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Research article

Influence of *in situ* biochar capping on microbial dynamics and ammonia nitrogen release in sediment

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ABSTRACT

To study the influence of *in situ* biochar (BC) capping technique on the release of ammonia nitrogen (NH_4^+-N) from sediments, a field mesocosm experiment was conducted in Baiyangdian Lake (BYDL), a critical water body often referred to as the "kidney of North China" where sediment pollution poses a significant threat to water quality. This study also assessed the impact of BC on sediment microorganisms. The results showed that the NH_4^+-N concentration in the overlying water of the BC-treated mesocosms was the lowest among four treatments, decreasing to 0.051 mg L^{-1} by the 60th day. More importantly, the BC treatment showed the least increase in NH_4^+-N concentrations in sediments compared to other treatments. For sediments capped with a 4 cm layer of BC, the potential release flux of NH_4^+-N was reduced from 1.84 mg m^{-2} d^{-1} to −0.76 mg m^{-2} d^{-1}. This reduction is likely due to the negatively charged surfaces of biochar, which enhance NH_4^+-N adsorption through electrostatic interactions. Additionally, BC modified the physical and chemical properties of the surface sediment, improving pH and increasing both organic content and the carbon/nitrogen (C/N) ratio. These changes influenced the microbial community structure within the sediments, enhancing NH_4^+-N removal. After 60 days, a significant alteration in the microbial community was observed in the BC-treated surface sediments. The addition of BC significantly increased the abundance of *Proteobacteria* and *Firmicutes* of the phyla in the sediments. Furthermore, BC enhanced the expression of functional genes including *amoA, amoB, nirK, nirS, hzsB, nrfA* and *ureC*, which are likely the primary microbial mechanisms promoting NH_4^+-N conversion in sediments for final removal.

1. Introduction

One major water quality issue in Chinese basins is the presence of oxygen-consuming pollutants. In recent years, ammonia nitrogen (NH_4^+-N) has overtaken chemical oxygen demand (COD) as the primary oxygen-consuming pollutant. Most surface water in China has been severely polluted by high concentrations of nitrogen (N). By the mid-1980s, nitrogen levels in most surface waters across Chinese provinces exceeded the water quality standard of 1.0 mg L^{-1}. Research indicates that the current discharge rate of anthropogenic N into freshwater is approximately 2.7 times higher than the estimated 'safe' nitrogen discharge threshold of 5.2 ± 0.7 megatonnes of N per year (Yu et al., 2019). In 2018, the Chinese government implemented new water protection policies to control anthropogenic N inputs and improve water quality (Bai et al., 2018). Despite these efforts, endogenous nitrogen sources in river and lake sediments have become a significant nutrient source, posing a threat to water quality. Nutrients are temporarily stored in these sediments and can be released to the overlying water through biological, physical, chemical, and mechanical processes, such as pH reduction, oxygen depletion, sediment resuspension, and benthic disturbance (Hu et al., 2001). The release of autochthonous N forms, such as NH_4^+-N and NO_3^--N, can significantly decrease water quality, accelerating eutrophication, promoting phytoplankton/algal blooms (Van Luijn et al., 1999), causing oxygen depletion (Mopper and Kieber,

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2002) and leading to fish kills. Additionally, excessive NH_4^+-N in the water bodies can cause acidification (Grennfelt and Hultberg, 1986) and produce toxic effects on aquatic organisms (Boyd, 2020).

To address the issue of NH_4^+-N pollution in lake and river sediments, various remediation techniques have been proposed (Table S1). These techniques are broadly categorized into two types: *in situ* and *ex situ* remediation techniques (Reible, 2014). *In situ* remediation techniques are superior to *ex situ* remediation methods. For instance, *in situ* methods offer advantages such as practicality, cost-effectiveness, and rapid implementation (Lofrano et al., 2017; Xu et al., 2022; Zhang et al., 2016). Additionally, *in situ* techniques treat the sediments in place, reducing the risk of further resuspension and release of contaminants compared to dredging or excavation methods (Zhang et al., 2016). The *in situ* approach encompasses several techniques, including natural recovery, capping, chemical treatment, and microbial treatment. Among these, the *in situ* capping technique offers several benefits, including significant reduction in pollutant fluxes from sediments through adsorption and chelation by capping materials (Ding et al., 2018; Knox et al., 2008; Talpur et al., 2024; Zimmerman et al., 2004), In addition, the cost of capping technique is lower compared to other methods, and it has been extensively used to mitigate nutrient pollution in sediments, particularly nitrogen and phosphorus (Xia et al., 2016; Xiong et al., 2018). Selecting an appropriate material for the capping layer is crucial. Traditional capping materials such as soil (Xu et al., 2012), coal ash (Kim et al., 2014), activated carbon (AC) (Gu et al., 2017), and natural and modified zeolites (Gibbs and Özkundakci, 2011a; Huang et al., 2018) have been used to inhibit the release of NH_4^+-N and other nutrients from sediments. More recently, researchers have applied biochar (BC) to remediate NH_4^+-N contaminated sediments (Wei et al., 2023; Zhang et al., 2021; Zhu et al., 2019b, 2019c). BC is a highly stable, carbon-rich aromatized solid material produced by the high-temperature pyrolysis (400–700 °C) of biomass (Ahmad et al., 2014). BC has demonstrated outstanding performance in remediating various contaminants in water, soil and sediments (Cao et al., 2011; De Rozari et al., 2018; Song et al., 2024; Uchimiya et al., 2011). Many studies have focused on the remediation effects and physicochemical mechanisms of BC capping (Gomez-Eyles et al., 2013; Lou et al., 2011; Wang et al., 2019). However, most of these studies mentioned were conducted under laboratory conditions rather than in the field (Gibbs and Özkundakci, 2011; Gu et al., 2017; Wei et al., 2023; Zhang et al., 2021). Additionally, these studies primarily focused on analyzing nutrient concentrations and fluxes in overlying water samples, with limited attention given to sediment samples (Gibbs and Özkundakci, 2011; Gu et al., 2017; Wei et al., 2023; Zhang et al., 2021). Furthermore, there has been limited research on the biological effects of BC on sediments, particularly studies that focus on the effects of BC on benthic organisms (Clements et al., 2015; Wei et al., 2023; Zhang et al., 2021) while often overlooking the impacts on microbial communities within sediments. Research shows that the addition of BC can influence the N-dependent microbes in the soil (Prommer et al., 2014; Xu et al., 2014). Therefore, it is important to investigate the impact of BC on N removal in both the overlying water and sediment, as well as the microbial communities involved in N cycling in contaminated sediments. This understanding could offer more comprehensive insights into the mechanisms of microbial remediation and aid in designing and implementing effective mitigation strategies for N pollution in sediments.

The aim of this study was to 1) determine the influence of BC capping on NH_4^+-N release from sediments through field experiments at Baiyangdian Lake (BYDL), the largest freshwater lake in North China; 2) investigate the effect of BC addition on the microbial community in sediments, and 3) understand the role of N-related functional genes and microbial mechanisms in remediating N-contaminated sediments.

