

Synergistic effects of soybean oligosaccharides and *Chlorella pyrenoidosa* on water quality and microbial community structure in biofloc system

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Abstract

This study explored the synergistic effects of soybean oligosaccharides (SBOS) and *Chlorella pyrenoidosa* on water quality and microbial community structure of aquaculture effluent in a biofloc system. Five experimental treatments were designed including CON group (control, no treatment), GLU group (glucose), SBOS group (2.5% SBOS and 97.5% glucose), CP group (glucose and *Chlorella pyrenoidosa*), and CS group (2.5% SBOS, 97.5% glucose and *Chlorella pyrenoidosa*). *Chlorella pyrenoidosa* were included at a concentration of 5.37×10^5 cells/mL for selected treatments. Each treatment had 5 replicates, and C/N ratio was 15. Over the nine-day experimental period, combination of SBOS and *Chlorella pyrenoidosa* demonstrated significant synergistic effects on water quality improvement and biofloc formation ($P < 0.05$). Specifically, in CS group, nitrate concentrations were significantly reduced on day 1, nitrite nitrogen concentrations exhibited a marked reduction on day 5, and both nitrite and total nitrogen concentrations showed significant reductions on day 7 compared to GLU group ($P < 0.05$). Biofloc volume (FV) in CS group showed a significant increase on day 3 compared to both CON and GLU groups ($P < 0.05$). Turbidity (NTU) was significantly lower in all experimental groups compared to CON group ($P < 0.05$). Compared with other experimental groups, the abundance of *Aeromonasaceae* decreased significantly, and *Bacillus* and *Mycobacteria* increased significantly in CS group, which contributed to the enhanced nitrogen cycling and degradation of organic matter degradation pathway ($P < 0.05$). These findings suggest that SBOS and *Chlorella pyrenoidosa* exhibit synergistic effects in treatment of effluent in biofloc system, and efficiently remove nitrogen and optimize microbial community structure.

1. Introduction

In recent years, the escalation of high-nitrogen wastewater sourced from industry and agriculture has intensified challenges in water management and has posed a serious threat to aquatic ecosystems (Conley et al., 2009). Biofloc technology (BFT), a microbial-based water purification technique, is extensively utilized in aquaculture due to its efficient nitrogen processing and cost-effectiveness (Avnimelech, 2009). This technology enhances the proliferation of heterotrophic bacteria through the addition of carbon sources (Avnimelech, 1999), effectively reduces excess nitrogen while ensuring the safety of aquatic life (Huang et al., 2023). BFT is widely recognized as a sustainable and effective method for treating aquaculture effluents (Wandana et al., 2024).

The health and growth of aquatic animal are directly influenced by microorganisms (Li et al., 2024), with changes in the microbial community structure in the water environment being closely linked to their overall health (Bruno et al., 2023). The immune system of aquatic animals can be activated by the complex interactions among microorganisms in the BFT system (Rajeev et al., 2023) with numerous studies consistently demonstrating its significant positive effects on the immune performance and overall health of aquatic animals ((El-Hawarry et al., 2021; Flores-Valenzuela et al., 2021; Yu et al., 2023). Functional oligosaccharides, serving as prebiotics, effectively enhance intestinal morphology and improve the microbial community structure in aquatic animals (Rajeev et al., 2021). Moreover, the incorporation of oligosaccharides into feed has been extensively reported to modulate the intestinal microbial community structure (Jia et al., 2017), enhanced intestinal functionality (Yang et al., 2018), and boost immune performance (Hahor et al., 2019).

Microorganism regulation in BFT systems is facilitated by addition of organic carbon sources, with oligosaccharides emerging as a viable option. Research has demonstrated that incorporating oligosaccharides as a carbon source in BFT systems enhances the intestinal microbiota of cultured aquatic animals by increasing beneficial bacteria and reducing harmful ones (Kishawy et al., 2020). Substituting 1%-5% of glucose with soybean oligosaccharides (SBOS) in BFT systems significantly improve the growth of *Carassius auratus* and promoted the proliferation of beneficial intestinal bacteria such as *Actinobacteria* (Qiu et al., 2023). Furthermore, replacing glucose with 2.5% SBOS in high-nitrogen water effectively optimized the microbial community structure, fostering the growth of beneficial bacteria (Zhou et al., 2024a). These findings indicate that oligosaccharides not only optimize bacterial communities in aquatic systems but also enhance the proliferation of beneficial bacteria growth (Zhou et al., 2024b). Therefore, the addition of SBOS may exert a positive influence on bacterial communities in aquaculture environments.

Microalgae possess a notable ability to reduce ammonia nitrogen ($\text{NH}_4^+\text{-N}$) and nitrite nitrogen ($\text{NO}_2^-\text{-N}$) in water, similar to heterotrophic bacteria (Nie et al., 2020), while also lowering the phosphate levels (Holanda et al., 2022). Additionally, polysaccharides from microalgae are metabolized and utilized as binders to facilitate flocculation (Watanabe et al., 2008). Yun et al. (2022) demonstrated that algal populations exhibited significantly greater proliferation compared to bacterial populations in shrimp farming systems, with algae being more profoundly affected by interactions with zooplankton and other associated organisms. These findings align with the recommendation by Ramanan et al. (2016) that algae-based BFT systems are more suitable for shrimp farming due to their enhanced ecological interactions and functional advantages over bacteria-based systems. Similarly, studies had demonstrated that nitrates and phosphates in high nitrogen effluents can be effectively reduced by using *Chlorella vulgaris* alone, highlighting the significant potential of microalgae treatment in mitigating water eutrophication (Pekkoh et al., 2022). Currently, research on BFT systems predominantly emphasizes bacterial interactions, while the functional role and contributions of algae remain insufficiently studied. This disparity highlights the need for a more balanced investigation to understand the underlying synergistic interactions between bacteria and algae within these systems.

Symbiotic interactions between microalgae and bacteria, both direct and indirect, accelerate microalgae growth, enhance wastewater removal, and promote microalgal flocculation (Ramanan et al., 2016). Several studies have indicated that bacteria in aerobic-activated sludge secrete abundant extracellular polysaccharides whose viscosity and rheological properties facilitate the capture of microalgae, thereby forming microalgae-bacteria bio-communities that effectively enhance the system's capacity to adsorb nitrogen and phosphorus from the water (Seviour et al., 2012, 2009; Wang et al., 2022). The polysaccharides secreted by microalgae promote the production of extracellular polymeric substances (EPS), a mixture composed of proteins, polysaccharides, humic-like substances, and nucleic acids (Wingender et al., 1999), which significantly influence the flocculating, settling, and dewatering properties of flocs (Dai et al., 2020; Kang et al., 2023; Meng et al., 2023). Therefore, the interactions between microalgae and bacteria are now regarded as a sustainable and more commercially viable approach (Subashchandrabose et al., 2011).

The competitive or synergistic interactions among different bacteria play an important role in influencing water quality. The addition of SBOS has been shown to significantly increase the abundance of specific bacterial communities, thereby optimizing the microbial structure of the water and enhancing bacterial-microalgal interactions. However, research focusing on optimizing bacterial community structures in water through the synergistic effects of carbon sources (particularly oligosaccharides) and algae remains limited. To address this gap, the present study aims to enhance the efficiency and practical application of BFT by investigating the combined effects of SBOS and *Chlorella pyrenoidosa* on high-nitrogen effluent purification and their influence on the microbial community in the water.

2. Materials and methods

2.1. Experimental Materials

The water effluent was collected from an aquaculture farm in Huzhou (China). The water contained 10.24 mg/L of total nitrogen (TN), 1.34 mg/L of $\text{NH}_4^+\text{-N}$, 0.54 mg/L of $\text{NO}_2\text{-N}$, and 8.12 mg/L of nitrate nitrogen ($\text{NO}_3^-\text{-N}$). Anhydrous glucose (AR, 99%) was purchased from China National Pharmaceutical Group Chemical Reagent Co., Ltd. SBOS (BR, 99%) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. The *Chlorella pyrenoidosa* algal liquid (8.6×10^7 cell/mL) was sourced from Hainan Yuanquan Biotechnology Co., Ltd.

Thirty high-density polyethylene (HDPE) tanks with a capacity of 300 L were used, each initially filled with 150 L of aquaculture effluent. No water exchange was performed during the 9-day experimental period, which was conducted under natural light. Five treatments were established: control group (Control), with no carbon source added; glucose group (GLU), receiving glucose; SBOS group (SBOS), receiving 2.5% SBOS and 97.5% glucose; *Chlorella pyrenoidosa* group (CP), receiving glucose and *Chlorella pyrenoidosa* at 5.37×10^5 cells/mL; *Chlorella pyrenoidosa* + SBOS group (CS), receiving 2.5% SBOS and 97.5% glucose, along with *Chlorella pyrenoidosa* at 5.37×10^5 cells/mL. In all treatments except the control, these additions served as carbon sources, with the C/N ratio adjusted to 15:1 (Avnimelech, 1999) and each treatment had 5 replicates.

