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Phosphorus mining from marine sediments adopting different carbon/nitrogen strategies driven by anaerobic reactors: The exploration of potential mechanism and microbial activities

Fengyi Zhu [a], Ece Kendir Cakmak [a], Federica D'Amico [b], Marco Candela [b], Silvia Turroni [b], Zeynep Cetecioglu [a,*]

[a] Department of Industrial Biotechnology, School of Engineering Sciences in Chemistry, Biotechnology and Health, KTH Royal Institute of Technology, SE-11421 Stockholm, Sweden
[b] Unit of Microbiome Science and Biotechnology, Department of Pharmacy and Biotechnology, University of Bologna, 40126 Bologna, Italy

HIGHLIGHTS

- Extra NH_4-N dosing inhibited the release of P under anaerobic condition.
- The carbon: NH_4-N imbalance in the anaerobic reactors reduced AP activity.
- The glucose-fed system favored better P release.
- Enriched OM-mineralizing organisms in glucose-fed system promoted the P release.
- The decreased PAO and SRB can potentially be the reason for P release suppression.

GRAPHICAL ABSTRACT
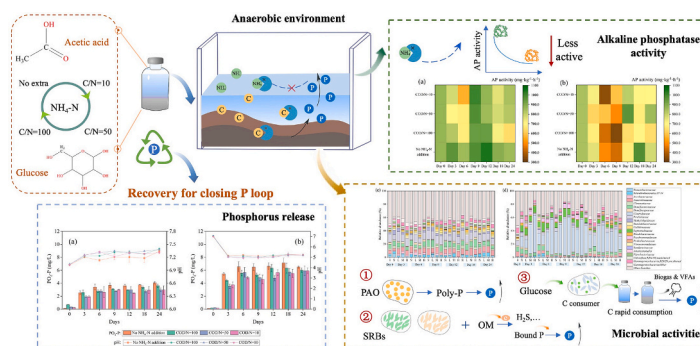
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ABSTRACT

To investigate the possibility of phosphorus (P) recovery from marine sediment and explore the role of the carbon: nitrogen ratio in affecting the internal P release under anaerobic conditions, we experimented with the external addition of carbon (acetic acid and glucose) and ammonia nitrogen (NH_4-N) to expose P release mechanisms. The 24-day anaerobic incubations were conducted with four different carbon: nitrogen dosing groups including no NH_4-N addition and COD/N ratios of 100, 50, and 10. The P release showed that extra NH_4-N loading significantly suppressed the decomposition of P ($p < 0.05$) from the marine sediment, the maximum P release was 4.07 mg/L and 7.14 mg/L in acetic acid- and glucose-fed systems, respectively, without extra NH_4-N addition. Additionally, the results exhibited that the imbalance of carbon: nitrogen not only failed to induce the production of organic P mineralization enzyme (alkaline phosphatase) in the sediment but also suppressed its activity under anaerobic conditions. The highest enzyme activity was observed in the group without additional NH_4-N dosage, with rates of 1046.4 mg/(kg•h) in the acetic acid- and 967.8 mg/(kg•h) in the glucose-fed system, respectively. Microbial data analysis indicated that a decrease in the abundance of P release-regulating bacteria, including polyphosphate-accumulating organisms (*Rhodobacteraceae*) and sulfate-reducing bacteria

* Corresponding author.
  E-mail address: zeynepcg@kth.se (Z. Cetecioglu).

(*Desulfosarcinaceae*), was observed in the high NH_4-N addition groups. The observed reduction in enzyme activity and suppression of microbial activity mentioned above could potentially account for the inhibited P decomposition in the presence of high NH_4-N addition under anaerobic conditions. The produced P-enriched solution from the bioreactors may offer a promising source for future recovery endeavors.

1. Introduction

Phosphorus (P), an essential nutrient for living organisms, has been industrially used for fertilizer production. Due to the limited availability and high dependency on the import of P rock, phosphate rock was identified as Critical Raw Materials in 2014 by the European Union (EU) (European Commission, 2014). Meanwhile, marine eutrophication has been a worldwide problem caused by the over-enrichment of nutrients for decades, especially in the Baltic Sea region. 97 % of the region was assessed to be below good eutrophication status, and the average total P content in the sediment surface layer was reported 1750 ± 780 mg P/kg in the Baltic Sea archipelagos (Puttonen et al., 2014). Moreover, the annual loads of P to the Baltic Sea for the period 1970–2010 were estimated to be 18,000 tons (Savchuk, 2018), while the economic losses triggered by eutrophication were estimated at € 3.8–4.4 billion/yr (HELCOM, 2021).

Therefore, recycling P from eutrophic sediments could be a practicable way for both marine remediation and resource recovery, while processes for marine P recovery should be further developed and studied. If part of the P can be reclaimed from the marine sediment, it will make a substantial contribution to global P recovery, especially in terms of recovering P from the natural environment and closing the P loop. However, there are currently few cases of P recovery from marine, especially from deep eutrophic sediments. Meanwhile, technologies to recover P from waste streams (e.g., wastewater, municipal sludge) have already been extensively applied and well established.

Feeding carbon (C) could potentially serve as an approach to stimulate P release under anaerobic conditions, drawing from previous experience in the wastewater treatment field. For example, in a batch experiment conducted by (Gerber et al., 1986), 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 eventual above 65 mg/L phosphate released from around 4 g/L MLSS (mixed liquor suspended solids) sludge system under anoxic-anaerobic conditions. More specifically, anaerobic conditions promote P release from polyphosphate-accumulating organisms (PAOs). Previous studies have demonstrated that PAOs can utilize organic C sources and subsequently release orthophosphates into the environment by hydrolyzing stored polyphosphate (poly-P) (Rey-Martinez et al., 2019).

The concept of "Redfield ratio" has been established to understand how organisms control the concentrations and distributions of nutrients in marine environments, and the C: nitrogen (N): P of 106:16:1 has served as the basis for biogeochemistry research (Steinberg et al., 2002). Ammonia nitrogen (NH_4-N) is an influential bioavailable N in sediments (Alexandre et al., 2015), but relevant studies on its role in influencing P internal release in anaerobic eutrophic environments are lacking. In the mesocosm experiment conducted by Ma et al. (2018), an excessive supply of NH_4-N led to N:P imbalance and stimulation of the alkaline phosphatase (AP) activity of the organism and hence, induced more P decomposition. Similar results were also reported by the observation of eutrophic lakes, with Li et al. (2016) finding that C and N enrichment can stimulate bioavailable P release through AP production, while this stimulation may accelerate lake eutrophication (Zhou et al., 2008).

However, there is a lack of relevant research on P recovery from the eutrophic environment, not to mention the exploration of stimulating P release through different C/N ratios. Meanwhile, gaining an understanding of the marine nutrient internal cycle under anaerobic conditions can also contribute to marine science. As we know, excess nutrient (including N and P) inputs are the main factors that trigger aquatic eutrophication, while the internal cycles for nutrient transformation in the sediment-water system show different pathways. For example, NO_3-N is capable of promoting P release from sediments by changing redox conditions, the P-binding capacity of iron, and by degrading organic matter under hypoxic conditions in nature (Ma et al., 2018).

