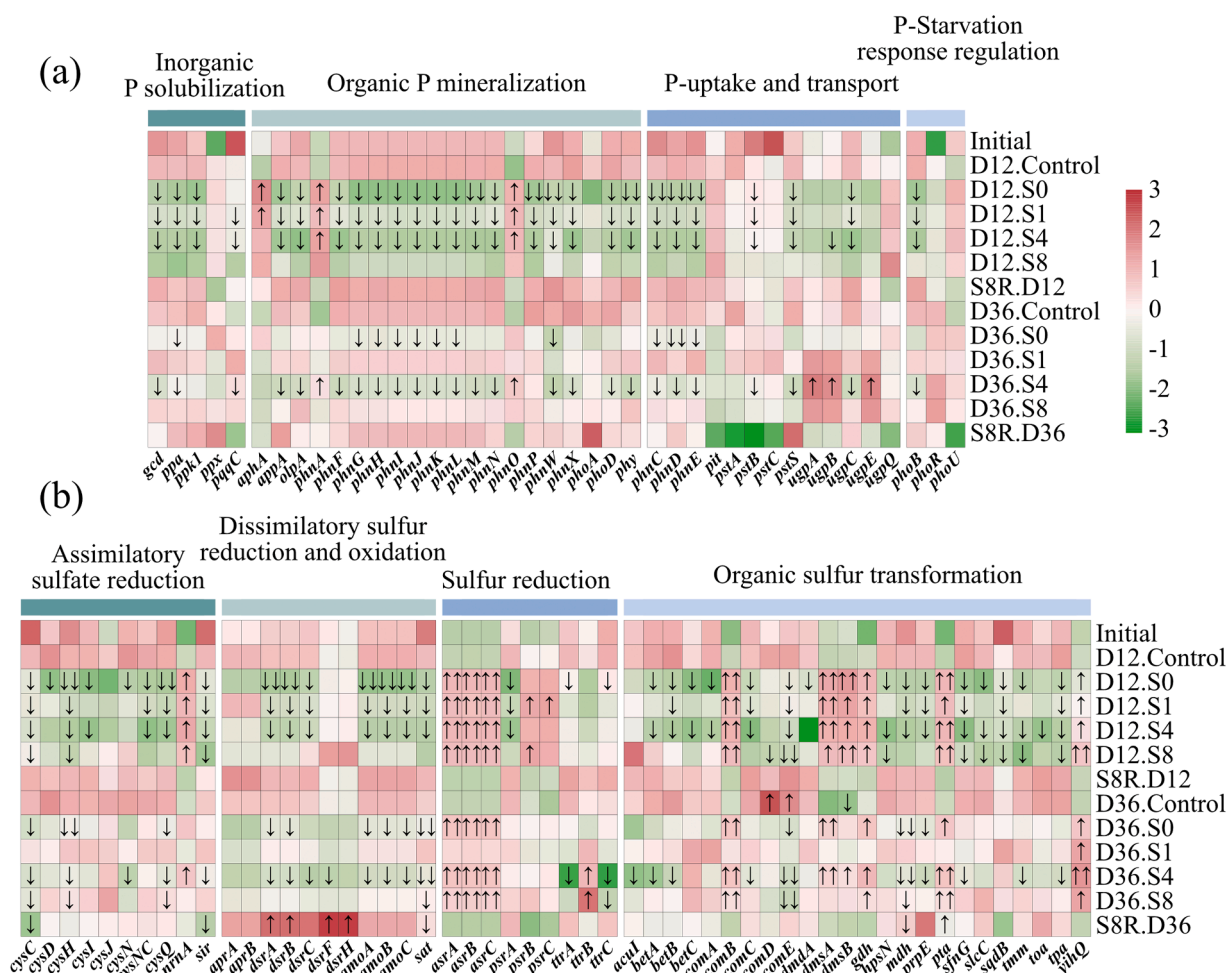


Fig. 6. Functional gene expression profiles of (a) phosphorus metabolism and (b) sulfur cycling. Heatmaps show strict log2-transformed KO abundances, with functional gene categories indicated by colored annotation bars. Arrows denote significant changes relative to the initial condition (Welch's t -test; BH-adjusted p -values): $\uparrow/\downarrow$ indicate significantly higher or lower expression (FDR-adjusted $p < 0.05$), while $\uparrow\uparrow/\downarrow\downarrow$ indicate stronger shifts (FDR-adjusted $p < 0.01$ and fold change > 1.5 or < 0.67).

electron donors for sulfate reduction in marine sediments, and that inhibition of sulfate reduction can lead to VFA accumulation, highlighting the tight coupling between fermentation and sulfate reduction (Finke and Jørgensen, 2008; Finke et al., 2007). The resulting sulfide production and redox changes likely facilitated Fe-bound P dissolution and enhanced organic P mineralization. Collectively, these findings suggest that sulfate-associated P mobilization in this system was closely linked to fermentative metabolism through its role in fueling sulfate reduction and subsequent sulfide-driven Fe-P transformation.