2.3. Water quality parameters

Key water quality parameters, including water temperature, pH, and dissolved oxygen (DO) of the water were monitored daily throughout the experimental using a YSI Pro Plus portable multi-parameter. Water samples (100 mL) were collected at the start of the experiment (day 0) and subsequently at two-day intervals (days 1, 3, 5, 7, and 9). Each water samples were filtered through a 0.45 μm membrane filter and nutrient concentrations, including $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, and TN in the water were measured using a QC8500 flow injection water quality analyzer. Biofloc volume (FV) was quantified using an Imhoff cone according to the method described by Avnimelech and Kochba (2009). A 1,000 mL water sample was allowed to settle naturally for 30 minutes in the Imhoff cone, which was positioned on a perfectly level surface, and the settled biofloc volume was recorded. Water Turbidity (NTU) was measured using a WTW 430 turbidity meter.

2.4. Microbial community analysis

At the start of the experiment (designated as the F0 group) and on day 9, 100 mL water samples were collected from 5 experimental tanks, filtered through a 0.22 μm membrane until visible particulates adhered to the membrane surface. The membranes were then placed in sterile centrifuge tubes and stored at -80°C for microbial diversity analysis. The DNA extraction and amplification were performed by Shanghai Meiji Bio-Pharmaceuticals Technology Co., Ltd., which conducted the 16S rDNA gene abundance sequencing analysis using Illumina sequencing. Specific kits and methods refer to previous studies (Deng et al., 2023). Primers 799F (5'-AACMGGATTAGATACCKG-3') and 1193R (5'-ACGTCATCCCACCTTCC-3') were used to amplify the hypervariable regions V5-V7, thereby avoiding the excessive influence of chloroplasts on the sequencing results in the V3-V4 regions. In phylogenetic and population genetic studies, Operational Taxonomic Unit (OTU) refers to a standard unit of classification, used to simplify the classification labeling process during analysis. The experimental results are presented at the phylum and genus levels, with OTUs clustered using a 97% similarity threshold. Community richness was reflected by ACE and Chao indices, community diversity was indicated by Shannon and Simpson indices, and community coverage was represented by the coverage index.

2.5. Statistical Analysis

IBM SPSS Statistics 26.0 was used for data analysis. One-way ANOVA was used to analyze the experimental data first, and then the synergistic effects of SBOS and *Chlorella pyrenoidosa* were analyzed by two-way ANOVA. A result is considered significant only both analysis methods show significance. Experimental results were expressed as mean $\pm$ standard deviation (mean $\pm$ SD). For high-throughput sequencing data, the Kruskal-Wallis rank-sum test was used for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Changes in Water Quality

Throughout the experiment, the temperature range from 27°C to 29°C (Fig. 1). The DO content in the experimental groups fluctuated significantly at the beginning of the experiment, dropping rapidly to its lowest point on day 1, which was significantly lower than that of the CON group ($P < 0.05$).

The content of ammonia nitrogen ($\text{NH}_4^+\text{-N}$) initially decreased, then increased, peaking on day 3, and subsequently decreased again. In contrast, the ammonia nitrogen content of the control group (CON) decreased and then stabilized (Fig. 2-a). On day 1, all groups experienced a rapid decline in $\text{NH}_4^+\text{-N}$

levels. By day 3, differences among the experimental groups were not statistically significant ($P > 0.05$), though the $\text{NH}_4^+\text{-N}$ remained significantly higher than the CON group ($P < 0.05$) (Table 1, Table S1). By day 5, the $\text{NH}_4^+\text{-N}$ content in the CS group was notably lower than those in the SBOS and GLU groups, with the latter showing higher content than the CP group ($P < 0.05$). A Significant synergistic effect between the SBOS and *Chlorella pyrenoidosa* on day 7, the $\text{NH}_4^+\text{-N}$ content in CS group was significantly higher than GLU group on days 7 and 9 ($P < 0.05$). However, $\text{NH}_4^+\text{-N}$ levels in all experimental groups were already in the healthy range.

Table 1
The effects of *chlorella pyrenoidosa* and soybean oligosaccharide on ammonia nitrogen

Algae	Carbon source	Groups	Day 1	Day 3	Day 5	Day 7	Day 9
-	GLU	GLU	0.17 ± 0.07	1.70 ± 0.21	0.24 ± 0.06 ^{Aa}	0.02 ± 0.00 ^b	0.08 ± 0.00 ^B
-	GLU + SBOS	SBOS	0.21 ± 0.08	1.86 ± 0.36	0.17 ± 0.02 ^{Ab}	0.03 ± 0.01 ^a	0.08 ± 0.00 ^B
<i>Chlorella pyrenoidosa</i>	GLU	CP	0.15 ± 0.06	1.66 ± 0.32	0.17 ± 0.08 ^{Ba}	0.03 ± 0.00 ^b	0.10 ± 0.01 ^A
<i>Chlorella pyrenoidosa</i>	GLU + SBOS	CS	0.19 ± 0.12	1.58 ± 0.35	0.12 ± 0.04 ^{Bb}	0.03 ± 0.01 ^a	0.09 ± 0.01 ^A
<i>P</i> -value							
Soybean oligosaccharide			0.31	0.78	0.04	0.05	0.81
<i>Chlorella pyrenoidosa</i>			0.64	0.28	0.03	0.47	0.00
S×C			0.99	0.41	0.61	0.01	0.41
Notes: GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and <i>Chlorella pyrenoidosa</i> added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and <i>Chlorella pyrenoidosa</i> added. S×C: the interaction between soybean oligosaccharide and <i>Chlorella pyrenoidosa</i> . Data are presented as means ± SD, n = 5. A, B, a, b Mean values with different letters were significantly different ($P < 0.05$).							

The content of nitrite nitrogen ($\text{NO}_2^-\text{-N}$) followed a similar fluctuating pattern to $\text{NH}_4^+\text{-N}$, with periods of increase and decrease observed throughout the experiment (Fig. 2-b). On day 3, $\text{NO}_2^-\text{-N}$ content in the GLU and SBOS groups was significantly lower than CP and CS groups ($P < 0.05$) (Table 2, Table S2). A distinct interaction between the SBOS and *Chlorella pyrenoidosa* significantly altered $\text{NO}_2^-\text{-N}$ concentrations on day 5, which significantly reduced the $\text{NO}_2^-\text{-N}$ content in the SBOS group ($P < 0.05$). By day 7, the $\text{NO}_2^-\text{-N}$ content in the SBOS group remained higher than that GLU group, while all experimental groups recorded higher levels than the control group ($P < 0.05$).

Table 2
The effects of *chlorella pyrenoidosa* and soybean oligosaccharide on Nitrite Nitrogen

Algae	Carbon source	Groups	Day 1	Day 3	Day 5	Day 7	Day 9
-	GLU	GLU	0.59 ± 0.20	0.15 ± 0.07 ^B	1.18 ± 0.24 ^A	0.17 ± 0.05 ^{Ab}	0.05 ± 0.03
-	GLU+ SBOS	SBOS	0.52 ± 0.31	0.16 ± 0.03 ^B	1.56 ± 0.18 ^A	0.29 ± 0.07 ^{Aa}	0.06 ± 0.03
<i>Chlorella pyrenoidosa</i>	GLU	CP	0.55 ± 0.39	0.34 ± 0.05 ^A	0.77 ± 0.24 ^B	0.17 ± 0.04 ^{Bb}	0.07 ± 0.02
<i>Chlorella pyrenoidosa</i>	GLU+ SBOS	CS	0.44 ± 0.45	0.25 ± 0.06 ^A	0.63 ± 0.34 ^B	0.15 ± 0.05 ^{Ba}	0.06 ± 0.02
<i>P</i> -value							
Soybean oligosaccharide			0.57	0.17	0.32	0.04	0.87
<i>Chlorella pyrenoidosa</i>			0.70	0.00	0.00	0.01	0.51
S×C			0.92	0.06	0.04	0.01	0.26
Notes: GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and <i>Chlorella pyrenoidosa</i> added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and <i>Chlorella pyrenoidosa</i> added. S×C: the interaction between soybean oligosaccharide and <i>Chlorella pyrenoidosa</i> . Data are presented as means ± SD, n = 5. A, B, a, b Mean values with different letters were significantly different ($P < 0.05$).							

The content of nitrate nitrogen ($\text{NO}_3^-\text{-N}$) initially decreased and then increased (Fig. 2-c, Table 3, Table S3). The $\text{NO}_3^-\text{-N}$ content remained relatively stable in the CON group, consistently higher than in the experimental groups ($P < 0.05$). A significant synergistic effect between SBOS and *Chlorella pyrenoidosa* was observed in reducing $\text{NO}_3^-\text{-N}$ levels on day 1, with the concentration in CS group significantly lower than other experimental groups ($P < 0.05$). By day 5, the

CP and CS groups exhibited higher NO₃⁻-N content compared to the GLU and SBOS groups ($P < 0.05$), although no significant differences were observed on days 7 and 9 ($P > 0.05$).