Therefore, to clarify the overall influence of NH_4-N loading on P decomposition from sediments and reach the goal of P recycling simultaneously, we conducted batch experiments under anaerobic conditions with different feeding ratios of C/N (COD/NH_4-N). Acetic acid and glucose, as prevailing C sources studied in the conventional biological P removal systems, were chosen and served as external C sources. The PO_4-P containing obtained after P release can potentially be precipitated later with added NH_4-N for future recovery in the form of struvite ($NH_4MgPO_4 \cdot 6H_2O$). Nevertheless, this experiment only focuses on investigating the P-release responses and does not involve the precipitation process. We hypothesized that the NH_4-N dosing would impact P release by influencing C consumption and the relevant microbial/enzymatic activity of the sediments under anaerobic conditions. Our study intends to: (1) investigate the P release capacity from marine sediment by dosing different C/N ratios in both acetic acid- and glucose-fed systems under anaerobic conditions; (2) clarify the NH_4-N dosing strategy for favorable P release from marine sediments; and (3) explore the potential microbial mechanism of P release in different NH_4-N loading systems.

2. Materials and methods

2.1. Anaerobic batch experiments

The sediments were collected in the eutrophic Baltic Sea area on 30th January 2022, Stockholm (58.476400 N, 17.456699 E). The characteristics of sediments and pore water are shown in Table S1. A series of batch experiments were conducted in 120-mL serum bottles in triplicate, a total of 168 bottles were required for two C-fed systems. Acetic acid and glucose were chosen as C sources, and the various of C/N experimental groups were set up with no extra NH_4-N addition (No NH_4-N group), low NH_4-N addition (C/N = 100 group), medium NH_4-N addition (C/N = 50 group), and high NH_4-N addition (C/N = 10 group) for investigating the influence of C/N on P release from sediment under anaerobic conditions.

In the acetic acid-fed system, 16 g of fresh sediments were dispensed in 80 mL synthetic seawater prepared with NaCl (6.5 psu, which is the average salinity of the water from the Baltic Sea). Later, acetate (1 g/L acetic acid) and NH_4Cl were added to the serum bottles, offering C and N sources. The corresponding NH_4-N concentrations within different C/N ratio groups were around 0, 11, 22, and 110 mg/L, respectively. The bioreactors were operated at 20 °C with daily manual mixing, where the reactors were shaken and overturned to ensure thorough mixing of water and sediment for at least 5 min. Sampling was conducted on days 0, 3, 6, 9, 12, 18, and 24. The experimental bottles were set up under an initial pH of 7.1 ± 0.1 and sealed with septa and aluminum caps. Each bottle was flushed with N_2 gas for at least 5 min to provide anaerobic condition. At each sampling day, the flasks from each treatment were analyzed (12 reactors on each day), PO_4-P, NH_4-N, pH, acetic acid/glucose concentrations in the bioreactors, AP activity, and microbial community structure of marine sediments were monitored during the 24-day bioreactor operation. We repeated the above batch operation for the glucose-fed system using the same initial COD.

2.2. Analytical methods

PO$_4$-P and NH$_4$-N in the supernatant were analyzed by the Murphy-Riley method (Cho and Nielsen, 2017) and cuvette tests (LCK 303 Hach Lange, Germany) using DR 3900 Hach Lange spectrophotometer. The variations of pH were monitored by the pH meter (Mettler Toledo FiveEasyTM pH bench meter, FE20). The concentrations and compositions of volatile fatty acids (VFAs, e.g., acetic acid) were measured with 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 as described by Khatami et al. (2021). The reducing glucose in the supernatant was determined by the dinitrosalicylic acid (DNS) method described by McKee (2017). The measurement of AP activity stated by Sayler et al. (1979) is based on a color change due to the production of p-nitrophenol through the cleavage of *p*-nitrophenyl phosphate. Other analytical methods for the characterization of marine sediments and pore water can be found in the Supplementary Material.

2.3. Microbial community analysis

2.3.1. DNA extraction

The sediment samples from the batch reactors were centrifuged at 4000 rpm (Hermle Z 206 A, Wehingen, Germany) for 10 min and then, stored at −20 °C. Microbial genomic DNA was isolated via DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany) by following the instructions of the manual protocol.

2.3.2. 16S rRNA amplicon sequencing and data processing

For each sample, the V3-V4 hypervariable regions of the 16S rRNA gene were amplified using primers 341F and 785R (Klindworth et al., 2013) with Illumina overhang adapter sequences. PCR products were purified using a magnetic bead-based clean-up system (Agencourt AMPure XP; Beckman Coulter, Brea, CA, USA), indexed by limited-cycle PCR using Nextera technology, and further cleaned up as above. Final libraries were pooled at equimolar concentration (4 nM), denatured with 0.2 N NaOH, and diluted to 5 pM prior to sequencing on an Illumina MiSeq platform with a 2 × 250 bp paired-end protocol, according to the manufacturer's instructions (Illumina, San Diego, CA, USA).

Raw sequences were processed using a pipeline combining PANDAseq (Masella et al., 2012) and QIIME 2 (Bolyen et al., 2019). After length and quality filtering, reads were binned into amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016). Taxonomy was assigned via the VSEARCH algorithm (Rognes et al., 2016), using the SILVA database (138.1 SSURef NR99) as a reference. Alpha diversity was measured using the number of observed ASVs, the Shannon index, and Faith's phylogenetic diversity. The sequencing raw data have been archived in the Sequence Read Archive (National Center for Biotechnology Information, US) under the BioProject accession number PRJNA1030998.

2.4. Statistical analysis

The analysis of variance (ANOVA) was conducted to assess the significance of the results ($p < 0.05$). Redundancy analysis (RDA) and Pearson's correlation were used to ascertain the relationship between bacterial relative abundance and several factors. To assess differences in alpha diversity and relative taxon abundances among study groups (i.e., acetic acid- and glucose-fed systems over time under different COD/N loading), the Kruskal-Wallis test followed by post-hoc Wilcoxon test (paired or unpaired as needed) were used. A *p*-value <0.05 was considered statistically significant, while a *p*-value <0.1 was considered a trend.

3. Results and discussion

3.1. Effects of carbon/nitrogen loading on phosphorus release

The dissolved PO$_4$-P released from sediments in acetic acid- and glucose-fed systems with different NH$_4$-N loadings are shown in Fig. 1(a) and (b). Collectively, glucose feeding performed significantly better than acetic acid in stimulating P release. Maximum PO$_4$-P was observed in the supernatant of acetic acid- and glucose-fed systems as 4.07 mg/L on day 24 and 7.14 mg/L on day 18 without extra NH$_4$-N addition, respectively. PO$_4$-P concentration increased markedly during the first 6 days in both C-fed systems, with 3.41 mg/L and 6.55 mg/L of P solubilization in acetic acid- and glucose-fed systems without extra NH$_4$-N addition, respectively. Although an increasing trend of P release was observed in both systems with the extension of operation time, the final P release increased by only 19.35 % and 8.26 %. Meanwhile, our results showed that P decomposition was significantly inhibited when more NH$_4$-N was dosed in the anaerobic systems ($p < 0.05$). With the increase of NH$_4$-N level from low (COD/N = 100) to high (COD/N = 10), the concentration of PO$_4$-P in the supernatant of the acetic acid-fed system decreased from 3.62 mg/L to 2.95 mg/L at the end of 24 days, while in the supernatant of the glucose-fed system, it decreased from 6.32 mg/L to 5.50 mg/L on day 18.