2. Material and methods

2.1. Field site and in situ capping method

BYDL is located approximately 100 km south of Beijing, in the Xiong'an District, China (115.99° E and 38.86° N). BYDL is the largest freshwater shallow inland lake in North China (Zhu et al., 2019a) and is part of the Daqing River system, a crucial water system within the Haihe River Basin. Often referred to as the "kidney of North China", BYDL faces significant water quality challenges due to sediment pollution (Mao et al., 2011). The field site was located in the north portion of the Beitian Village in the middle of BYDL. Water (n = 3) and sediment (n = 3) samples were collected from the sampling site between June and September 2019 (summer), during which the average temperature was approximately 26.8 °C. The field experimental area and site are shown in Fig. 1 and S2.

The field experiment was conducted using mesocosms which were made from polyvinyl chloride (PVC) pipes, each with a diameter of 0.9 m and a length of 2.0 m. Eight PVC pipes were vertically inserted into the sediment, extending approximately 50 cm above the water surface and 30 cm below the sediment-water interface (SWI). The mesocosms were positioned over 5 m from the shore to minimize anthropogenic influences. Four treatments were applied in this study, including a control (Treatment 1), sediments capped with 4 cm of BC (Treatment 2), AC (Treatment 3) and mixed BC/soil (1:1, w/w, Treatment 4) (Fig. 1c). Each treatment was conducted in two replicates. Water and sediment quality parameters, including pH, temperature, oxidation-reduction potential (ORP), and dissolved oxygen (DO), were monitored in one mesocosm, while microbiological analysis was conducted in a separate mesocosm to avoid any potential disturbance from the water and sediment quality measurements.

The detailed procedure of preparing BC for this experiment was described in our previous study (Zhu et al., 2019b). Briefly, the *Phyllostachys pubescens* bamboo was pyrolyzed at 500 °C for 3 h. The prepared BC was ground and sieved to 80 mesh, then rinsed with deionized water to remove any grease and fine suspensions, and dried before use. The AC (First grade, GB/T 13,803.2-1999), with the same particle size as BC, was purchased from Zhejiang Rongxing Carbon Industry Co., LTD. The clean soil was collected from the shore at the field site, subsequently dried and sieved to 80 mesh. This soil was mixed with BC (1:1, w/w) before being applied in the field.

2.2. Sampling and analysis

After the mesocosms were deployed, 100 mL of the overlying water sample (n = 3) was collected from each treatment on days 0, 1, 3, 5, 10, 15, 30, 45 and 60. Water quality parameters, including pH, temperature, ORP and DO, were recorded using a calibrated combined meter (YSI ProPlus Multiparameter, the US). 70 mL of each water sample was immediately filtered through membrane filters with a pore size of 0.45 μm (PVDF, Millipore). The filtered samples were kept at 4 °C during transport and were stored at −20 °C upon returning to the laboratory. On day 60, sediment cores were collected from the same mesocosm using a gravity stainless steel sampler with a PVC pipe (12 cm diameter). The top 20 cm of the sediments were selected, sliced into 1 cm intervals, transferred into 50 mL centrifuge tubes, and centrifuged at 5000 $r \cdot min^{-1}$ to extract the pore water. The pore water was filtered (0.45 μm, PVDF, Millipore) before analyzing the concentration of NH_4^+-N and NO_3^--N in the samples.

The concentrations of NH_4^+-N and NO_3^--N in the water samples were determined using a flow injection analyzer (Skalar Analytical, Breda, Netherlands). The total nitrogen (TN) concentrations in unfiltered water were measured using alkaline potassium persulfate digestion with an ultraviolet spectrophotometry (HJ 636–2012). NH_4^+-N concentrations in the pore water were analyzed with a chemical analyzer (Smart Chem, 2000; AMS-WESTCO, Italy). All analysis was conducted in triplicate, and

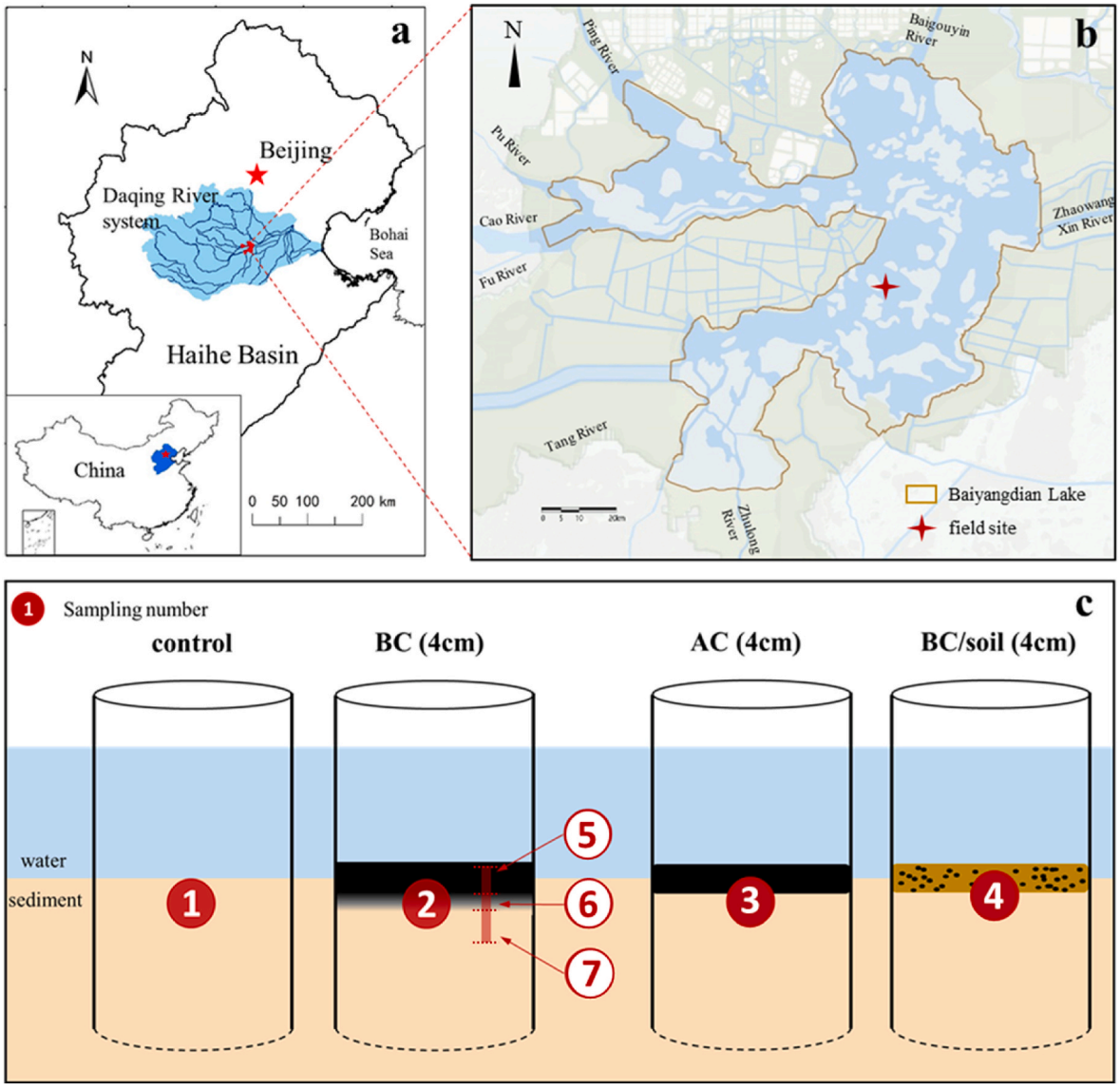

Fig. 1. (a) The location of Baiyangdian Lake. (b) The field mesocosms experiment site. (c) Schematic diagram of four mesocosms and sampling position.

the mean values were recorded.