Table 3
The effects of *Chlorella pyrenoidosa* and soybean oligosaccharide on Nitrate Nitrogen

Algae	Carbon source	Groups	Day 1	Day 3	Day 5	Day 7	Day 9
-	GLU	GLU	1.47 ± 0.10 ^{Aa}	0.35 ± 0.06 ^B	2.84 ± 0.72 ^B	5.37 ± 0.81	6.03 ± 0.44
-	GLU+ SBOS	SBOS	1.43 ± 0.05 ^{Ab}	0.28 ± 0.06 ^B	2.13 ± 0.42 ^B	4.92 ± 0.73	5.66 ± 0.82
<i>Chlorella pyrenoidosa</i>	GLU	CP	0.58 ± 0.06 ^{Ba}	0.53 ± 0.24 ^A	3.68 ± 0.95 ^A	5.60 ± 0.96	5.89 ± 0.90
<i>Chlorella pyrenoidosa</i>	GLU+ SBOS	CS	0.26 ± 0.04 ^{Bb}	0.46 ± 0.18 ^A	3.19 ± 1.03 ^A	5.03 ± 0.60	5.70 ± 0.66
<i>P</i> -value							
Soybean oligosaccharide			0.00	0.35	0.12	0.16	0.40
<i>Chlorella pyrenoidosa</i>			0.00	0.02	0.02	0.63	0.87
S×C			0.00	0.94	0.77	0.87	0.79
Notes: GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and <i>Chlorella pyrenoidosa</i> added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and <i>Chlorella pyrenoidosa</i> added. S×C: the interaction between soybean oligosaccharide and <i>Chlorella pyrenoidosa</i> . Data are presented as means ± SD, n = 5. A, B, a, b Mean values with different letters were significantly different ($P < 0.05$).							

The content of total nitrogen (TN) showed a similar trend to NO₃⁻-N, with an initial decrease followed by an increase (Fig. 2-d, Table 4, Table S4). The TN content in the CON group remained relatively stable and was significantly higher than that in the experimental groups throughout the experiment ($P < 0.05$). On day 1, the TN content was significantly higher in the GLU group compared to the SBOS group, and the CP group recorded higher content than the CS group ($P < 0.05$). The content of TN on days 3, 5, and 9 showed no significant differences among the experimental groups ($P > 0.05$).

Table 4
The effects of *Chlorella pyrenoidosa* and soybean oligosaccharide on Total Nitrogen

Algae	Carbon source	Groups	Day 1	Day 3	Day 5	Day 7	Day 9
-	GLU	GLU	5.07 ± 0.69 ^{Aa}	3.30 ± 0.37	4.60 ± 0.52	7.15 ± 0.86 ^B	8.43 ± 0.30
-	GLU+ SBOS	SBOS	3.63 ± 1.17 ^{Ab}	3.41 ± 0.47	4.24 ± 0.33	8.71 ± 1.10 ^B	8.18 ± 0.80
<i>Chlorella pyrenoidosa</i>	GLU	CP	3.71 ± 0.51 ^{Ba}	3.29 ± 0.60	4.53 ± 0.46	9.40 ± 1.15 ^A	8.08 ± 1.05
<i>Chlorella pyrenoidosa</i>	GLU+ SBOS	CS	3.41 ± 0.57 ^{Bb}	3.05 ± 0.21	4.53 ± 0.32	8.59 ± 0.88 ^A	8.18 ± 0.95
<i>P</i> -value							
Soybean oligosaccharide			0.02	0.73	0.35	0.42	0.84
<i>Chlorella pyrenoidosa</i>			0.04	0.37	0.56	0.03	0.63
S×C			0.12	0.38	0.35	0.02	0.64
Notes: GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and <i>Chlorella pyrenoidosa</i> added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and <i>Chlorella pyrenoidosa</i> added. S×C: the interaction between soybean oligosaccharide and <i>Chlorella pyrenoidosa</i> . Data are presented as means ± SD, n = 5. A, B, a, b Mean values with different letters were significantly different ($P < 0.05$).							

3.2. Changes in the biofloc formation

Biofloc was not observed in the CON group during the experiment. Biofloc formation began in all experimental groups on day 2. The volume of the bioflocs gradually increased, reaching a peak on day 3 and then decreased (Fig. 3-a). On day 3, there was a significant interaction between SBOS and *Chlorella pyrenoidosa* ($P < 0.05$) (Table 5, Table S5). Additionally, the results on days 3 and 9 were similar, the FV content in each experimental group was significantly higher than that in control group ($P < 0.05$). The FV content in SBOS group was significantly higher than in GLU group ($P < 0.05$). On day 5, no significant differences were observed among the experimental groups ($P > 0.05$).

Table 5
The effects of *Chlorella pyrenoidosa* and soybean oligosaccharide on Floc Volume

Algae	Carbon source	Groups	Day 1	Day 3	Day 5	Day 7	Day 9
-	GLU	GLU	-	35.00 ± 12.37 ^{Ab}	8.40 ± 2.88	3.26 ± 0.73	2.38 ± 0.19 ^{Ab}
-	GLU+ SBOS	SBOS	-	79.00 ± 11.92 ^{Aa}	10.80 ± 1.79	3.13 ± 0.38	3.20 ± 0.39 ^{Aa}
<i>Chlorella pyrenoidosa</i>	GLU	CP	-	44.80 ± 3.42 ^{Bb}	7.76 ± 3.27	3.75 ± 0.48	1.69 ± 0.19 ^{Bb}
<i>Chlorella pyrenoidosa</i>	GLU+ SBOS	CS	-	47.40 ± 11.61 ^{Ba}	8.00 ± 4.69	3.30 ± 0.67	2.85 ± 0.23 ^{Ba}
<i>P</i> -value							
Soybean oligosaccharide			-	0.00	0.39	0.28	0.00
<i>Chlorella pyrenoidosa</i>			-	0.03	0.26	0.23	0.00
S×C			-	0.00	0.48	0.56	0.17
Notes: GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and <i>Chlorella pyrenoidosa</i> added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and <i>Chlorella pyrenoidosa</i> added. S×C: the interaction between soybean oligosaccharide and <i>Chlorella pyrenoidosa</i> . Data are presented as means ± SD, n = 5. A, B, a, b Mean values with different letters were significantly different (<i>P</i> < 0.05).							

The NTU in each group initially increased and then decreased, with the control group showing a smaller decrease (Fig. 3-b). On day 1, the NTU in the SBOS group was significantly higher than GLU group (*P* < 0.05) (Table 6, Table S6). In the following days, there were no significant differences in NTU among the groups (*P* > 0.05), but all groups exhibited significantly lower NTU compared to the control group (*P* < 0.05).

Table 6
The effects of *Chlorella pyrenoidosa* and soybean oligosaccharide on Turbidity

Algae	Carbon source	Groups	Day 1	Day 3	Day 5	Day 7	Day 9
-	GLU	GLU	45.74 ± 6.38 ^b	34.1 ± 13.25	9.65 ± 4.00	4.35 ± 0.87	5.82 ± 1.60
-	GLU+ SBOS	SBOS	67.84 ± 7.99 ^a	35.64 ± 17.98	9.02 ± 1.77	3.95 ± 1.53	5.71 ± 1.54
<i>Chlorella pyrenoidosa</i>	GLU	CP	54.82 ± 9.92 ^b	37.17 ± 21.28	8.95 ± 1.71	3.51 ± 1.18	4.48 ± 0.41
<i>Chlorella pyrenoidosa</i>	GLU+ SBOS	CS	55.4 ± 9.18 ^a	27.99 ± 3.88	5.47 ± 2.07	3.67 ± 1.98	7.22 ± 2.14
<i>P</i> -value							
Soybean oligosaccharide			0.01	0.59	0.09	0.86	0.08
<i>Chlorella pyrenoidosa</i>			0.66	0.75	0.08	0.40	0.91
S×C			0.01	0.45	0.23	0.67	0.06
Notes: GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and <i>Chlorella pyrenoidosa</i> added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and <i>Chlorella pyrenoidosa</i> added. S×C: the interaction between soybean oligosaccharide and <i>Chlorella pyrenoidosa</i> . Data are presented as means ± SD, n = 5. A, B, a, b Mean values with different letters were significantly different (<i>P</i> < 0.05).							

3.3. The microbial diversity and abundance in the water of BFT system

The results of microbial community diversity index for each group are shown in Table 7. Compared to the control group, the community diversity (Simpson index) was significantly increased, and community richness (OTU) was decreased in GLU group (*P* < 0.05). The community richness (ACE and Chao indexes) was significantly reduced in SBOS group compared to the control group (*P* < 0.05). The community diversity (Shannon index) was significantly lowered in CP group compared to the control group (*P* < 0.05). Both the CS and CP groups significantly enhanced community diversity (Shannon indexes) compared to the GLU and SBOS groups (*P* < 0.05). These results indicate that the experimental groups effectively modulated bacterial community diversity and impacted the microbial community structure. The Venn diagram also showed that the control group had more unique bacterial communities compared to the experimental groups (Fig. 4).