Fig. 1 also illustrates the profiles of pH variations. The pH in the acetic acid-fed system remained overall stable during the 24-day experiments, varying from 7.0 ± 0.1 at the beginning to 7.3 ± 0.1. On the contrary, the pH in the glucose-fed system decreased considerably from 7.0 ± 0.1 to 5.1 ± 0.1 at the beginning of day 3, followed by a very slight increase until a final pH of 5.2 ± 0.1 in all glucose-fed reactors on day 24. The drop of pH in the glucose-fed system may be related to anaerobic fermentation; in the acid-producing stage, the bacteria converted the soluble monomers into end-products, including VFAs (seen in Fig. 3(c)-(f)), and the accumulated VFAs led to the pH decrease (Liang et al., 2021).

In this study, organic P (OP) constituted the predominant P fraction in the initial sediments (Table S1), accounting for 58.3 % of the total P, while the inorganic fraction represented 41.7 % of the total P (1.27 mg/g). Notably, inorganic P (IP) is the primary source of bioavailable P, with major contributions from Fe/Al-P and Ca-P to the IP supply within the sediment (Wang et al., 2005). Previous research has demonstrated a significant positive correlation between anaerobic P-release rates and the initial Fe/Al-P fraction (Adhikari et al., 2015). This suggests that anoxic sediments primarily release P bound within the inorganic fraction associated with Fe. Despite the relatively small contribution of the Fe/Al-P fraction, approximately 4 % of the total P, it plays a crucial role in P regeneration under anaerobic conditions (Adhikari et al., 2015). Moreover, the high-frequency monitoring data of P release from sediments detected by Ma et al. (2023) also supports the idea that the reduction of Fe-P in sediment leads to the simultaneous release of Fe and variable or mobile P. In summary, under anaerobic conditions, the release of P is likely influenced by the decomposition of Fe-P. To validate this, the release of Fe from sediment was monitored (seen in Fig. S1). As depicted, the Fe release in the acetic acid- and glucose-fed systems were notably distinct. After 24-day anaerobic incubation episodes, only a minimal amount of Fe (<1 mg/L) was released in the acetic acid-fed systems, suggesting that the Fe-P decomposition process may not be predominant in the P release when acetic acid is the carbon source. In contrast, a substantial increase was observed in the glucose-fed system, where Fe release rose from an initial 0.038 mg/L to 18.4 mg/L in the group without additional NH$_4$-N. This could be one of the reasons why the stimulating effect on P release is more pronounced when glucose is fed as the carbon source. Although our research did not directly investigate the variations in P fractions, it can be projected that the inorganic component, Fe/Al-P, might also decompose and decrease due to the increase in Fe release.
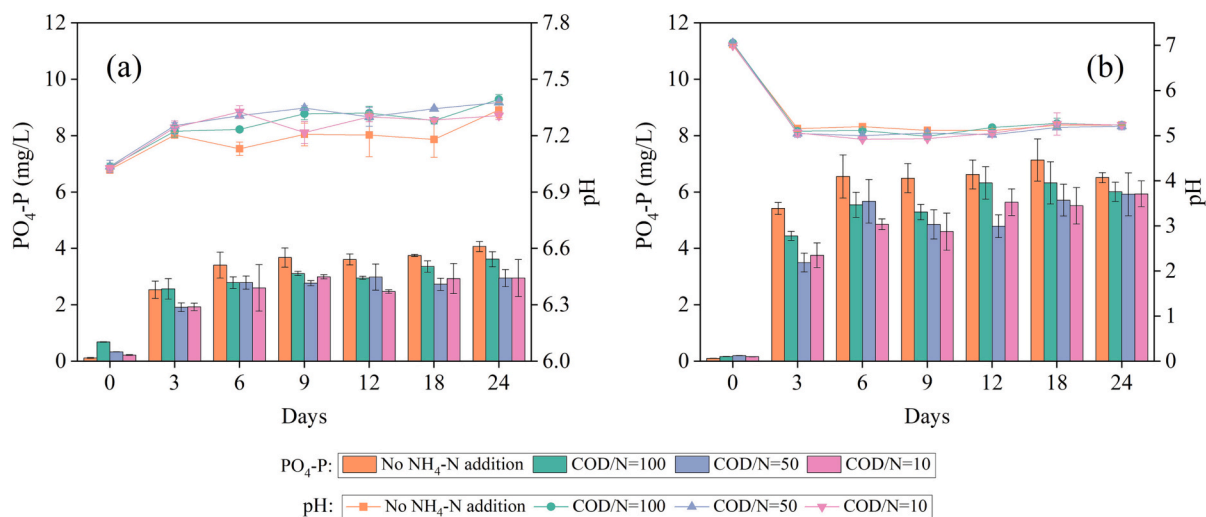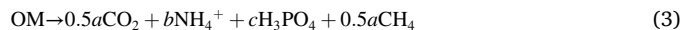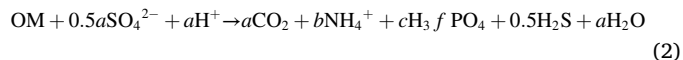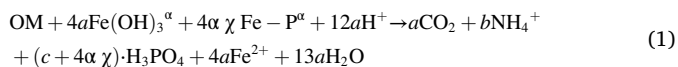
Fig. 1. Variations of PO$_4$-P and pH: (a) in the acetic acid-fed system; (b) in the glucose-fed system during the 24-day anaerobic operation under different COD/N loading.

The P release from sediment under anaerobic conditions is not a process driven by a singular mechanism, as other factors, including the dissolution of Fe/Ca-P, anaerobic OM decomposition, and microbially mediated P release, can all influence the P regeneration process. Consequently, there is a need to optimize conditions and investigate more parameters affecting P cycling in order to develop bioengineering approaches for further P recovery. In addition to the above, we should also take into account that the pH variations and sediment acidification can also lead to an increase in P release. However, the additional NH$_4$-N introduced in the acetic acid-fed reactors did not significantly influence pH variations (Fig. 1(a)). In contrast, the acidification induced by glucose resulted in a noticeable pH drop (from 7 to 5.2), which might potentially enhance the P release, partly attributed to the dissolution of IP in the sediment (e.g., Ca-P: detrital apatite) (Ma et al., 2023).

3.2. Variations of ammonia nitrogen in the acetic acid/glucose-fed system

NH$_4$-N concentration in the acetic acid-fed system showed a small increasing trend. On day 24, 3.76 mg/L, 14.71 mg/L and 26.19 mg/L NH$_4$-N were measured in the group of no addition, COD/N = 100 and COD/N = 50 (Fig. 2(a)), corresponding to an increase of 3.53 mg/L, 2.33 mg/L and 4.04 mg/L NH$_4$-N, respectively. Meanwhile, a clear depletion in NH$_4$-N was observed in the group of COD/N = 100 and COD/N = 50 within the first 6 days in the glucose-fed system (Fig. 2(b)).