To investigate the impact of different capping materials on the microbial community in sediments, surface sediments were collected from the mesocosms of all treatments for microbial analysis. Approximately 10 cm surface sediment samples were collected using a Van Veen Grab Sampler (Eijkelamp, Netherlands). To further examine the vertical distribution of microorganisms in sediments under BC capping, sediment cores were obtained from the mesocosm of Treatment 2 using a gravity stainless steel sampler equipped with a 9 cm diameter PVC pipe. For microbiological analysis, three distinct layers were identified: the capping layer (0–4 cm, sediment sample 5), the transition layer (4–6 cm, sediment sample 6), and the deeper sediment layer (6–10 cm, sediment sample 7). The samples were stored at −80 °C for DNA extraction.

2.3. DNA extraction, PCR amplification and sequencing

DNA was extracted from frozen sediments in duplicate using the Powersoil®DNA Isolation kit for sediments (MoBio, USA) follwing the manufacturer's protocols. The duplicate DNA extracts were then combined for PCR amplification. The V4–V5 region of the bacterial 16S rRNA gene was amplified by PCR using the barcoded primer set 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) (Zhang et al., 2017). PCR amplification was conducted in triplicate with each 20 μL reaction mixture containing 4 μL 5 × FastPfu Buffer, 2 μL 2.5 mM dNTPs, 0.8 μL each Primer (5 μM), 0.4 μl FastPfu Polymerase, 0.2 μL Bovine SerumAlbumin (BSA) and 10 ng template DNA. The thermocycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s (denaturation), 55 °C for 30 s (annealing), 72 °C for 45 s (extension), and a final extension at 72 °C for 10 min.

The amplicons were purified using the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor TM-ST (Promega, USA). After the purified amplicons were pooled in equimolar amounts, they were paired and sequenced (2 × 250) on an Illumina MiSeq platform according to standard protocols (Majorbio, Shanghai, China).

2.4. Real-time PCR

Real-time qPCR was carried out for the measurements of total bacteria (16S rRNA gene), *anoA, amoB, nirK, nirS, hzsB, nrfA and ureC* gene copy abundance as described in previous studies (Mao et al., 2011; Palmer et al., 2012; Song et al., 2015). Quantification of all the gene transcripts was performed on a 9600 Plus QPCR system (Bioer,

Hangzhou, China). PCR was performed at initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s. Each 20 μL reaction mixture contained 2 μL of template DNA, 16.5 μL of 2 × ChamQ SYBR Color qPCR Master Mix and 0.8 μl of each primer at a concentration of 5 μM. Each sample was measured in triplicate. PCR was performed at initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 56 °C (nrfA, 60 °C) for 30 s, and extension at 72 °C for 40 s. Plasmid pMD18-T (2692 bp, Takara, Dalian, China) was used in cloning gene fragments to establish standard curves for quantitative PCR.

2.5. Biodiversity analysis and phylogenetic classification

Rarefaction curves were established using MOTHUR program with an operational taxonomic unit (OUT) defined at 97% similarity. To compare the microbial diversity and richness of the seven sediment samples, the diversity indices of bacterial communities in sediment samples were performed using the PAST 3.0 (Liu et al., 2015).

2.6. Statistical analysis

One-way ANOVA, Tukey's post hoc test was performed to assess the significance of differences between the various treatments. A significant difference was considered to be present where $p < 0.05$. Statistical analyses were conducted using IBM SPSS Statistics 29.

3. Results

3.1. Nitrogen concentrations in the overlying water

The NH_4^+-N concentration in the overlying water displayed distinct patterns across the different treatments, except for the BC and AC treatments, which exhibited similar trends, as shown in Fig. 2a. Among these, the control showed the highest NH_4^+-N concentrations compared to other treatments ($p < 0.05$, one-way ANOVA). In the control, NH_4^+-N concentration increased significantly during the first 10 days ($p < 0.05$, one-way ANOVA), followed by a substantial decrease until day 20. After day 20, the concentration gradually declined until the end of the study period on day 60. In contrast, in the three capping treatments, NH_4^+-N concentration initially decreased within the first 3 days, with the most pronounced reduction observed in the BC/soil capping treatment, before the concentration began to rise. The overall pattern of changes in NH_4^+-N concentration for the BC and AC capping treatments were similar throughout the experiment. After day 10, the NH_4^+-N concentrations in these treatments continued to decline, with the BC capping treatment recording the lowest concentration, reaching 0.05 mg L^{-1} by day 60 ($p < 0.05$, one-way ANOVA). However, for the BC/soil capping treatment, NH_4^+-N concentration increased after day 3 and then fluctuated around 0.20 mg L^{-1} until day 60.

The concentration of NO_3^--N in the overlying water of different mesocosms showed fluctuations during the first 15 days, as shown in Fig. 2b. After this period, the concentration of NO_3^--N in the capping treatment mesocosms exhibited varying degrees of increase, followed by a decrease after 30–40 days. By day 60, the concentration of NO_3^--N in the control, BC and AC treatments ranging from 0.10 mg L^{-1} to 0.21 mg L^{-1}.

Regarding TN in the overlying water, the concentration in the control remained elevated, peaking at 2.90 mg L^{-1} by day 60. In contrast, TN concentrations in the capping treatment mesocosms were consistently lower than those in the control, with relatively similar levels among the three capped treatments as shown in Fig. 2c. The concentration of TN in the three mesocosms fluctuated during the first 15 days and then stabilized. At day 60 day, the concentration of TN in the overlying water of mesocosms 2, 3, and 4 were 0.95, 1.35, and 1.48 mg L^{-1}, respectively.

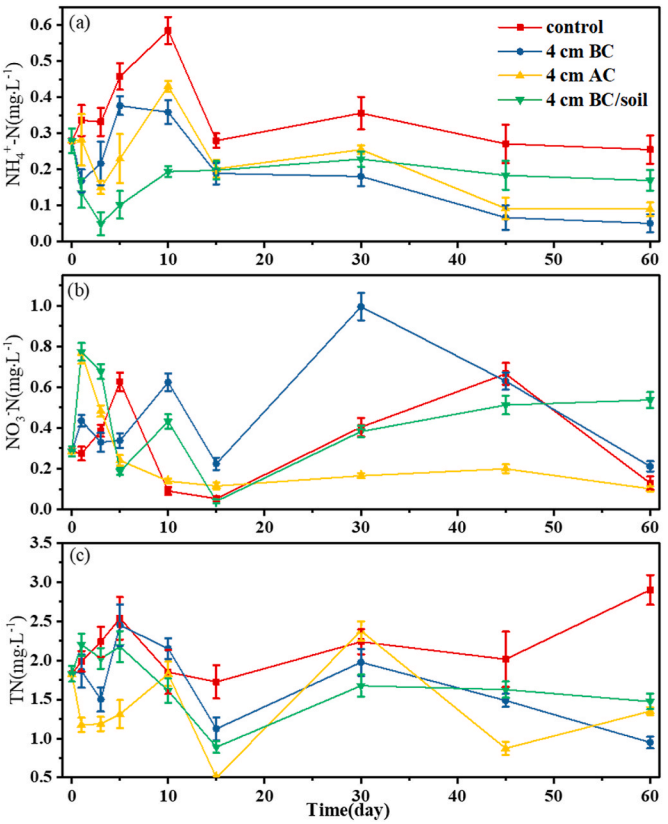

Fig. 2. Concentrations of NH_4^+-N (a), NO_3^--N (b) and TN (c) in the overlying water of different mesocosms. Each value represents the average ± standard deviation (n = 3).