Table 7 Microbial diversity and abundance in bioflocs

Groups	ace	chao	shannon	coverage	OTU		
F0	1379.00±78.91	1311.00±67.16	4.66±0.07*	0.99±0.00	1166.00±63.04		
CON	1606.00±359.50	1538.00±331.20	4.30±0.38 ^a	0.99±0.00	1364.00±328.50 ^a		
GLU	1238.00±205.00	1186.00±196.10	3.17±0.37 ^{cd}	0.99±0.00	1014.00±172.90 ^b		
SBOS	1168.00±203.90	1128.00±187.50	2.96±0.39 ^d	0.99±0.00	945.60±164.20 ^b		
CP	1426.00±136.80	1358.00±136.10	3.63±0.38 ^{bc}	0.99±0.00	1176.00±129.00 ^{ab}		
CS	1448.00±91.87	1383.00±80.60	3.78±0.29 ^b	0.99±0.00	1195.00±61.22 ^{ab}		
Algae	Carbon source	Groups	ace	chao	coverage	shannon	OTU
-	GLU	GLU	1238.00±205.00	1186.00±196.10	0.99±0.00	3.17±0.37 ^b	1014.00±172.90
-	GLU+SBOS	SBOS	1168.00±203.90	1128.00±187.50	0.99±0.00	2.96±0.39 ^b	945.60±164.20
<i>Chlorella pyrenoidosa</i>	GLU	CP	1426.00±136.80	1358.00±136.10	0.99±0.00	3.63±0.38 ^a	1176.00±129.00
<i>Chlorella pyrenoidosa</i>	GLU+SBOS	CS	1448.00±91.87	1383.00±80.60	0.99±0.00	3.78±0.29 ^a	1195.00±61.22
<i>P</i> -value							
Soybean oligosaccharide			0.93	0.86	0.77	0.90	0.79
<i>Chlorella pyrenoidosa</i>			0.09	0.15	0.77	0.00	0.07
S×C			0.64	0.74	0.81	0.23	0.70

Notes: The upper section presents the pairwise comparison results between the CON and F0 groups, the middle section shows the results of Kruskal-Wallis rank-sum test, and the lower section displays the interaction effects between SBOS and *Chlorella pyrenoidosa*. F0: original water sample collected before the experiment started; CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added. S×C: the interaction between soybean oligosaccharide and *Chlorella pyrenoidosa*. Data are presented as means ± SD, n = 5. a, b Mean values with different letters were significantly different ($P < 0.05$). * indicates a significant difference between CON group and F0 group.

3.4. The microbial community composition in the BFT system

Compared to the F0 group, the abundance of *Actinobacteriota* increased in the CON group, with an overall similar structural proportion. The microbial community structures in all experimental groups were effectively altered in comparison to the CON group. At the phylum level, Actinobacteriota and Proteobacteria dominated in the CON group. In the experimental groups, the top five dominant bacteria were Bacteroidota, Firmicutes, Actinobacteriota, and Proteo (Fig. 5). Additionally, the GLU and SBOS groups had a higher abundance of Bacteroidota and a lower abundance of Actinobacteriota when compared to the CP and CS groups. At the genus level, all experimental groups effectively increased the abundance of *Flavobacterium* and *Bacillus* while decreasing the abundance of *hgeL_clade* and *unclassified_o__Saccharimonadales* (Fig. 6).

3.5. Differences in Bacterial Community Composition in BFT Systems

In this experiment, the microbial community structure of the experimental group was significantly different from CON group ($P < 0.01$), suggesting that the experimental groups significantly altered the bacterial community structure in the water (Fig. 7-a). Similarly, significant differences were observed among the microbial communities of the experimental groups ($P < 0.05$), with the CS group exhibiting a greater degree of divergence from the other groups (Fig. 7-b). The microbial community structure in the water was significantly affected by different treatments in the experimental groups. At the phylum level, the abundance of Bacteroidota was significantly increased in GLU, SBOS, and CP treatments compared to the CON group ($P < 0.05$) (Fig. 8-a). The abundance of Actinobacteriota was significantly reduced in all experimental groups ($P < 0.05$) (Fig. 8-b), while the abundance of Firmicutes was significantly increased ($P < 0.05$) (Fig. 8-c). At the genus level, the abundance of *Flavobacterium* and *Bacillus* increased, and the abundance of *hgeL_clade* decreased in experimental groups generally ($P < 0.05$) (Fig. 8-d). The abundance of *Terrimonas* was significantly increased and the abundance of *Chryseobacterium*, *Parvibaculum*, and *Neochlamydia* was significantly decreased in the SBOS group compared to the GLU group ($P < 0.05$) (Fig. 8-e). The abundance of *Saprospiraceae*, *Pseudomonas*, and *Chlamydiales* was significantly increased and the abundance of *Chryseobacterium* was significantly decreased in the CP group compared to the GLU group ($P < 0.05$) (Fig. 8-f). The abundance of *Saprospiraceae*, *norank_f_67_14*, and *Limnobacter* was significantly increased and the abundance of *Flavobacterium* decreased in the CS group compared to the GLU group ($P < 0.05$) (Fig. 8-g). Significant community differences were also observed among experimental groups. The abundance of *Hydrogenophaga*, *Mycobacterium*, *Saprospiraceae*, and *NS9_marine_group* were significantly increased in the CP group compared to the SBOS group ($P < 0.05$) (Fig. 8-h). Similarly, the abundance of *Phenylobacterium*, *Pseudomonas*, *Hyphomicrobium*, and *Acidibacter* was significantly increased in the CS group compared to the SBOS group ($P < 0.05$) (Fig. 8-i). A significant increase in the abundance of *Roseomonas*, *Phenylobacterium*, *Sphingomonas*, and *Solimonadaceae* in CS group compared to the CP group ($P < 0.05$) (Fig. 8-j). Linear Discriminant Analysis Effect Size (LefSe) analysis showed that Myxococcota was significantly enriched in the GLU group. The Bacteroidota was significantly enriched in the SBOS group, and Firmicutes was significantly enriched in CS group ($P < 0.05$) (Fig. 9-a). At the genus level, LDA value of 3.5

was used. *Flavobacterium* was significantly enriched in the SBOS group, *Hydrogenophaga* was significantly enriched in CP group, and *Bacillus* and *Mycobacterium* were significantly enriched in CS group ($P < 0.05$) (Fig. 9-b).

3.6. FAPROTAX prediction

FAPROTAX, a manually constructed database, is considered one of the most reliable methods for predicting microbial ecological functions (Zhang et al., 2023). Microbial function predictions based on the experimental results showed that the functional structure of the microorganisms was significantly altered in the experimental groups. The function involved in degrading aromatic hydrocarbons was significantly increased in the SBOS group compared to the GLU group ($P < 0.05$) (Fig. 10-a). The function related to nitrogen cycle conversion was significantly increased in the CP group ($P < 0.05$) (Figs. 10-b), while functions related to symbionts and nitrogen cycle conversion were significantly upregulated in the CS group ($P < 0.05$) (Fig. 10-c). The function of chemoheterotrophy was significantly increased in the SBOS group compared to the CP group ($P < 0.05$) (Fig. 10-d). The nitrogen cycle conversion ability was significantly increased in the CS group compared to the SBOS group ($P < 0.05$) (Fig. 10-e), and the abilities for nitrogen fixation and cellulolysis were significantly increased in the CS group compared to the CP group ($P < 0.05$) (Fig. 10-f).

3.7. The interactions of microorganisms in BFT systems and their impact on the environment

The distribution of samples and species was illustrated by the collinearity network (Fig. 11-a). The results showed that the proportion of shared bacteria between the experimental groups and the CON group was low. The microbial compositions of GLU and SBOS groups were quite comparable, whereas those of CS and CP groups also shared similarities. *Flavobacterium* and *Bacillus* were connected with every experimental group. *Rhizobiales* was connected only with SBOS group, and *Mycobacterium* was connected only with CS group. The univariate network diagram demonstrates the positive and negative correlations between bacterial communities, with red lines indicating positive correlations, green lines indicating negative correlations, and the thickness of the lines representing the correlation coefficient (Fig. 11-b). The results denoted a strong degree of correlation among bacteria in the BFT system. All lines connected to *Flavobacterium* were green, indicating negative correlations with bacteria such as *Mycobacterium*. In contrast, most lines linked to *Bacillus* were red, representing positive correlations, except for a single green line, which highlighted a negative correlation with *hgcL* clade. Overall, the red lines predominated over the green ones, suggesting a general trend of mutual promotion among bacterial communities within the system. The correlation between experimental bacterial communities and various environmental factors was also demonstrated (Fig. 12), with FV showing positive correlations with $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$, and negative correlations with $\text{NO}_3^-\text{-N}$, TN, and NTU. *Flavobacterium* showed a significant positive correlation with FV ($P < 0.01$) and significant negative correlations with $\text{NO}_3^-\text{-N}$, TN, and NTU ($P < 0.05$). *Bacillus* exhibited significant positive correlations with $\text{NH}_4^+\text{-N}$ and FV, and significant negative correlation with $\text{NO}_3^-\text{-N}$, TN, and NTU ($P < 0.05$). *HgcL* clade was significantly negatively correlated with FV and pH, while showing a significant positive correlation with NTU ($P < 0.05$).