The concentration of NH$_4$-N in the COD/N = 50 group decreased from initial 20.73 mg/L to 8.32 mg/L on day 6, and the decrease of NH$_4$-N in the COD/N = 100 group was even more evident in the initial 3 days, equal to 97 % (from initial 12.05 mg/L to 0.36 mg/L).

$$OM + 4aFe(OH)_3^\alpha + 4\alpha \chi Fe - P^\alpha + 12aH^+ \rightarrow aCO_2 + bNH_4^+$$
$$+ (c + 4\alpha \chi)\cdot H_3PO_4 + 4aFe^{2+} + 13aH_2O \tag{1}$$

$$OM + 0.5aSO_4^{2-} + aH^+ \rightarrow aCO_2 + bNH_4^+ + cH_3fPO_4 + 0.5H_2S + aH_2O \tag{2}$$

$$OM \rightarrow 0.5aCO_2 + bNH_4^+ + cH_3PO_4 + 0.5aCH_4 \tag{3}$$

The variations of NH$_4$-N in the anaerobic reactors could partly be attributable to the decomposition of OM in marine sediments, particularly the release of NH$_4$-N. Previous studies showed that P regeneration from sediment is closely coupled with the C cycle and subsequently with N, S, and Fe cycles (Biche et al., 2017), while N and P concentrations depend on the decomposition of OM. When the environment lacks oxygen, NH$_4$-N can be released from sediments through the following processes Eqs. (1)–(3) (Kraal et al., 2012). Therefore, in the acetic acid-fed system, the NH$_4$-N concentration can be increased with OM decomposition.

The OM is of the form (CH$_2$O)$_a$(NH$_4^+$)$_b$(H$_3$PO$_4$)$_c$ and χ represents the molar ratio between P and Fe in Fe (oxyhydr)oxides with adsorbed phosphate.
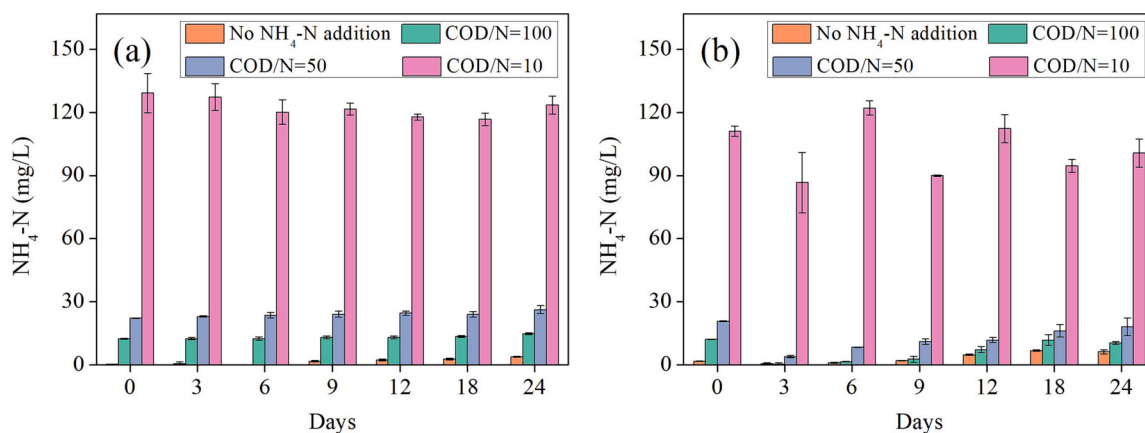

Fig. 2. Variations of NH$_4$-N in two different carbon-fed systems during the 24-day anaerobic operation under different COD/N loading: (a) acetic acid-fed system; (b) glucose-fed system.

At the same time, the microbial anabolism may also take place. The microbial synthesis process involves the consumption of a certain amount of NH_4-N. This could potentially account for the initial decrease in NH_4-N concentration in glucose-fed bioreactors. For instance, Oliveira et al. (2017) developed a C and N feeding strategy for polyhydroxyalkanoate (PHA) production from cheese whey, and NH_4-N consumption was found to be coupled with C uptake; when C (50 g/L COD) in the system was depleted, NH_4-N was also almost completely consumed from around 20 mg/L in 5 h. As a result of the higher energy yield in the anaerobic complete oxidation of glucose compared to an acetate-fed system (Kleerebezem and Van Loosdrecht, 2010), the probability of anabolism occurring in the glucose system is higher due to the availability of energy supply. Thereafter, continuous decomposition of OM (Eqs. (1)–(3)) and anaerobic fermentation led to an increase of soluble N from day 6, where solubilization of N from anaerobic fermentation was potentially attributed to the hydrolysis of nitrogenous OM (e.g., proteins) (Zou et al., 2018). The high concentration of NH_4-N dosing may inhibit OM decomposition redox reactions, since NH_4-N could act as a weak electron donor under anaerobic conditions, thereby competing with carbon donor (Kleerebezem and Van Loosdrecht, 2010). As illustrated in Eqs. (1)–(3), the decomposition of OM also results in phosphate release (in the form of H_3fPO_4). Therefore, if the decomposition of OM is hindered, it can potentially suppress P decomposition, especially with extra NH_4-N dosage compared to no extra NH_4-N addition group.

3.3. Variations of carbon in the acetic acid/glucose-fed system

As illustrated in Fig. 3(a), the concentration of acetic acid remained overall stable in all acetic acid-fed groups, with a slight decrease of 8.5 % (from 1136 mg/L to 1039 mg/L) and 6.8 % (1160 mg/L to 1081 mg/L) after 24 days, in the group of no extra NH_4-N and COD/N = 100, respectively. Unlike acetic acid, glucose rapidly decreased in the glucose-fed system, with over 95 % of the glucose consumed in all reactors in the first 6 days.