3.4. Potential metabolic activities exploration

As shown in Fig. 5(a), the RDA analysis revealed that RDA1 (78.6%) and RDA2 (13.1%) together explained 91.7% of the total constrained variance, indicating that the selected environmental factors were closely associated with the variation in microbial community structure. Among these, P_{release}, Sulfate_{consumption}, and IP_{reduction} had the longest arrows, indicating the closest associations with community shifts, and were strongly linked to the glucose-addition treatments (S0, S1, S4, S8). In contrast, pH pointed in the opposite direction to P_{release}, suggesting a negative correlation between pH and P release, while N_{release} was oriented toward the Control group, indicating that higher nitrogen release primarily occurred under control groups.

Regarding microbial families, *Clostridiaceae* was positioned near P_{release} and Sulfate_{consumption}, implying that it may promote P release and sulfate consumption through acid production during anaerobic fermentation. *Lachnospiraceae*, *Prolixibacteraceae*, and *Spirochaetaceae* were also aligned with P_{release}. *Chlorobiaceae* was primarily associated with late-stage samples from the “no Glc + S8” treatment, consistent with the ecological traits of anaerobic phototrophic sulfur bacteria.

Fig. 5(b) shows the Spearman correlation heatmap among environmental factors, where Sulfate_{consumption} showed a weak positive association (a marginal trend) with P_{release} ($p < 0.1$) and significantly correlated with acetate and propionate production ($p < 0.05$), suggesting that sulfate consumption may be potentially coupled with organic acid accumulation and P release. Fig. 5(c) presents the Mantel test results showing significant associations ($p < 0.05$) between microbial families and environmental variables. No families exhibited significant negative correlations. Notably, *Prolixibacteraceae* showed a strong positive correlation with P_{release} ($r = 0.76$) and acetate production, while *Spirochaetaceae* was positively correlated with P_{release} ($r = 0.64$), acetate and propionate production, and Sulfate_{consumption}. These findings reinforce the hypothesis that both families may participate in secondary fermentation by utilizing primary fermentation products (VFAs) after glucose depletion, thereby enhancing P release.

As shown in Fig. 6 and Fig. S6, the predicted abundances of functional genes related to P, sulfur, and carbon metabolism exhibited pronounced temporal and treatment-specific dynamics. For P metabolism (Fig. 6(a)), in the early stage (Day 12), most functional genes involved in inorganic/organic P mineralization (e.g., *gcd*, *ppa*, *phnP*, *phnE*, *pit*) showed markedly reduced predicted abundance in glucose-addition groups (S0, S1, S4, S8). This suggests that, during the initial response phase, extra carbon input may have shifted microbial metabolism toward pathways less favorable for P release, consistent with previous composting studies in which glucose addition increased humus-bound P content and reduced its immediate mineralization potential (Li et al., 2023). By the later stage (Day 36), the predicted abundance of these genes increased significantly in those treatments ($p < 0.05$), indicating that, as labile carbon was depleted and system redox conditions became more reducing, P acquisition and mineralization processes were reactivated (Shaheen et al., 2021).

For sulfur cycling (Fig. 6(b)), functional gene prediction suggested significant shifts in sulfur metabolism, including changes in key marker genes associated with dissimilatory sulfate reduction, supporting the occurrence of active sulfate reduction under glucose-amended conditions. Specifically, the predicted abundance of sulfate reduction-related functional genes (e.g., *asrA*, *asrB*, *asrC*, *psrB*, *psrC*) increased markedly only in the glucose-fed groups, suggesting that such conditions may promote sulfate reduction; however, most other sulfur-related functions, such as dissimilatory sulfur reduction and oxidation as well as organic sulfur transformation, tended to decrease during the same period. For carbon metabolism (Fig. S6), at Day 12, glucose-fed groups were characterized by increased predicted abundance of genes related to glycolysis (e.g., *glk*, *pgm*, *pfkA*) and glycogen metabolism (e.g., *glgP*), coupled with reduced abundance of genes related to carbon storage and entry into the tricarboxylic acid cycle (e.g., *aceE*, *pgl*). This reflects a metabolic preference for rapid fermentation and glycogen accumulation during the early stage. By Day 36, carbon metabolism-related gene abundance tended to stabilize or decline, whereas P mineralization and transport-related genes increased, probably suggesting a metabolic shift from early-stage “rapid carbon utilization-fermentation” toward “nutrient cycling and mineralization regeneration”. A previous study reported that organic inputs (e.g., fertilization) can restructure microbial functional traits by suppressing P uptake and transport genes while enhancing P mineralization genes in soils (Hu et al., 2023).