4. Discussion

Bacteria and microalgae are crucial components of aquatic ecosystems, playing a crucial role in enhancing system stability (Vale et al., 2023). This stability is particularly important in BFT systems, where maintaining stable and healthy bacterial communities can effectively reduce harmful nitrogen levels in the water and positively influence the growth of aquatic animals (Lu et al., 2014). Harmful nitrogen in water can be effectively reduced by the combined action of microalgae and heterotrophic bacteria (Mahari et al., 2024). Additionally, various types of aquaculture effluents can be effectively treated through synergistic interaction of algae and bacteria (Lee et al., 2015; Li et al., 2023; Zhang et al., 2020), which also establishes symbiotic relationships within the water (Bhatt et al., 2024). In this symbiosis, bacteria supply nutrients such as nitrogen to algae, while algae provide fixed organic carbon to bacteria (Cho et al., 2015). Although microalgae and carbon sources are often used independently, their simultaneous application can overcome the limitations of individual use, optimizing bacterial communities and improving water purification (Shi et al., 2024).

During the experiment, DO and pH were observed to decline rapidly on day 1, followed by a sharp increase on day 3. Simultaneously, $\text{NH}_4^+\text{-N}$ content increased on day 3 before decreasing, whereas $\text{NO}_2^-\text{-N}$ nitrogen content increased on the day 5 and subsequently decline. These fluctuation may be attributed to the growth and proliferation of bacteria in the water. Following the addition of carbon sources, heterotrophic bacteria proliferate rapidly and metabolize, consuming large amounts of DO and producing acidic substances (Deng et al., 2021). This process creates a temporary hypoxic environment and causes rapid drop in pH. Under these conditions, the activity of nitrifying bacteria was inhibited, leading to the accumulation of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ (Crab et al., 2012). Subsequently, as the biofloc system stabilizes, DO levels gradually recover, allowing the nitrification process to resume. Simultaneously, as the carbon is gradually depleted, bacterial metabolites transition from acidic to neutral or alkaline, resulting in a gradual increase in pH (Espeleta et al., 2017). Additionally, the results indicate that the CS group outperformed the SBOS and CP groups in reducing nitrogen, suggesting that the combined use of SBOS and *Chlorella pyrenoidosa* may be more effective in removing nitrogen compared to their separate use. The CS group consistently maintained a relatively low content of nitrogen and NTU levels while sustaining high FV content. In contrast, the CP group was found to be less effective than the CS group in reducing nitrogen content and maintaining system stability. This may be attributed to the partial digestion absorption of SBOS by beneficial bacteria using specialized enzymes (Roberfroid et al., 2010). This process increases the abundance of beneficial and core bacteria while reducing harmful bacteria through bacterial competition, which promotes microalgae-bacteria interactions. The types of bacteria are crucial for microalgae-bacteria interactions (Crespo et al., 2023). For instance, the growth rate of microalgae (*Chlorella sorokiniana*) is significantly enhanced by *Azospirillum brasilense* and *Bacillus pumilus* through the secretion of growth hormones or the provision of essential nutrients (Amavizca et al., 2017). This demonstrates that the yield and quality of biomass can be significantly improved by specific bacteria in aquaculture systems (Kang et al., 2021). Conversely, c bacteria may inhibit the growth of specific microalgae; for example, the growth of *Nannochloropsis gaditana* is significantly inhibited by *Muricauda* sp., likely due to bacterial

density and the chemical substances they release (Han et al., 2016). Previous studies also highlighted that an optimized bacterial community can enhance algal-bacterial symbiosis (Kim et al., 2014), thereby enhancing the overall productivity of aquatic systems.

In this experiment, α -diversity index in the experimental groups was lower than CON group. This reduction in diversity and richness of the microbial community may be due to the addition of carbon sources. Microbial diversity and richness were observed to decrease in high-carbon environments, and heterotrophic bacteria were found to rapidly adapt to the high-carbon conditions and proliferate quickly (Chen et al., 2023). The abundance of *Terrimonas*, a bacterium with a unique nitrogen fixation system belonging to the *Rhizobiales*, was found to be significantly increased by the addition of SBOS (Huang et al., 2022). Meanwhile, the abundance of the harmful bacterium *Chryseobacterium* was significantly decreased by SBOS (Zamora et al., 2012). This is consistent with previous findings, where the number of harmful bacteria such as *Vibrio* in the intestines of crucian carp was effectively reduced by a small amount of SBOS as a carbon source (Qiu et al., 2023), and the abundance of harmful bacteria like *Aeromonadaceae* in the water was also reduced by a small amount of fructooligosaccharides as a carbon source (Zhou et al., 2024b). Similarly, the abundance of beneficial bacteria such as *Bacillus* in the intestines of Nile Tilapia (*Oreochromis niloticus*) was significantly increased by using mannanoligosaccharides as a carbon source in BFT, while the abundance of harmful bacteria such as *Vibrio* and *Aeromonas* was reduced (Kishawy et al., 2020). This aligns with the previous hypothesis that the addition of a small amount of SBOS as a carbon source can effectively increase the abundance of beneficial bacteria in the water while reducing the abundance of harmful bacteria. A previous study also demonstrated that the addition of *Chlorella pyrenoidosa* significantly increased the abundance of *Saprospiraceae* and *Pseudomonas*. *Saprospiraceae*, a core genus in activated sludge, is known for its ability to metabolize glucose, galactose, and specific proteins (Wu et al., 2019). Free-living *Pseudomonas* has also been shown to effectively remove nitrogen from the water (Yi et al., 2023). *Chlorella pyrenoidosa* may supply these microorganisms with glucose, galactose, or specific amino acids and proteins (Ummat et al., 2024), thereby promoting their growth. The abundance of bacteria that can effectively degrade nitrogen in the water, such as *Hydrogenophaga* (Fan et al., 2023), *Mycobacterium* (Dong et al., 2022), and *Saprospiraceae* (McIlroy and Nielsen, 2014), was significantly increased in the CP group compared to the SBOS group. Nitrogen accumulation was more effectively reduced under low C/N conditions by a BFT system with algal-bacterial symbiosis compared to a system dominated solely by bacteria (Markou et al., 2023). Similarly, the CS group exhibited the same increase in *Pseudomonas* and *Hydrogenophaga* as seen in the CP group compared to the SBOS group, while a significant increase in the abundance of *Roseomonas* and *Sphingomonas* was observed in the CS group compared to the CP group. Research has shown that an inverse relationship exists between *Roseomonas* and nitrite content, indicating that nitrite reduction is positively influenced by *Roseomonas* (Mang et al., 2024). *Sphingomonas* has also been found to effectively reduce the accumulation of $\text{NH}_4^+\text{-N}$ and nitrite in aquaculture (Yun et al., 2019). The significant increase in *Solimonadaceae* may be related to the presence of HDPE materials (Nguyen et al., 2021), and the combined effects of SBOS and *Chlorella pyrenoidosa* on the microbial community may drive this change, although the underlying mechanisms remain unclear.

LEfSe analysis revealed that differentially enriched bacteria were present in each experimental group. The observed changes in bacterial communities may have been influenced by these differentially enriched bacteria. *Flavobacterium* was found to be significantly enriched in the SBOS group, *Hydrogenophaga* was significantly enriched in the CP group, and *Bacillus* was significantly enriched in the CS group. This result is consistent with the environmental factor analysis, where nitrogen transformation in the water was significantly influenced by the bacteria affected by each experimental group. Additionally, the overall functionality of the microbial community was considerably impacted by the significantly enriched bacteria helping maintain its balance and stability (Guo et al., 2022). The microbial function prediction results for each experimental group showed that nitrite-related metabolism was significantly increased in the CP group, which can reduce toxic nitrite levels (Wang et al., 2020). The ability for nitrogen fixation was significantly increased in the CS group compared to the CP group, possibly due to the significant enrichment and high abundance of *Mycobacterium* in the CS group. *Mycobacterium* has been found to have a positive impact on nitrogen fixation (Pajares and Bohannan, 2016). Compared to the SBOS group, nitrite-related metabolism was significantly increased in the CS group, likely due to the symbiosis between *Chlorella pyrenoidosa* and many denitrifying bacteria, greatly enhancing this function.

The correlation between environmental factors and bacterial communities shows that *Flavobacterium*, as a core genus, was negatively correlated with $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, and positively correlated with $\text{NO}_2^-\text{-N}$. This suggests that $\text{NH}_4^+\text{-N}$ is effectively converted by *Flavobacterium*, and the positive correlation between *Flavobacterium* and FV indicates that harmful nitrogen in the water is efficiently converted into biofloc. These findings align with previous research, where $\text{NO}_3^-\text{-N}$ is effectively converted to $\text{NO}_2^-\text{-N}$ by *Flavobacterium*, a denitrifying bacterium (Bernardet and Bowman, 2006). A significant positive correlation was observed between *Bacillus* and $\text{NH}_4^+\text{-N}$ and FV, and a significant negative correlation was found between *Bacillus* and $\text{NO}_3^-\text{-N}$. This may be due to sufficient organic matter for *Bacillus* growth being provided by $\text{NH}_4^+\text{-N}$, and biofloc formation being promoted by the physiological activities of *Bacillus*, effectively reducing $\text{NO}_3^-\text{-N}$ content (He et al., 2023). *Hgcl_clade*, a bacterium used to gauge water quality (Tong et al., 2023), shows no significant differences with the three forms of nitrogen but is significantly negatively correlated with FV. This may be due to substances such as nitrogen being removed from the water by biofloc, inhibiting the growth of *hgcl_clade*. The changes in microbial community structure caused by SBOS and *Chlorella pyrenoidosa* indicate that microbial activity and water quality are closely linked.