Meanwhile, an amount of biogas (data not available) and VFAs were produced in the glucose-fed system (seen in Fig. 3(c)–(f)). All VFAs accumulated over time, with acetic and butyric acids being the dominant VFAs for all groups; 795.56 mg/L, 721.91 mg/L, 742.50 mg/L and 761.94 mg/L of the final VFAs were produced by the group of no NH_4-N addition, COD/N = 100, COD/N = 50 and COD/N = 10, respectively. Although the highest production was measured in the no extra NH_4-N addition group, no significant differences were found between NH_4-N dosages ($p = 0.153$) in final VFA production. It has been confirmed that an increase in the organic loading rate can stimulate the production of total VFAs. For example, higher production of VFAs was observed with increasing organic loading; when the organic loading rate was 15 kg COD/m^3·d, over 2 g/L VFAs could be generated in the thermophilic anaerobic bioreactor (Wijekoon et al., 2011). Therefore, the VFA production capacity was mainly influenced by C loading. P decomposition correlated significantly with VFA yield ($p < 0.01$), which demonstrated that P release in the glucose-fed system may be partly driven by the
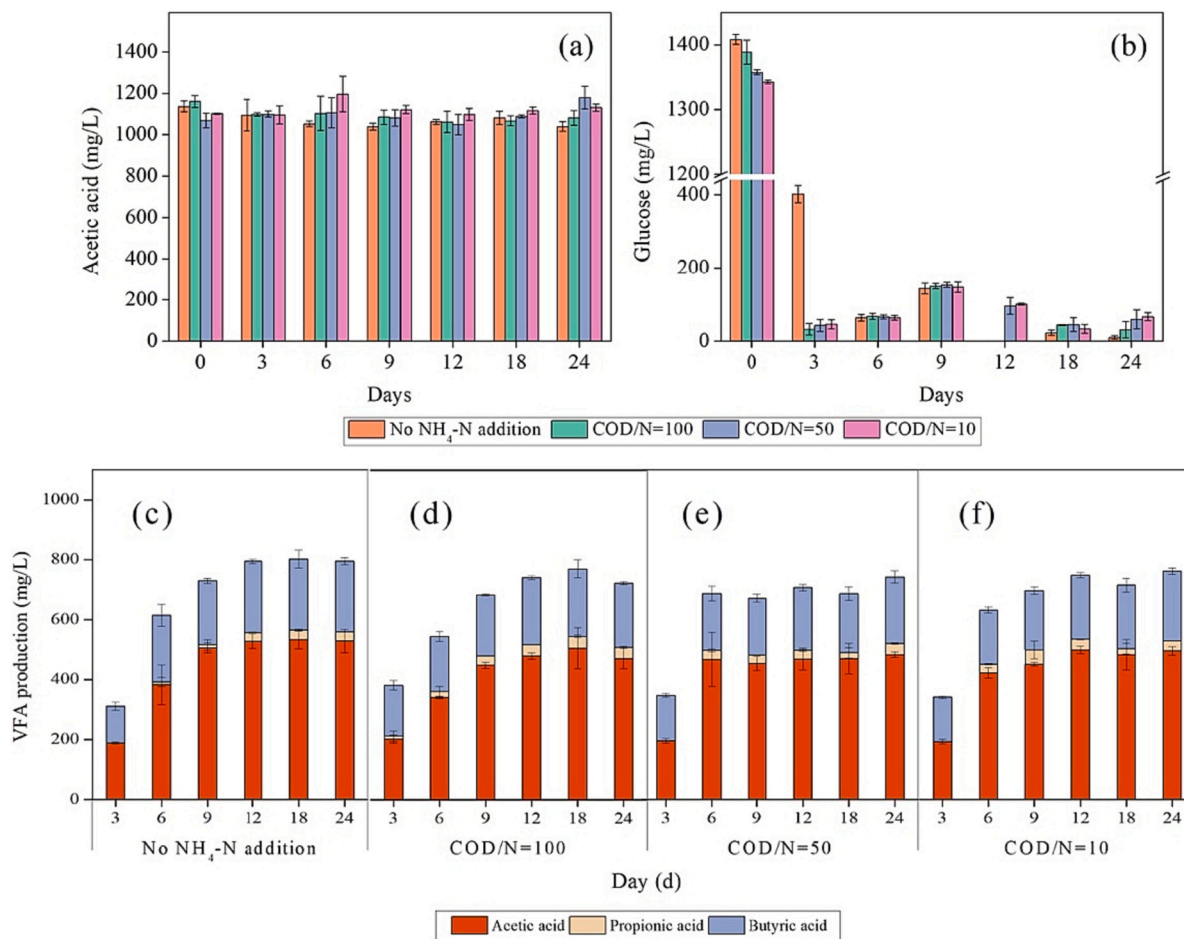

Fig. 3. Variations of (a) acetic acid consumption in the acetic acid-fed system, (b) glucose consumption, and (c)–(f) VFA production in the glucose-fed system during the 24-day anaerobic operation under different COD/N loading.

fermentation process, and it has been reported that inorganic P can be easily released during anaerobic fermentation (Yan et al., 2021).

Poly-P hydrolysis is driven by C catabolism, with the C source contributing as an electron donor for anaerobic oxidation, and subsequently leads to anaerobic orthophosphate release (Cakmak et al., 2022). In our experiments, acetic acid was the unlimited organic substrate that should be utilized by the biological reduction process and result in P release (Wu et al., 2012). However, only a small amount of acetic acid (<9 %) was consumed after 24 days, indicating that acetic acid is not a preferred C source for the bio-P bacteria of the sediments. In this case, the bacteria are probably forced to utilize their internal C sources for denitrification and drive the hydrolysis of poly-P (Hu et al., 2003; Wang et al., 2002). In the glucose-fed system, through C balance calculation, we found that the amount of C fed into the system was greater than that of C produced (mainly VFAs) via fermentation conversion, suggesting that part of the dosed C is probably stored or converted through synthesis of other C compounds (i.e., PHAs and glycogen) in the sediment cells.

3.4. Variations of alkaline phosphatase activity

AP as hydrolase has been identified as a key enzyme in organic P mineralization (Chen et al., 2011). The variations of AP activity are shown in Fig. 4. The AP activity in the acetic acid-fed system first increased over time and subsequently decreased. The maximum activity of 1046.97 mg/(kg·h) was reached on day 12 from an initial 885.90 mg/(kg·h) in the group of no NH_4-N addition group, and subsequently, this value fell back to 860.66 mg/(kg·h) on day 24. The opposite phenomenon was observed in the glucose-fed system, where the AP activity decreased from 838.11 mg/(kg·h) (day 0) to 490.67 mg/(kg·h), 447.86 mg/(kg·h), 584.45 mg/(kg·h) and 354.75 mg/(kg·h) on day 6 in the no NH_4-N addition, COD/N = 100, COD/N = 50, and COD/N = 10 group, respectively. However, the enzyme activity of the no NH_4-N addition group increased up to 967.79 mg/(kg·h) on day 18, being higher than the initial one.

Ammonia acts as an essential nutrient for the growth of microorganisms and, therefore, it may promote/inhibit P release from sediment by influencing metabolic processes/activity (Li et al., 2016; Ma et al., 2018). Previous studies have reported that excessive loading of NH_4-N can induce N:P imbalance and consequently lead to AP production, which accelerates the release of organic P in mesocosm experiments (Li et al., 2016; Ma et al., 2018). However, this phenomenon was not found in our experiment, where the highest enzymatic activities were contributed by the no NH_4-N addition group in both C-fed systems. Yu et al. (2010) investigated the changes in the phosphatase activities of

excess sludge during anaerobic fermentation at pH 10 within 20 h, and AP activities were significantly suppressed with fermentation time (dropped from ~20 to ~1 μg/(mg·h)). Similarly, the enzyme activity of waste-activated sludge was also reduced from ~47 μg/(mg·h) volatile solid (VS) to ~2 μg/(mg·h) VS with 4 days of anaerobic fermentation at pH 10 (Huang et al., 2014). Considering the P release results presented in Fig. 1, the above case might help us infer that the biotic effect derived from AP activity could play a role in influencing P decomposition, while excess NH_4-N loading to a certain extent suppressed the enzymatic activity under anaerobic environments, and consequently, inhibited P release from the sediment.

3.5. Analysis of microbial community structure

As illustrated in Fig. 5 (a) and (b), the initial sediment mainly comprised of the following phyla: Proteobacteria (21.4 % in acetic acid-fed and 32.0 % in glucose-fed system), Planctomycetota (16.0 % in acetic acid-fed and 13.5 % in glucose-fed system) and Chloroflexi (9.9 % in acetic acid-fed and 11.8 % in glucose-fed system). Proteobacteria dominated the sediment microbial community in both systems. This phylum comprises various species, which have been implicated in microbial C, N, and S cycling in a variety of water bodies (Wang et al., 2021). Planctomycetota, another prevalent phylum widely present in the sediment, has been found to be involved in many biological processes such as ammonia and carbohydrate metabolism (Wang et al., 2022). Chloroflexi has been reported to be abundant in organic-rich sediments where it can contribute to OM degradation (Hug et al., 2013). In our experiments, the abundance of Chloroflexi phyla in the acetic acid-fed system without extra NH_4-N addition increased from 9.9 % to 15.6 % on day 24. Meanwhile, the relative abundance of all three phyla in the glucose-fed system declined over the 24 days of anaerobic operation, from 32.0 % to 24.9 % for Proteobacteria, from 13.5 % to 8.4 % for Planctomycetota, and from 11.8 % to 6.3 % for Chloroflexi. Variations in phylum proportions could be associated with organic C utilization within the systems, the enrichment of Chloroflexi phylum was favored in the system fed with acetic acid, where acetate consumption was not significant (Fig. 3). Conversely, rapid glucose consumption is detrimental to the proliferation of organic-decomposing phyla (e.g., Proteobacteria, Planctomycetota, and Chloroflexi).