The RDA and Mantel analyses (Fig. 5) indicated that P release, sulfate consumption, and IP reduction were the environmental factors most strongly associated with community shifts, particularly in glucose-added groups. Families such as *Clostridiaceae*, *Prolixibacteraceae*, and *Spirochaetaceae* were closely aligned with these factors, suggesting potential involvement in anaerobic fermentation and the utilization of VFA for further metabolism, thereby promoting P mobilization.

Consistent with these taxonomic inferences, functional gene predictions (Fig. 6) revealed a staged carbon-sulfur-P coupling process: in the early stage, glucose addition stimulated microbial fermentation, resulting in VFA accumulation; subsequently, the excess carbon served as an electron donor for sulfate reduction, driving sulfide generation and Fe-P desorption; finally, in the late stage, the predicted abundance of P mineralization and transport genes increased, further facilitating P release, especially OP, from sediments. The alignment between community composition and predicted metabolic potential strengthens the conclusion that functional shifts, rather than major taxonomic restructuring, played a dominant role in driving the observed P release under sulfate-addition, carbon-rich conditions.

3.5. Environmental implications

This study demonstrates that the coupling of external carbon input with sulfate reduction under anaerobic conditions can substantially enhance P release from sediments, providing a promising approach for P mobilization toward subsequent recovery. The results indicate that labile carbon addition stimulates rapid microbial fermentation, producing VFAs that serve as efficient electron donors for sulfate reduction. Sulfate reduction leads to sulfide generation, which reacts with Fe-P, promoting its desorption into the dissolved phase. In the later stage, the reactivation of P mineralization and transport processes further accelerates P mobilization.

From an applied perspective, this mechanism could be exploited in controlled engineering systems such as in situ sediment reactors, constructed wetlands, or dredged sediment treatment facilities. By regulating the type, dosage, and timing of carbon and sulfate inputs, P release can be optimized for subsequent recovery via precipitation, adsorption, or biological uptake. In practical field-scale applications, glucose can be regarded as a model labile carbon source used to elucidate mechanisms, whereas more cost-effective and readily available organic substrates may be preferable. Potential alternatives include organic wastes and waste-derived streams (e.g., food-waste, and wastewater-derived carbon sources), which can provide electron donors to support anaerobic reactors and subsequent sulfate reduction.

Furthermore, the strategy is particularly relevant to eutrophic lakes and aquaculture pond sediments, where targeted stimulation of microbial carbon-sulfur-P interactions could both mitigate long-term internal P loading risks and provide a renewable source of P. In practical applications, once phosphate is mobilized into the aqueous phase, it could be recovered using established capture/separation technologies. For instance, dissolved phosphate can be concentrated and recovered via chemical precipitation/crystallization (e.g., struvite) after pH adjustment and Mg addition, or immobilized by adsorption onto reactive sorbents. These recovery options highlight that enhancing P mobilization may serve as a critical front-end step for downstream P recovery in engineered sediment treatment systems.

However, the present results were obtained under anaerobic, well-mixed batch conditions, whereas natural sediments and practical applications (e.g., constructed wetlands) often exhibit heterogeneous and dynamic dissolved oxygen regimes (oxic-anoxic interfaces), which may affect sulfate reduction intensity and Fe-sulfur-P cycling. Importantly, sediment P mobilization is generally favored under anaerobic/reducing conditions; therefore, the mechanisms identified here are most applicable to anoxic zones or engineered settings where anaerobic conditions can be locally promoted (e.g., deeper sediments and organic-rich microzones).

Future research should expand on these findings by exploring strategies to further enhance the extent and rate of P release, including the integration of additional electron acceptors/donors, optimization of microbial community succession, and coupling with cost-effective P recovery technologies. Such developments could facilitate the transition from laboratory-scale validation to large-scale, field-applicable solutions for sustainable P management.

4. Conclusions

This study demonstrated that the co-addition of glucose (1 g/L) and sulfate (8 mM) under anaerobic conditions substantially enhanced P release from sediments compared with sulfate-only and control treatments. This enhancement was driven by coupled carbon-sulfur-P interactions, where glucose fermentation supplied electron donors that supported sulfate reduction, promoting Fe transformation and facilitating P mobilization. A temporal shift was observed, with early-stage IP release followed by enhanced OP mineralization, while partial re-retention of IP likely occurred in later stages.

Microbial community analysis revealed that, under glucose addition, sulfate supplementation did not drastically alter the overall taxonomic structure but did induce functional shifts. Overall, these findings demonstrate that co-supplementation of labile carbon and sulfate effectively enhances sediment P mobilization and supports its potential recovery and reuse.

CRedit authorship contribution statement

Chen Chen: Data curation. **Frederico Marques Penha:** Supervision. **Fengyi Zhu:** Writing – original draft, Methodology, Investigation, Data curation. **Maria Cuartero:** Supervision. **Zeynep Cetecioglu:** Writing – review & editing, Supervision, 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. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eti.2026.104856](https://doi.org/10.1016/j.eti.2026.104856).

Data availability

Data will be made available on request.

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