3.2. Nitrogen concentrations in the sediments

The physical and chemical properties of the sediment samples from the three mesocosms are detailed in Table 1. The surface sediments of the field site displayed weak alkalinity, with a pH of 8.07. With the addition of BC, the pH of surface sediments decreased slightly, approaching neutrality. Conversely, the addition of AC resulted in a higher pH value of the sediments. DO remained approximately 11 mg L^{-1} in the water until day 15, after which it declined sharply until day 20, eventually stabilizing between 4 and 8 mg L^{-1}, with the lowest DO recorded in the control. Conversely, ORP gradually increased from −240 mV on day 0 to a range of −120 to −60 mV, with the highest ORP observed in the BC treatment (Fig. S1). The introduction of capping

Table 1
The physical and chemical properties of different sediment samples (LOI: loss of ignition, TC: total carbon, TN: total nitrogen, C/N: carbon to nitrogen ratio, TP: total phosphorus).

	1[a]	2[a]	3	4[a]	5	6	7
pH	8.07	7.89	8.26	7.63	7.48	7.91	7.94
LOI (%)	2.4	4.2	14.0	4.7	29.7	3.9	2.0
ρ (g·cm^{-3})	1.23	1.33	1.25	1.32	1.30	1.34	1.36
Porosity (%)	60.2	53.4	50.2	55.4	51.8	56.7	61.3
TC (g·kg^{-1})	18.1	411.7	451.9	398.8	803.5	157.8	19.6
TN (g·kg^{-1})	2.1	4.1	2.3	4.9	6.9	3.1	2.6
C/N	8.6	100.4	200.1	81.4	116.4	50.8	7.5
NH_4^+-N (mg·kg^{-1})	254.8	200.2	216.7	213.9	36.5	188.4	276.3
NO^{3-}-N (mg·kg^{-1})	0.84	1.17	1.09	0.90	1.21	0.95	0.80
TP (g·kg^{-1})	0.86	0.69	0.72	0.75	0.61	0.71	0.78

[a] The mesocosms was designed with other field experiments at same time, this part of the data has been published (Zhu et al., 2019b).

materials also led to an increase in the organic content of the surface sediments to varying extents. Being carbonaceous materials, both BC and AC significantly increased the total carbon (TC) and carbon to nitrogen ratio (C/N) of the sediments. TC and C/N in the surface sediments capped with AC reached 451.9 g kg^{-1} and 200.1, respectively, whereas those capped with BC were 411.7 g kg^{-1} and 100.4. After the application of different capping materials, the releasing of NH_4^+-N from the surface sediments decreased from 254.8 mg kg^{-1} to 200.2 mg kg^{-1}, 216.7 mg kg^{-1} and 213.9 mg kg^{-1}, respectively. In the BC capped layer, NH_4^+-N leaching was significantly reduced to a very low value of 36.5 mg kg^{-1}.

Sediment samples were collected and analyzed to determine the distribution of NH_4^+-N in pore water. In the control, AC, and BC/soil treatments, the concentrations of NH_4^+-N increased significantly from the SWI to greater depths. In contrast, the BC treatment exhibited only a slight increase in NH_4^+-N concentrations from 0 to 4 cm below the SWI, aligning with the depth of the BC capping layer. For instance, NH_4^+-N concentrations in this layer ranged from 0.01 mg L^{-1} to 1.20 mg L^{-1}, whereas in the control, concentrations at the same depth ranged from 0.87 mg L^{-1} to 2.12 mg L^{-1}. In addition, the BC treatment exhibited the smallest increase in NH_4^+-N concentrations compared to other treatments ($p < 0.05$, one-way ANOVA) (Fig. 3a and b). Furthermore, Fig. 3b illustrates the potential release flux of NH_4^+-N on day 60. All treatments, except for the BC capping, demonstrated a positive NH_4^+-N flux from the sediments. No significant difference was observed between the control and AC treatment ($p > 0.05$, one-way ANOVA), but a significant difference was found between the control and the BC/soil treatment ($p < 0.05$, one-way ANOVA). The control showed the highest positive NH_4^+-N flux at 1.84 mg m^{-2} d^{-1}, followed by AC capping at 1.05 mg m^{-2} d^{-1} and BC/soil capping at 0.74 mg m^{-2} d^{-1}. Conversely, the BC capping demonstrated a negative flux of NH_4^+-N of −0.76 mg m^{-2} d^{-1}.

3.3. Effects of BC on microbial community

A total of 37 phyla with a relative abundance ratio greater than 0.1% were identified among the 7 sediment samples collected. For this study, Fig. 4 presents 11 bacterial phyla with abundances exceeding 1%, visualized using Circos (Krzywinski et al., 2009). Among these, *Proteobacteria* had the highest relative abundance, surpassing 31.5%, followed by *Chioroflexi* (18.3–24.9%), *Bacteroidetes* (9.7–11.6%), *Firmicutes* (6.4–11.4%), *Actinobacteria* (2.5–6.9%) and *Acidobacteria* (3.0–4.8%). Other phyla, including *Planctomycetes* and *Aminicenantes*, ranged between 1.0% and 2.3%, while the remaining phyla were below 1%. Differences in microbial community composition were observed across sediment samples, particularly in samples containing BC, which showed more distinct community shifts. In the BC capping layer (S5), the abundance of *Proteobacteria* increased to 44.0%, compared to 31.5–36.8% in other samples, while surface sediments with BC (S2) had the highest *Firmicutes* abundance at 11.4%.

Additionally, several dominant bacterial families with relative abundances above 1% were identified for further analysis. The family *Anaerolineaceae* (within the *Chloroflexi* phylum) was consistently most abundant across all samples, ranging from 12.7% to 20.0%. It is noteworthy that *Comamonadaceae* from *Proteobacteria* phylum was significantly more abundant in BC-capped sediment samples, with abundances ranging from 6.2% to 10.6%, compared to 3.2%–3.5% in other samples. The BC capping layer (S5) also showed distinct shifts in microbial family composition relative to the control, with increased abundances of *Desulfobulbaceae* (3.1%), *Clostridiaceae* (1.8%), *Hydrogenophilaceae* (2.5%), *Rhodocyclaceae* (2.7%), *Methylococcaceae* (1.1%), *Peptostreptococcaceae* (1.2%), *Cyanobacteria* (1.2%), *Methylophilaceae* (1.5%), and *Saprospiraceae* (1.0%), among others.