The most abundant family was initially *Pirellulaceae* with relative abundances of 12.2 % and 8.2 % in sediment from the acetic acid- and glucose-fed system, respectively (seen in Fig. 6(c) and (d)). In the glucose-fed system, the *Clostridiaceae* family (the most represented of Firmicutes) increased up to 57.4 % on day 9 ($p < 0.001$). A parallel decrease in *Desulfosarcinaceae* (from Desulfobacterota) and
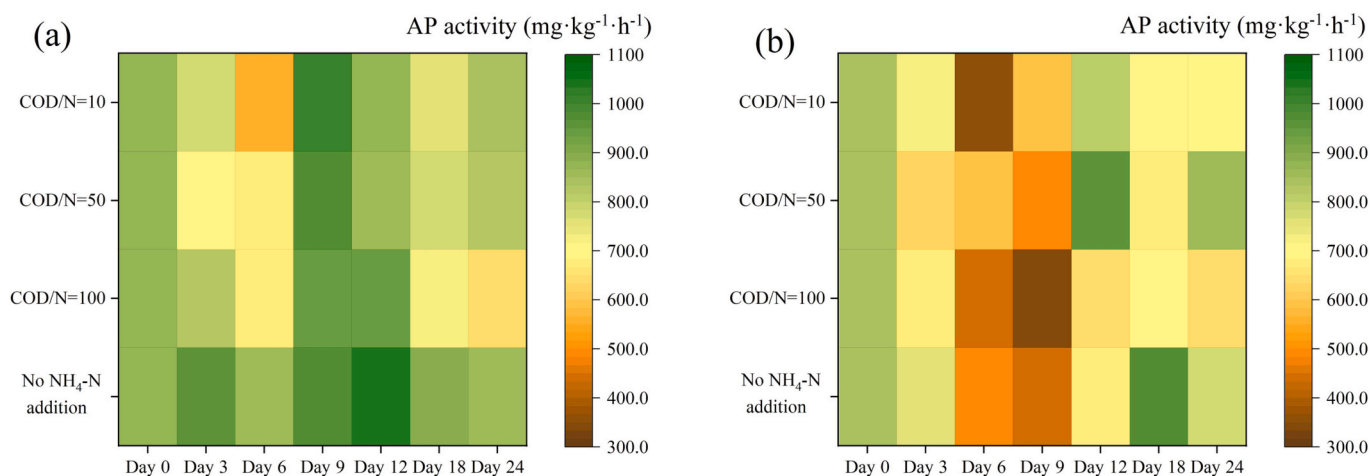

Fig. 4. Variations of alkaline phosphatase activity of marine sediment during the 24-day anaerobic operation under different COD/N loading: (a) acetic acid-fed system; (b) glucose-fed system.
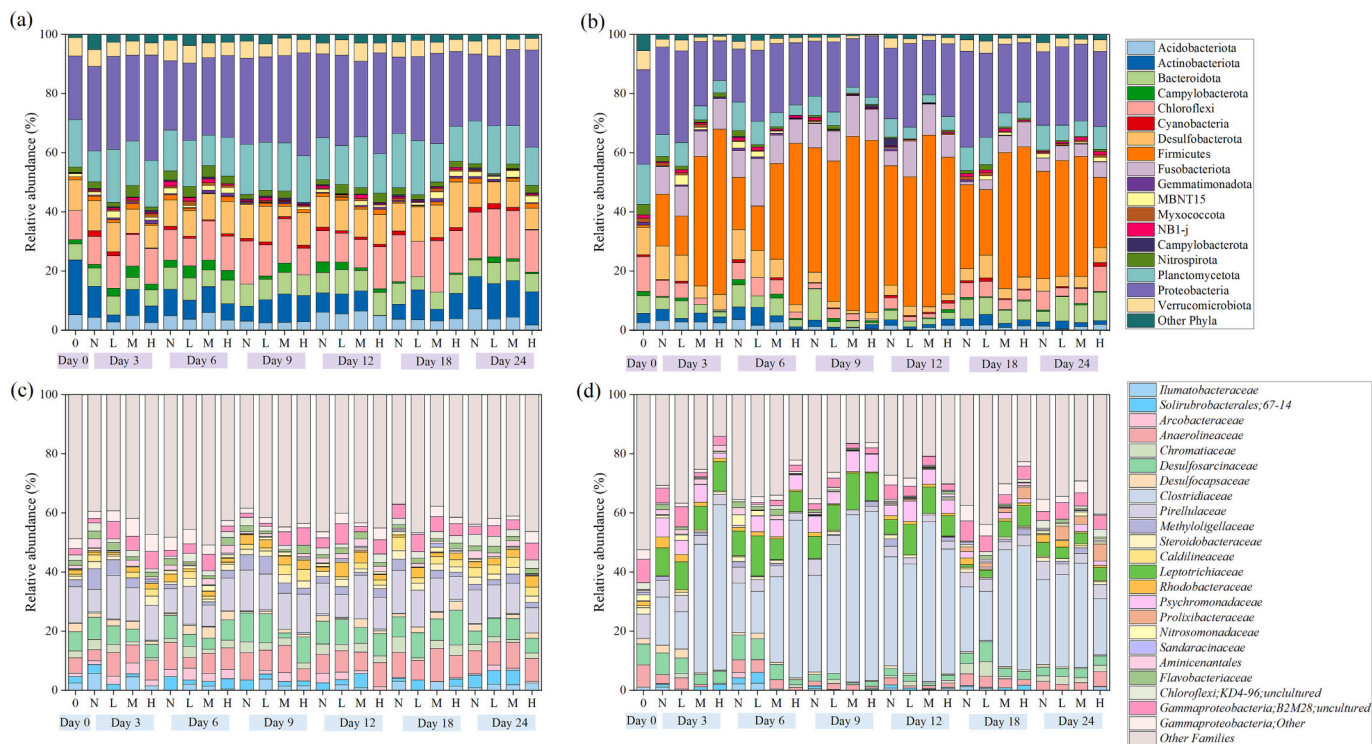
Fig. 5. Compositional structure of microbial communities during the 24-day anaerobic operation under different COD/N loading at the phylum level: (a) in the acetic acid-fed system; (b) in the glucose-fed system; and at the family level: (c) in the acetic acid-fed system; (d) in the glucose-fed system (N, no extra NH₄-N addition; L, COD/N = 100; M, COD/N = 50; H, COD/N = 10).
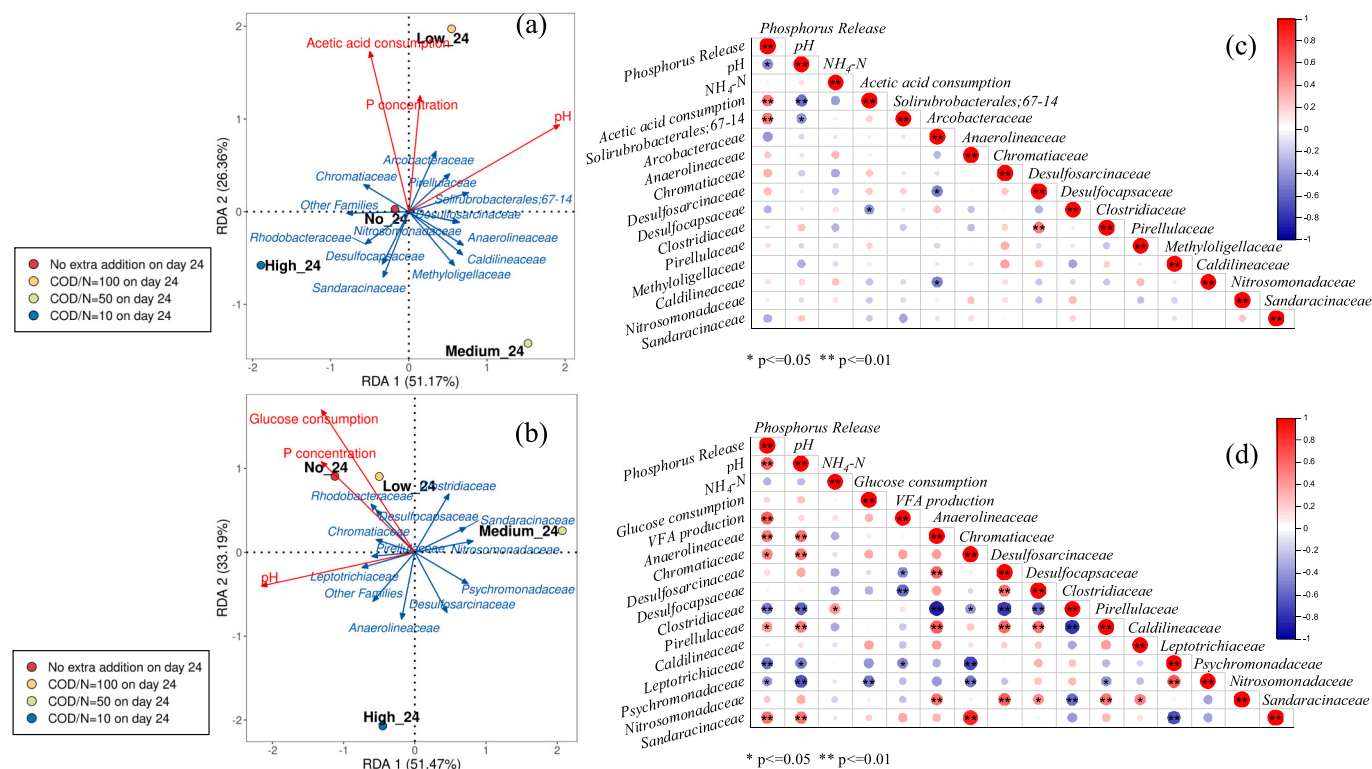