5. Conclusion

The research demonstrates that both SBOS and *Chlorella pyrenoidosa*, whether used individually or in combination, effectively promote the growth of heterotrophic bacteria, facilitating nitrogen absorption and biofloc formation. The addition of SBOS significantly increases the abundance of *Flavobacterium*, while the addition of *Chlorella pyrenoidosa* significantly increases the abundance of *Hydrogenophaga*. When used together, they further boosted the abundance of *Bacillus* and *Mycobacterium*, promoting the expression of nitrogen cycle-related functional genes. This highlights a synergistic effect between SBOS and *Chlorella pyrenoidosa* in promoting the growth of beneficial bacteria, enhancing nitrogen absorption, and contributing positively to the reduction of nitrogen accumulation and the stability of the aquatic ecosystem in aquaculture environments.

Declarations

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Competing interests

The authors declare no competing interests

Author Contributions

Hangxian Zhou: Conceptualization, Methodology, Formal analysis, Resources, Data curation, Writing – original draft. **Mengsha Lou:** Conceptualization, Methodology, Writing – original draft. **Clement de Cruz:** Writing – review & editing. **Jie Wei:** Conceptualization, Methodology, Writing – original draft. **Mingwei Tao:** Conceptualization, Methodology, Writing – original draft. **Jianhua Zhao:** Formal analysis. **Rongfei Zhang:** Formal analysis. **Qiyu Xu:** Conceptualization, Writing – review & editing, Supervision.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Figures

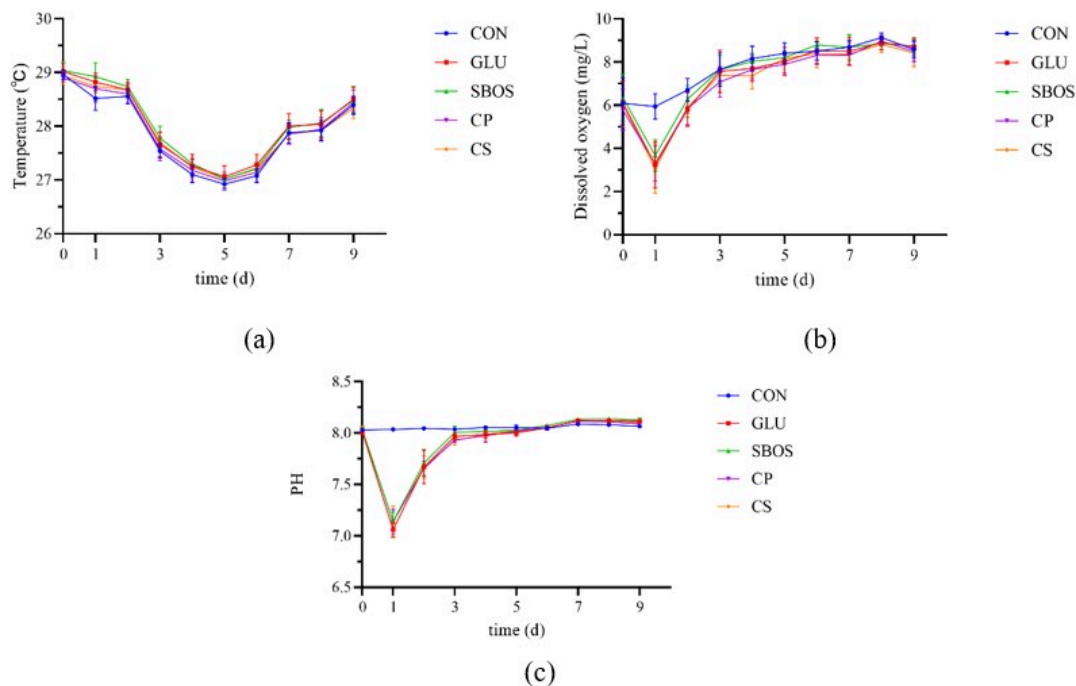


Figure 1

The variations in Temperature (a), Dissolved oxygen (b), and pH(c)

Note: CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added.

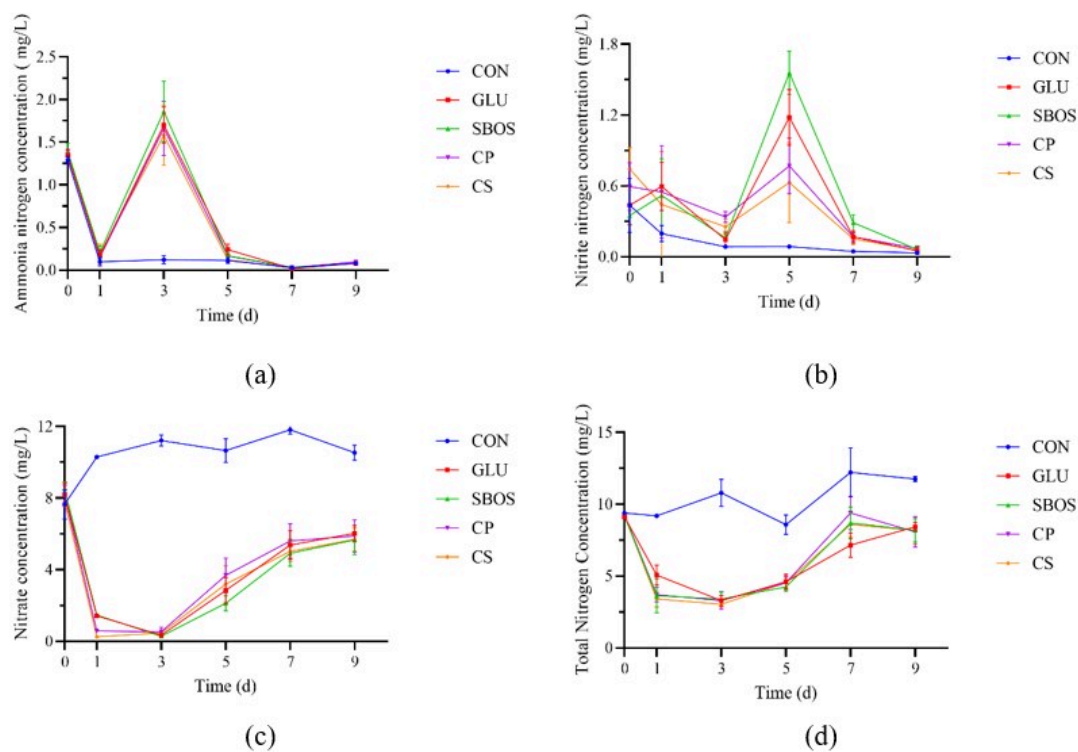


Figure 2

The variations in Ammonia Nitrogen (a), Nitrite Nitrogen (b), Nitrate Nitrogen (c), and Total Nitrogen (d)

Note: CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added.

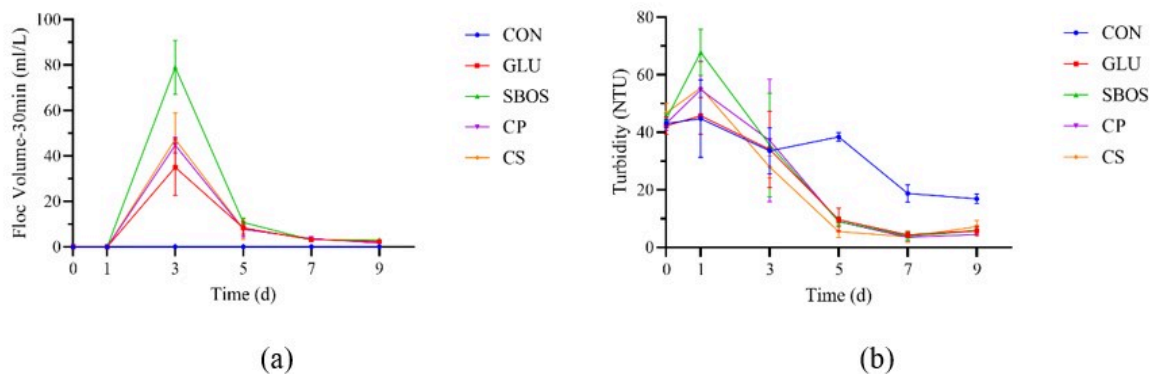


Figure 3

The variations in Floc Volume (a) and Turbidity (b)

Note: CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added.