3.4. Transcript abundances of 16S rRNA and functional genes related to nitrogen cycle

Fig. 5 presents different function gene transcript copies as determined by qPCR. The gene copy numbers in cDNA, produced from the reverse transcription of RNA, were used to demonstrate the active expressions of total archaea, total bacteria, ammonia-oxidizing archaea, ammonia-oxidizing bacteria and denitrifiers. Similarly, the transcripts of *amoA, amoB, nrfA, nirS, nirK, hzsB* and *ureC* genes showed responses of microorganisms to BC. Significant differences in the transcription of *amoA, nirK*, and *hzsB* genes were observed in the BC treatment compared to other treatments ($p < 0.05$, one-way ANOVA), while the AC treatment

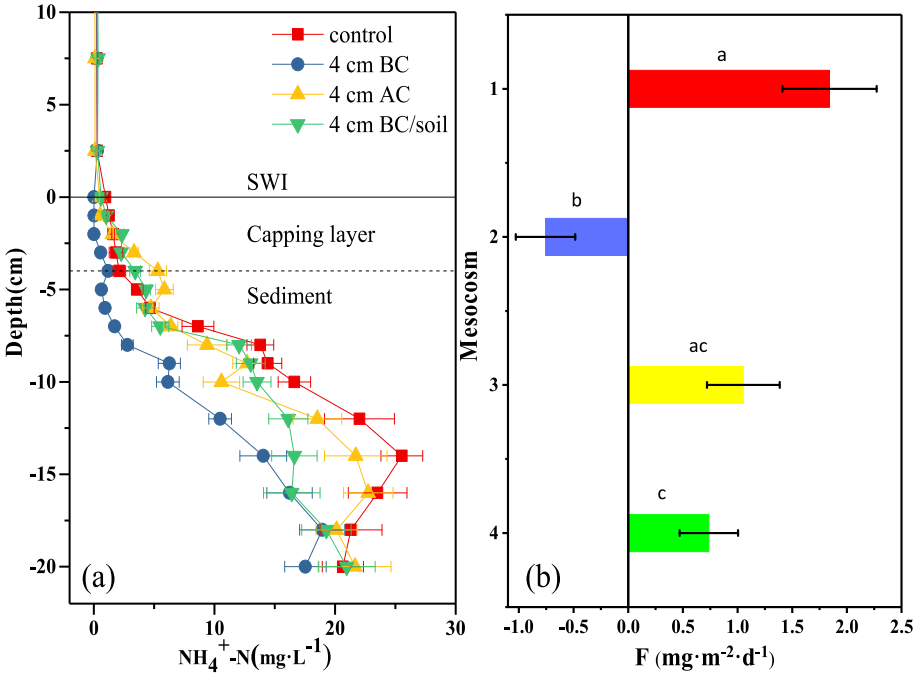

Fig. 3. (a) Downcore distributions of NH_4^+-N in different sediment sections in four mesocosms. (b) The potential NH_4^+-N release flux on day 60. Each value represents the average ± standard deviation (n = 3). Different letters (e.g., a, b, c) within each column in (b) indicate significant differences between treatments ($p < 0.05$, one-way ANOVA).

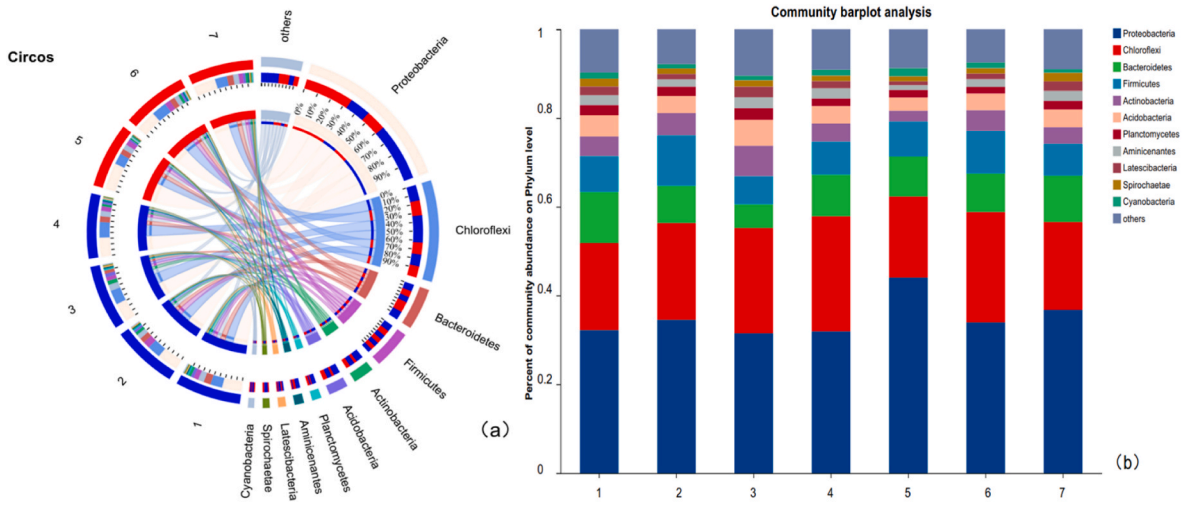

Fig. 4. (a) Distribution of microbial community for each sample at phylum level. (b) Relative abundance of the main families identified in the sediment samples.

recorded the lowest transcription levels of *amoA, nirK*, and *hzsB*. Additionally, the gene copy numbers in the BC/soil treatment sediment sample were similar to those observed in the control ($p > 0.05$, one-way ANOVA). In the surface sediment (S2) capped with BC, transcriptions of different genes, except for *nrfA*, were 1.9–4.1 times that of the control. Transcription copies in the BC capping layer (S5) were significantly higher than those of other experimental samples ($p < 0.05$, one-way ANOVA), reaching 7.0×10^9, 2.9×10^4, 7.3×10^6, 2.4×10^5, 1.6×10^8, 2.2×10^6, 1.8×10^5 and 6.4×10^6 transcript copies per gram dry sediment, respectively.

4. Discussion

4.1. The effect of in situ biochar capping on the release of ammonia nitrogen from sediments

As shown in Fig. 2a, the concentrations of NH_4^+-N in overlying water of the capping mesocosms showed a significant decrease within the first 3 days. The results suggested that NH_4^+ in the overlying water was removed through absorption by BC. The surfaces of BC are often negatively charged, which enhances this adsorption process through electrostatic interactions (Huang et al., 2018; Nguyen et al., 2017). *Phyllostachys pubescens* BC has been demonstrated to effectively adsorb NH_4^+-N from aqueous solution (Zhu et al., 2019c). Initially, as the material settled from the water column to the sediment surface, the adsorption by BC and AC was the dominant factor influencing NH_4^+-N in the water. This decrease was not observed in the control. Similar findings were observed in field experiments where biozeolite capping inhibited the release of NH_4^+-N from sediments (Huang et al., 2012; Zhou et al., 2016). The increase in NH_4^+-N concentrations observed after day 3 is likely due to enhanced ammonification/mineralization within the sediment, induced by the addition of capping materials. This finding aligns with previous studies (Nguyen et al., 2017; Zhang et al., 2021) and is further supported by the observed increase in *ureC* gene abundance, which is associated with ammonification/mineralization processes. However, the lower concentrations of NH_4^+-N in the water of the mesocosms treated with three different capping layers suggest that the capping significantly inhibited the release of NH_4^+-N from the sediments. After 10 days, the concentration of NH_4^+-N in the water of each mesocosm decreased and later stabilized, suggesting that the impact of microbial activity became more dominant, which will be explored further in Section 4.2. Previous studies have demonstrated that incorporating BC into the soil can enhance rates of denitrification (Castaldi et al., 2011), nitrification (Prommer et al., 2014) and anaerobic ammonia oxidation (Pan et al., 2017). Therefore, the addition of BC and AC likely contributed to the enhancement of these processes in the sediments, facilitating the removal of NH_4^+-N.