Fig. 6. Redundancy analysis for the relationships between environmental factors and microbial community for (a) acetic acid-fed system and (b) glucose-fed system; Pearson correlation analysis between the relative abundance of bacteria at the family level and different factors for (c) acetic acid-fed system and (d) glucose-fed system. For these analyses variables on day 24 were considered.

Anaerolineaceae (from Chloroflexi, $p < 0.01$) was observed during the anaerobic culture. The alpha diversity of glucose-fed groups tended to be higher overall with no extra NH₄-N addition until about day 12 of the experiment ($p \leq 0.08$) (Fig. S2). When looking at the compositional changes over time under different COD/N loading, we found that in the glucose-fed system, the group with no extra NH₄-N addition differed from the others, being discriminated by specific taxa (at specific time points) (Fig. S3). In particular, on day 3, this group was characterized by an overabundance of *Desulfocapsaceae* compared to the COD/N = 50 and COD/N = 10 groups ($p < 0.08$).

The *Clostridiaceae* family comprises a significant proportion of anaerobic fermentative bacteria (Zhang et al., 2018). In the glucose-fed system, the rapid increase in the relative abundance of the *Clostridiaceae* family can be observed (Fig. 5(d)); especially on day 9, the proportions rose from an initial around 0 % to 32.7 %, 43.6 %, 56.6 % and 57,4 % for the groups of no extra NH₄-N addition, COD/N = 100, COD/N = 50 and COD/N = 10, respectively. Another family that showed significant enrichment in the glucose-fed system, with the highest relative abundance on day 9, is *Leptotrichiaceae*, which also includes anaerobic organisms that can metabolize in anoxic environments (Lory, 2014; Thompson and Pikis, 2012). The proportions of *Leptotrichiaceae* increased from almost 0 % initially to 7.5 %, 8.6 %, 12.5 %, and 9.4 % for the groups with no extra NH₄-N addition, COD/N = 100, COD/N = 50, and COD/N = 10 on day 9, respectively. As glucose in the system was depleted after day 9 (Fig. 5(b)), the relative abundance of *Leptotrichiaceae* gradually declined. However, the abundance of these two mentioned families has consistently remained low in the acetic acid-fed system. The diverse variations in microbial communities in sediments from both acetic acid- and glucose-fed systems indicate a significant influence of the predominant carbon source on the composition of microbial communities.

Fig. 6(a) and (b) show the RDA results for the relationship between family-level microbial community composition and environmental factors on day 24. The interpretation rates of the two RDA axes were 51.17 % and 26.36 % in the acetic acid-fed system, 51.47 % and 33.19 % in the glucose-fed system, and the cumulative explanation rate was 77.53 % and 84.66 %, respectively. In both systems, the relative abundance of *Desulfocapsaceae* was significantly correlated with the no extra NH₄-N group, which also showed better P release. This family includes sulfate-reducing bacteria (SRB), which have already been previously identified in marine sediments. P release from sediment is closely associated with SRB since these bacteria can facilitate P release in sediment through anaerobic respiration, thereby promoting the formation of FeS, the reduction of Fe(III), and indirectly triggering the release of Fe-P in sediment (Yang et al., 2021). As illustrated in Fig. 5, the addition of extra NH₄-N led to the reduction in the proportions of the phylum Desulfobacterota, and the families *Desulfosarcinaceae* and *Desulfocapsaceae*, especially during the first 9 days. The reduction of SRB may potentially inhibit sulfate reduction under anaerobic conditions, leading to the suppression of the binding of Fe and S (Yang et al., 2021). Consequently, this led to a decrease of P decomposition from Fe-P which could be another potential reason for inhibiting P release, especially in the presence of high NH₄-N addition. This result is consistent with the previously discussed results of Fe release, as illustrated in Fig. S1, where the highest release of Fe (possible Fe-P dissolution) was from the no additional NH₄-N dosing group.

Additionally, the family *Rhodobacteraceae*, functioning as typical PAOs, and participating in C and sulfur metabolism bioprocesses, deserves more investigation concerning their fluctuations within anaerobic reactors (Zhang et al., 2020). As illustrated in Fig. 5, there is a slight increase in the abundance in both systems. The abundance of *Rhodobacteraceae* got increased from 1.5 % and 1.3 % to the maximum of 4.8 % and 3.7 % in acetic acid and glucose-fed, respectively. The two-way ANOVA analysis demonstrates a significantly higher abundance in the no extra NH₄-N addition group compared to the groups with COD/N ratios of 50 and 10 ($p < 0.05$) during the 24-day operation. It is worth

noting that the family *Rhodobacteraceae* and *Desulfosarcinaceae* exhibited similar behavior in the RDA analysis, particularly within the glucose-fed system. Indeed, the family *Rhodobacteraceae* plays a crucial role in the transformation and release of P as PAO under anaerobic conditions. Therefore, the addition of NH₄-N might potentially lead to a reduction in bio-P release through influencing the activities of PAO.