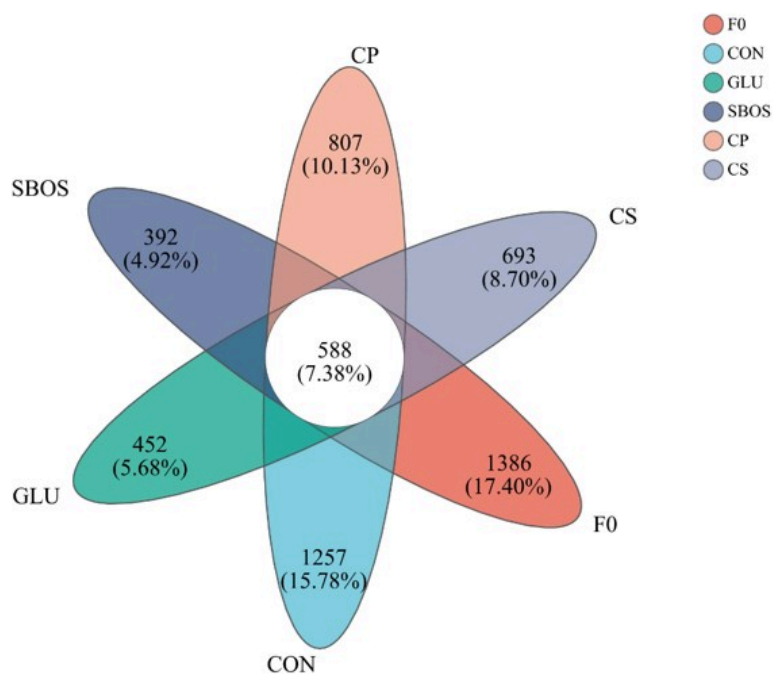


Figure 4

Venn diagram of microbial OTU in water

Note: F0: original water sample collected before the experiment started; CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added.

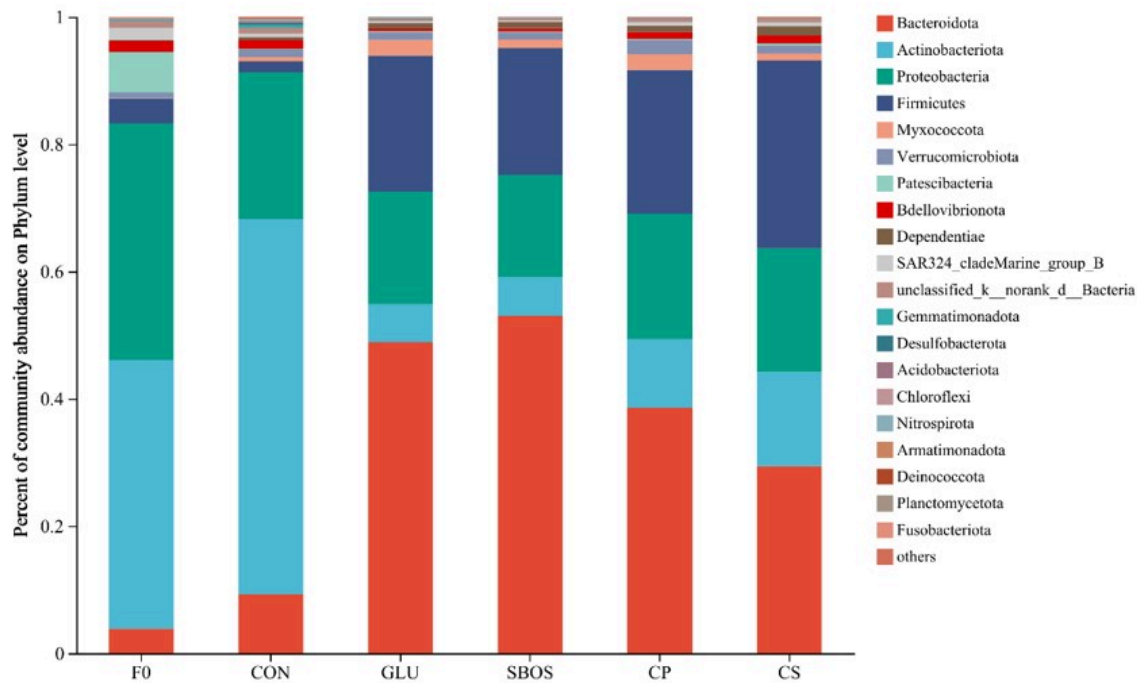


Figure 5

Community structure histogram at phylum level

Note: F0: original water sample collected before the experiment started; CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added.

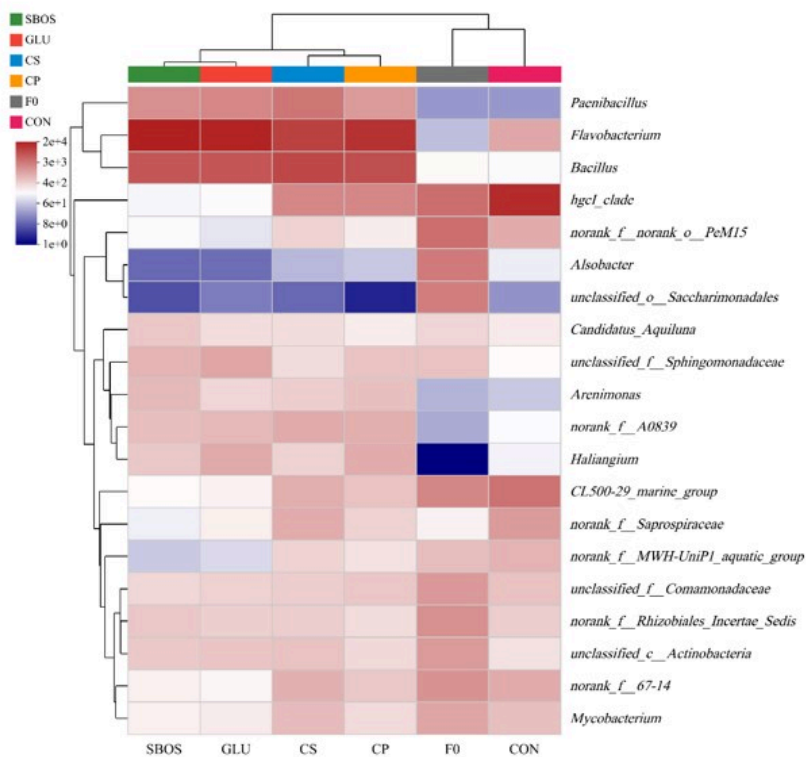


Figure 6

Heatmap of community structure at genus level

Note: F0: original water sample collected before the experiment started; CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added.

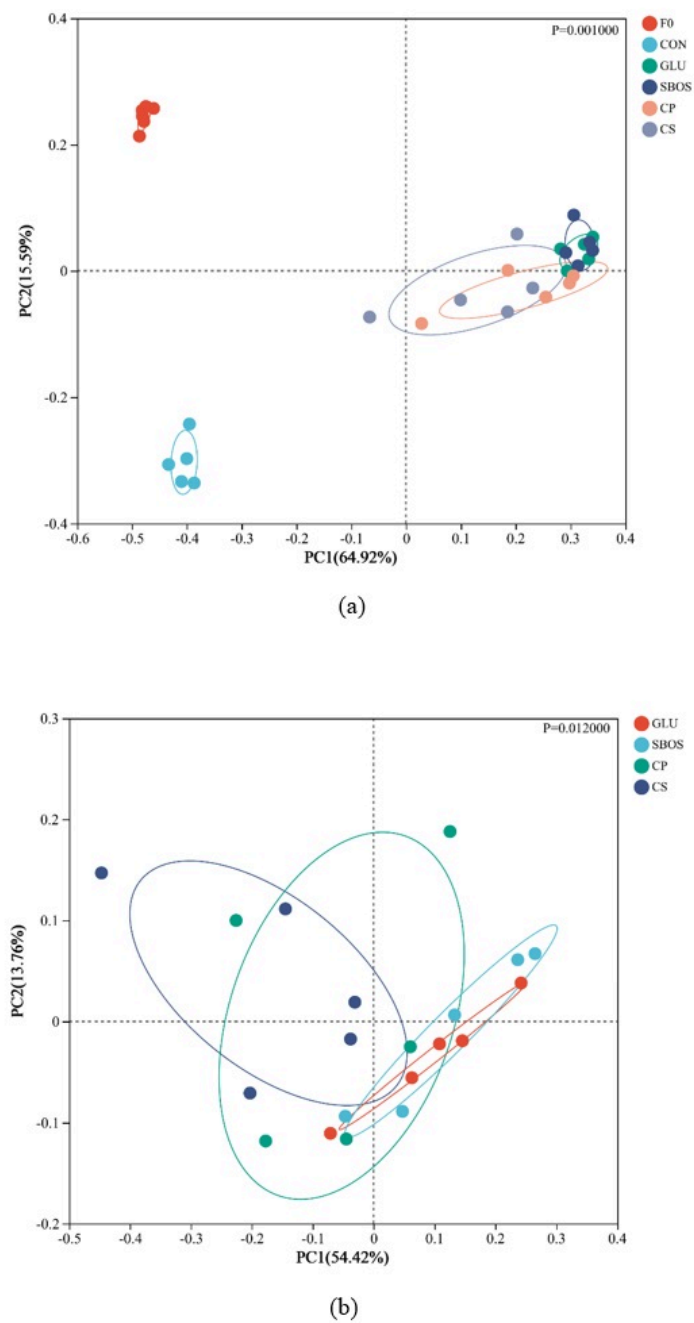


Figure 7

PCoA analysis of bacterial community (a): PCoA analysis of all treatment groups and initial samples; (b): PCoA analysis of all experimental groups

Note: F0: original water sample collected before the experiment started; CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added.