The rise in NO_3^--N concentration observed during the first 3 days across all four mesocosms (Fig. 2b) was primarily attributed to the rise in ORP at the sediment-water interface (Figs. S1 and S2). The porous capping materials contributed to this rise in ORP, shifting the environment from reducing to oxidizing conditions, which subsequently enhanced nitrification (Gao et al., 2009). Similar effects were noted in studies that used AC and nonwoven fabric mats as capping agents for the remediation of lake sediments enriched with nitrogen (Gu et al., 2017). Consequently, the NO_3^--N concentrations were higher in the mesocosms with capping treatments. After 10 days, an increase in microbial activity likely contributed to the further rise in NO_3^--N concentrations due to nitrification, coinciding with changes in NH_4^+-N concentrations. Over time, NO_3^--N accumulated in the sediment. After 40 days, as oxygen was depleted, denitrification became the dominant process, leading to a reduction in NO_3^--N concentrations in the water.

NH_4^+-N and NO_3^--N are important inorganic components of TN. Therefore, fluctuations in NH_4^+-N and NO_3^--N concentrations can influence the overall concentration of TN. The results indicated that all three types of capping materials had some degree of effectiveness in reducing TN concentrations in water. At the end of the field experiment, the TN concentration was lowest in the water of the mesocosm capped with BC. This mesocosm also recorded the lowest NH_4^+-N concentration, suggesting that among the capping materials, BC was the most effective in inhibiting the autochthonous release of NH_4^+-N from sediments.

The concentrations of NH_4^+-N in the sediment pore water across four mesocosms appeared to increase with depth. However, the concentrations NH_4^+-N in the pore water of mesocosms treated with BC capping showed the smallest increase. This can be attributed to several factors. Firstly, adsorbing NH_4^+-N through electrostatic interactions by BC limited the diffusion of NH_4^+-N to the overlying water. Secondly, previous results showed a low resupply of NH_4^+-N from the sediment after BC capping (Zhu et al., 2019c). Thirdly, the addition of carbon materials influenced the activity of microorganisms in the surface sediments, enhancing the processes of nitrification and denitrification, which will be discussed further in Section 4.2. This enhancement tends to increase the rate of NH_4^+-N consumption, thereby reducing its concentration in the pore water. Evidence of this effect is demonstrated by the change in NH_4^+-N flux from positive in the control to negative under BC treatment. This finding was consistent with our previous laboratory sediment core incubation experiments (Zhu et al., 2019c). In the other two capping treatment mesocosms, the release flux of NH_4^+-N over the SWI was also reduced, though the decrease was not as significant as with the pure BC capping.

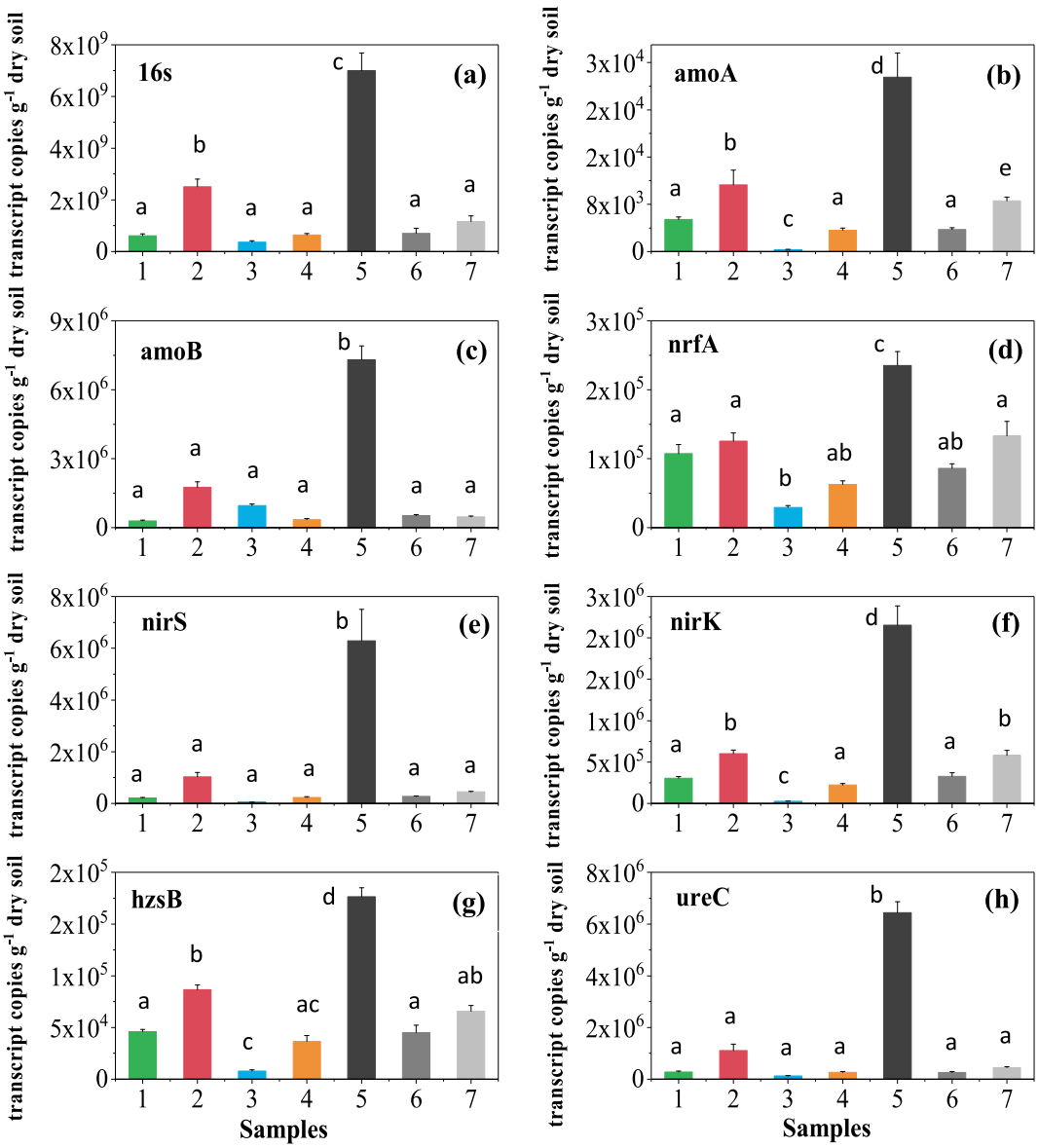

Fig. 5. Quantitative analysis of genes related to the nitrogen cycle in 7 sediment samples. Each value represents the average ± standard deviation (n = 3). Different letters (e.g., a, b, c) within each column indicate significant differences between treatments ($p < 0.05$, one-way ANOVA).