The analysis indicated a significant correlation ($p < 0.01$) between P-release and acetic acid consumption (Fig. 6a). While in the glucose-fed system, P release was directly correlated with the VFA production ($p < 0.01$), and relative abundance of families *Anaerolineaceae* and *Sandaracinaceae* ($p < 0.01$) (seen in Fig. 6d). The *Anaerolineaceae* family, primarily composed of anaerobic fermenters, has been demonstrated to play a key role in the mineralization and hydrolysis of OM, displaying high biodegradation efficiency (Zhang et al., 2022); *Sandaracinaceae* members are heterotrophic consumers of organic compounds, such as ethanol, hydrogen, and acetate (Probandt et al., 2017). This indicates that these families that consume organic compounds play a crucial role in influencing the release of P from sediment.

Overall, in the acetic acid-fed system, organisms capable of mineralizing organic compounds were not significantly enriched in the anaerobic environment, resulting in only a slight consumption of acetate. Conversely, glucose dosing could promote the enrichment of organisms, which could rapidly assimilate organic C, produce acids and decompose P from marine sediments. The analysis indicates that the addition of NH₄-N reduced the abundance of PAO and SRB thereby inhibiting P release. The potential processes and mechanisms influencing P release, as discussed in the previous sections, along with relevant microbial activities, have been summarized in Fig. 7.

3.6. The implication of the study

Our experiment aims to assess the feasibility of P mining from the Baltic Sea sediments, while concurrently addressing the eutrophication pollution stemming from excess P. Inspired by resource recovery techniques from waste streams, anaerobic C stimulation with different C/N ratios was conducted with batch reactors. The results indicate that no extra NH₄-N allows higher P release under the anaerobic bioreactors, when fed with glucose, the system is also accompanied by organic acid production. Meanwhile, the released P and NH₄-N in the system can be potentially recovered through struvite precipitation, along with the magnesium released from the marine sediment (>10 mM detected in the pore water).

Concerning P recovery, future research should be adopted for P precipitation. Although the P concentration that we obtained from the P-containing solution in our research is only 7.14 mg/L with the total solid (TS) content of 4 % sediment. However, obtaining a higher concentration of P solution is essential for a more effective P harvest from liquids. Achieving this can be explored by increasing the TS content of the sediment, scaling up the reactor, or implementing alternative approaches such as membrane separation. Additionally, it is crucial to prioritize and enhance P release from sediments to improve recovery efficiency. The release of P under anaerobic conditions is a complex process influenced by various factors (i.e., decomposition of OM and dissolution of IP). Hence, further research, especially concerning variations OM and P fraction of sediment, should be undertaken to gain a more comprehensive understanding. The microbial activities in this study evidenced that the abundance of key functional bacteria related to P release, such as PAOs and SRBs, are relatively low. Therefore, if these functional organisms could be enriched in the innovation-designed larger-scale bioreactors to improve the organic P release, the enhanced strategy could be widely spread for practical P recovery from the sediment.

Our results also offer insights into the internal cycling of C, N, and P within marine sediments, particularly in the deep-sea anoxic/anaerobic environment. This contributes to a more comprehensive understanding of the dynamic balance of nutrients in marine systems. Additionally, our experiment revealed that under anaerobic conditions, the presence of
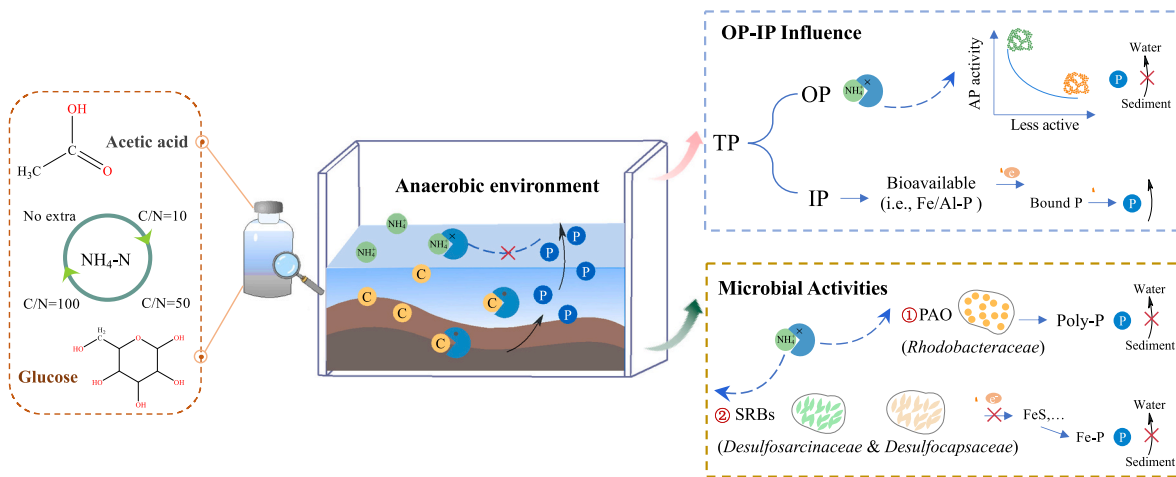
Fig. 7. The potential processes and mechanisms of the P release from sediment in response to NH$_4$-N loading strategies.

NH$_4$-N inhibits the release of P from sediment, consequently reducing the amount of P discharged into the water. Actively supporting the alleviation of issues associated with eutrophication by curbing marine P release. This information is crucial for developing precise nutrient management plans and approaches, thereby contributing to marine nutrient control, especially in mitigating eutrophication triggered by P and sustaining the health of ecosystems.

4. Conclusion

The strategy of C/N feeding can be crucial in affecting the P dynamics of eutrophic sediments since it can influence the growth of microorganisms and their metabolic activity, and subsequently P decomposition. Our study investigated the influence of different COD/NH$_4$-N dosing on P release for future P recycling simultaneously by anaerobic batch reactors. The results suggest that additional NH$_4$-N loading can significantly inhibit P decomposition under anaerobic conditions in both acetic acid- and glucose-fed systems, with the release of 27.5 % and 22.8 % less P, respectively, compared to the no extra NH$_4$-N loading group. Under anaerobic operation, the imbalance of C and NH$_4$-N did not stimulate the enzymatic AP activity, leading to an increase in P decomposition; instead, the enzyme activity decreased, which could be the potential inhibitory reason for OP release.

The glucose-fed system performed better than the acetate-fed system, with up to 7.12 mg/L P obtained through 18-day anaerobic batch reactors compared to only 4.07 mg/L in the acetic acid-fed system. Additionally, microbial community analysis demonstrated the families *Rhodobacteraceae* and *Desulfosarcinaceae*, as typical PAO and SRB, showed higher relative abundance with no extra NH$_4$-N addition, which might further elucidate the inhibition mechanism under high-concentration NH$_4$-N addition.

CRediT authorship contribution statement

Fengyi Zhu: Data curation, Investigation, Methodology, Visualization, Writing – original draft. **Ece Kendir Cakmak:** Project administration, Writing – review & editing. **Federica D'Amico:** Formal analysis, Investigation. **Marco Candela:** Formal analysis. **Silvia Turroni:** Formal analysis, Writing – review & editing. **Zeynep Cetecioglu:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.169902.

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