4.2. The effect of in situ biochar capping on microbial community and functional gene in sediments

The study shows that the *Proteobacteria* constituted the largest proportion of bacterial phyla across the seven analyzed sediment samples. Most ammonia-oxidizing bacteria were identified within the *Betaproteobacteria* and *Gammaproteobacteria* classes of the *Proteobacteria* phylum. These bacteria can oxidize ammonia to nitrite (Arp and Stein, 2003). The proportion of *Proteobacteria* was highest in the BC capping layer (S5), enhancing nitrification, which involves the conversion of NH_4^+-N into NO_2^--N or NO_3^--N. Additionally, the relative abundance of the *Proteobacteria* in S2, treated with BC capping, was the highest among all treatments. In addition, nitrite oxidation is a key biochemical pathway dominated by aerobic nitrite-oxidizing bacteria, which belong to the *Proteobacteria, Chloroflexi, Nitrospinae and Nitrospirae* phyla (Daims et al., 2016; Zhang et al., 2021). These bacteria accounted for 57.5%–63.2% of the microbial communities in sediment samples containing BC, with the highest percentage observed in S5, compared to 53.1% in the control. This indicates that BC significantly influences microbial community composition and may suggest a potential impact on nitrite oxidation. However, direct measurements of nitrite oxidation rates would be necessary to confirm any enhancement. *Bacteroidetes*, which were relatively abundance were high in each sample, had the capability to reduce nitrite to nitric oxide (Maia and Moura, 2014). Furthermore, the sediment samples containing BC, particulate S2, exhibited a higher relative abundance of the *Firmicutes* compared to other samples. This increase might be associated with the presence of BC, as the relative abundance of *Firmicutes* has also been observed to increase in soil following wildfires (Cobo-Díaz et al., 2015), a natural process for producing BC. Additionally, many microorganisms involved in nitrogen fixation processes are classified within the *Firmicutes* phylum (Henson et al., 2004).

Additionally, this study showed the influence of BC capping material on the microbial community structure in sediments, particularly regarding nitrogen transformations within the nitrogen cycle. Predominant bacterial families such as *Anaerolineaceae* and *Comamonadaceae* have important roles to play. *Anaerolineaceae* could degrade macromolecular organics via fermentation, providing readily available carbon sources in anaerobic conditions favorable for denitrification processes (Miao et al., 2016; Narihiro et al., 2012). *Comamonadaceae*, known for

their physiological diversity, were involved in both the assimilation of inorganic carbon autotrophically and the decomposition of organic carbon compounds, thereby facilitating further microbial metabolic activities and enhancing nitrogen removal (Kersters et al., 2006). Additionally, *Comamonadaceae* included dominant denitrifying bacteria that utilized poly (L-lactic acid) as a carbon source, enriching these communities in the BC-treated sediments and promoting nitrogen conversion (Takahashi et al., 2009). Similarly, *Rhodocyclaceae* contributed to nitrite/nitrate reduction using fermented carbon sources. Alongside *Saprospiraceae*, these families were integral to the fermentation coupled with the denitrification process, showing higher relative abundance in the BC capping layers (Guo et al., 2016b).

Other families, such as *Desulfobulbaceae*, were involved in sulfur hydrolysis and facilitated both denitrification and denitritation processes (Kostrytsia et al., 2018). *Clostridiaceae* species were noted for their nitrogen-fixing capabilities (Wiegel et al., 2006), while *Hydrogenophilaceae* played a role in autotrophic desulfurization-denitrification, demonstrating significant denitrification capacity (Chen et al., 2016; Guo et al., 2016a). *Methylococcaceae*, active aerobic methanotrophs, not only played a role in methane (CH_4) oxidation but also were capable of nitrogen fixation, linking them to nitrogen cycle dynamics in coastal marshes (Deng et al., 2019). *Methylophilaceae* connected methanol oxidation to denitrification in freshwater lake sediments, further supported by metagenomic analysis showing potential denitrification capabilities within the *Methylotenera* genus (Kalyuhznaya et al., 2009). *Saprospiraceae* members, including *Haliscomenobacter* and an unclassified genus of *Saprospiraceae* were identified as potentially capable of N_2 fixation, particularly under nitrogen-deficient conditions (Li et al., 2017). Lastly, *Cyanobacteria* played an important role in ammonium and organic nitrogen assimilation, exemplified by species like *Prochlorococcus marinus* (Gruber, 2008). The presence of these microorganisms in the BC capping layers highlights the potential of BC to enhance microbial processes essential for the effective transformation and removal of NH_4^+-N in sediment environments.

BC remediation has been observed to alter microbial diversity and the relative abundances of taxa within sediment environments, with potential implications for nitrogen cycling processes such as nitrification and denitrification (Zhang et al., 2021). This study showed that the addition of BC led to significant increases in gene transcript copies associated with the nitrogen cycle. A large increase in the number of 16S rRNA gene copies in the BC layer suggests an increased microbial population. This attributed to its porous structure, which includes an average pore diameter of 60 nm, total pore volume of 0.15 $cm^3\ g^{-1}$, and the large specific surface area with 260.7 $m^2\ kg^{-1}$, all of which enhance microbial attachment and proliferation (Zhu et al., 2019b). In contrast, AC, despite being a similarly porous material, did not show significant microbial enrichment. This is likely because AC undergoes activation processes involving high temperatures and various chemicals, which make it less suitable for microbial propagation in sediments (Shan et al., 2015). In addition, the presence of low molecular weight hydrocarbons on BC surfaces may serve as a carbon source for microorganisms (Farrell et al., 2013), and the NH_4^+-N adsorbed on BC could be consumed by a large number of bacteria attaching to BC, similar to the effect seen with zeolite attaching biofilm (Huang et al., 2011).

Furthermore, an increase in microbial abundance does not automatically enhance the conversion and removal of NH_4^+-N in the sediments. To investigate further, seven functional genes crucial for the nitrogen cycle were analyzed, showing significant increases in the BC layer. For instance, *amoA/B* genes, facilitating the initial oxidation of NH_4^+ to NH_2OH, were increased, thus promoting the nitrification process (Van Zwieten et al., 2014). *nrfA* gene involved in reducing NO_2^- to NH_4^+ during dissimilatory nitrate reduction to ammonium (DNRA), showed higher transcript copies, suggesting increased DNRA rates (Welsh et al., 2014). Increased transcript copies of *nirS* and *nirK* genes, which encode nitrite reductases, suggested enhanced denitrification capabilities. The *hzsB* which promotes anaerobic ammonium oxidation, was also significantly higher, aiding in NH_4^+ removal (Pan et al., 2017). The *ureC* gene, encoding urease that converts urea to NH_4^+, showed higher abundance, indicating increased ammonification (Bastida et al., 2017; Gresham et al., 2007). Further analysis comparing transcript copies to 16S RNA (as shown in Fig. 6) revealed increases in *nirS* and *ureC* in the BC layer compared to other samples ($p < 0.05$, one-way ANOVA). This suggests that BC not only supports an increase in microbial numbers but also enhances their nitrogen-related metabolic activity, which is crucial for the nitrogen cycle in sediments.

4.3. Benefits of using BC as the capping material for remediation

Compared to traditional capping materials, BC offer numerous benefits. The comparison between BC, AC and zeolite is presented in Table S2. BC has high adsorption capacities which effectively reduce NH_4^+-N in sediments and have demonstrated significant remediation effectiveness in reducing the bioavailability and bioaccumulation of metals in sediments, as evidenced by our previous studies (Zhang et al., 2018). BC, is a low-cost carbonaceous material and is emerging as an economical alternative to AC for removing diverse organic and inorganic contaminants (Oliveira et al., 2017). Distinct from other capping materials like zeolite, BC increased the TC content and C/N of the surface sediments. This alteration in the C/N ratio can influence microbial communities in the sediments, which has been demonstrated in this study.

Additionally, BC materials were generally weakly alkaline (Fidel et al., 2017), providing a neutralizing effect on surface sediments. BC also has a large surface area of 260 $m^2\ g^{-1}$, which is higher than that of zeolite (52.1 $m^2\ g^{-1}$ from Gu et al., 2017), and a relatively low density, much lower than that of zeolite that are commonly used for the remediation of NH_4^+-N contaminated sediments. In our field experiment, only 7.1 kg of BC was required to achieve a 4 cm thick capping layer per square meter. In contrast, approximately 27 kg of zeolite would be needed for the same coverage (Gu et al., 2017). Furthermore, China is rich in *Phyllostachys pubescens*, providing an abundant resource for BC production (Zhang, 2023). More importantly, compared to AC, which is the most commonly used method for sediment remediation, biochar exhibits fewer toxic effects on benthic organisms, making it a promising material for ecological restoration (Clements et al., 2015; Libralato et al., 2018). The application of a biochar layer creates a new benthic environment with reduced toxicity, which is advantageous for the long-term restoration of sediments. However, there are still some challenges and uncertainties that need to be addressed in future research. For example, the potential influence of benthic organisms on the structural integrity of the biochar layer is not yet fully understood, necessitating further investigation. Additionally, determining the optimal thickness of the biochar layer for effective nitrogen removal requires more research. It will be important to measure the nitrification and denitrification rate in the future to confirm he overall impact of biochar on nitrification and denitrification within sediments. Given that denitrification is a primary mechanism for nutrient removal in aquatic environments, it is crucial to measure denitrification rates using ^{15}N isotope labeling in future studies. Addressing these issues will be essential for enhancing the efficacy and sustainability of biochar-based technologies for nutrient removal in sediments of Baiyangdian Lake.

5. Conclusion

The effectiveness of biochar (BC) capping in mitigating NH_4^+-N contaminated sediments was assessed through a field mesocosm experiment. Within 60 days, NH_4^+-N concentrations in overlying water with BC capping showed a significant reduction, achieving the lowest concentrations (0.051 mg L^{-1}) compared to the control and other treatments (activated carbon [AC] and BC/soil mixture). Additionally, sediments capped with a 4 cm layer of BC achieved the greatest

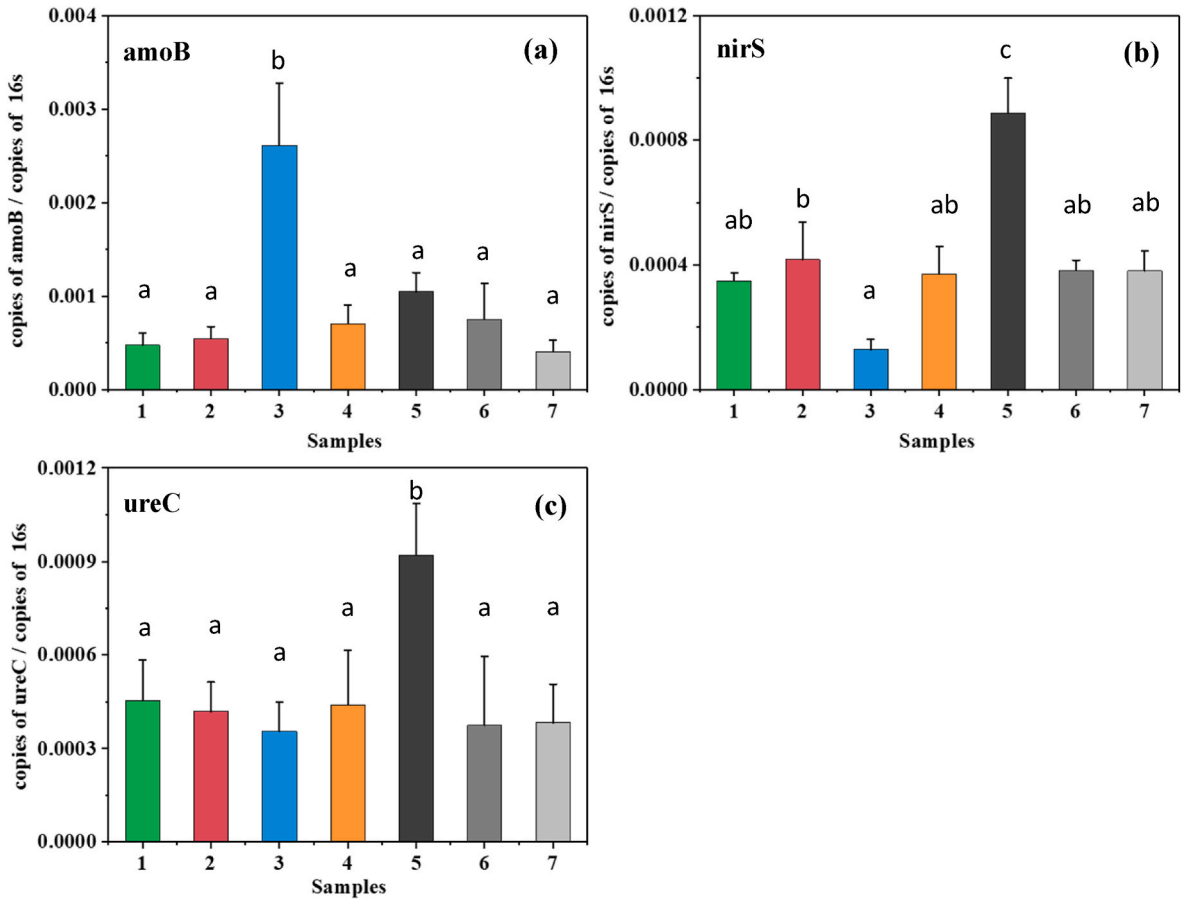

Fig. 6. Relative abundance of function genes related to nitrogen cycle. Each value represents the average ± standard deviation (n = 3). Different letters (e.g., a, b, c) within each column indicate significant differences between treatments ($p < 0.05$, one-way ANOVA).

reduction in NH_4^+-N release, with the flux reduced from 1.84 mg m^{-2} d^{-1} to −0.76 mg m^{-2} d^{-1}. This reduction can be attributed to changes in the physical and chemical properties of the sediment surface due to the addition of BC. Furthermore, BC altered the microbial community structure within the sediments, enhancing the removal of NH_4^+-N. The study indicated the increased abundance of *Proteobacteria* and *Firmicutes* from phylum in the sediments. The abundances of microorganisms in many families also increased, such as *Comamonadaceae*, *Desulfobulbaceae* and *Clostridiaceae*. The addition of BC was associated with a rise in functional microbial genes, including *amoA, amoB, nirK, nirS*, and *hzsB*, which are crucial for the microbial conversion processes that facilitate the effective removal of NH_4^+-N from the sediments.

CRediT authorship contribution statement

Yu Zhao: Writing – review & editing, Formal analysis. **Yaoyao Zhu:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Jianyin Huang:** Writing – original draft, Conceptualization. **Zhixin Song:** Writing – review & editing, Methodology, Investigation. **Wenzhong Tang:** Writing – review & editing, Methodology, Investigation.

Data availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

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Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled“The effect of *in situ* biochar capping on microorganisms during inhibiting the release of ammonia nitrogen from sediments”.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.123524.

Data availability

Data will be made available on request.

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