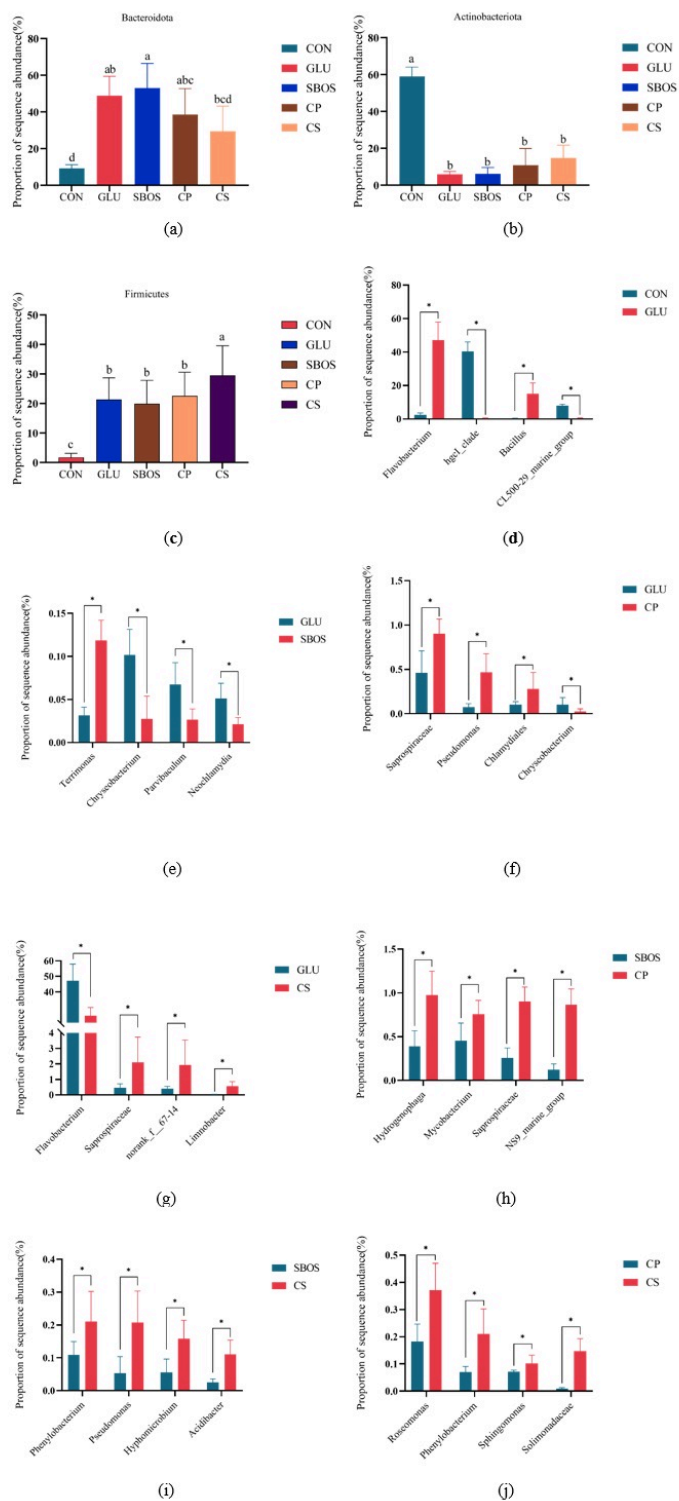
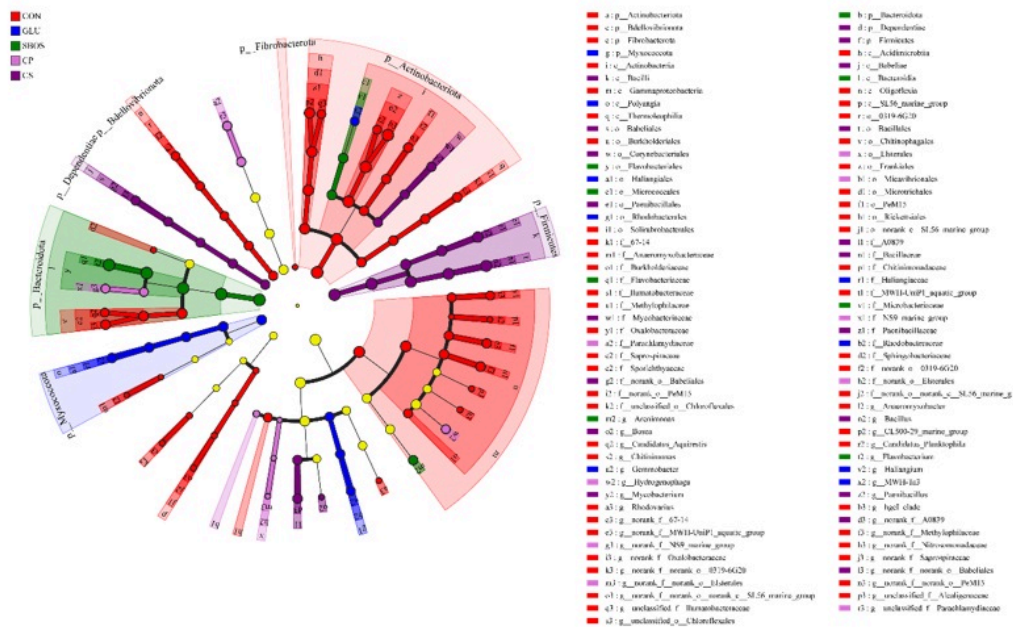


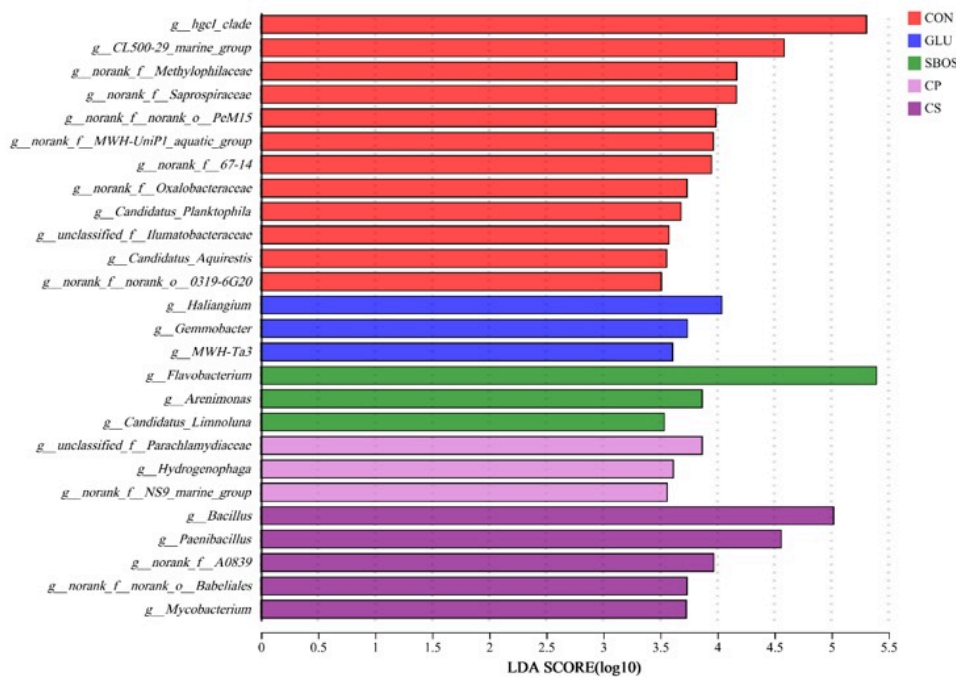
Figure 8

Differences of bacterial community at gene level

Note: CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added. (a), (b), (c): The most abundant bacterial phylum with significant differences at the phylum level ($P < 0.05$). (d), (e), (f), (g), (h), (i), (j): The most abundant bacterial genera with significant differences at the genus level in pairwise comparison ($P < 0.05$).



(a)



(b)

Figure 9

Bacterial community taxa with significant intergroup differences were identified through LEfSe analysis. (a): LEfSe analysis the differential bacteria between phylum to genus level. (b): histogram of LDA values at the genus level (LDA > 3.5)

Note: CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added. (a): The most abundant bacterial phylum with significant differences at the phylum level ($P < 0.05$). (d), (e), (f), (g), (h), (i), (j): The most abundant bacterial genera with significant differences at the genus level in pairwise comparison ($P < 0.05$).

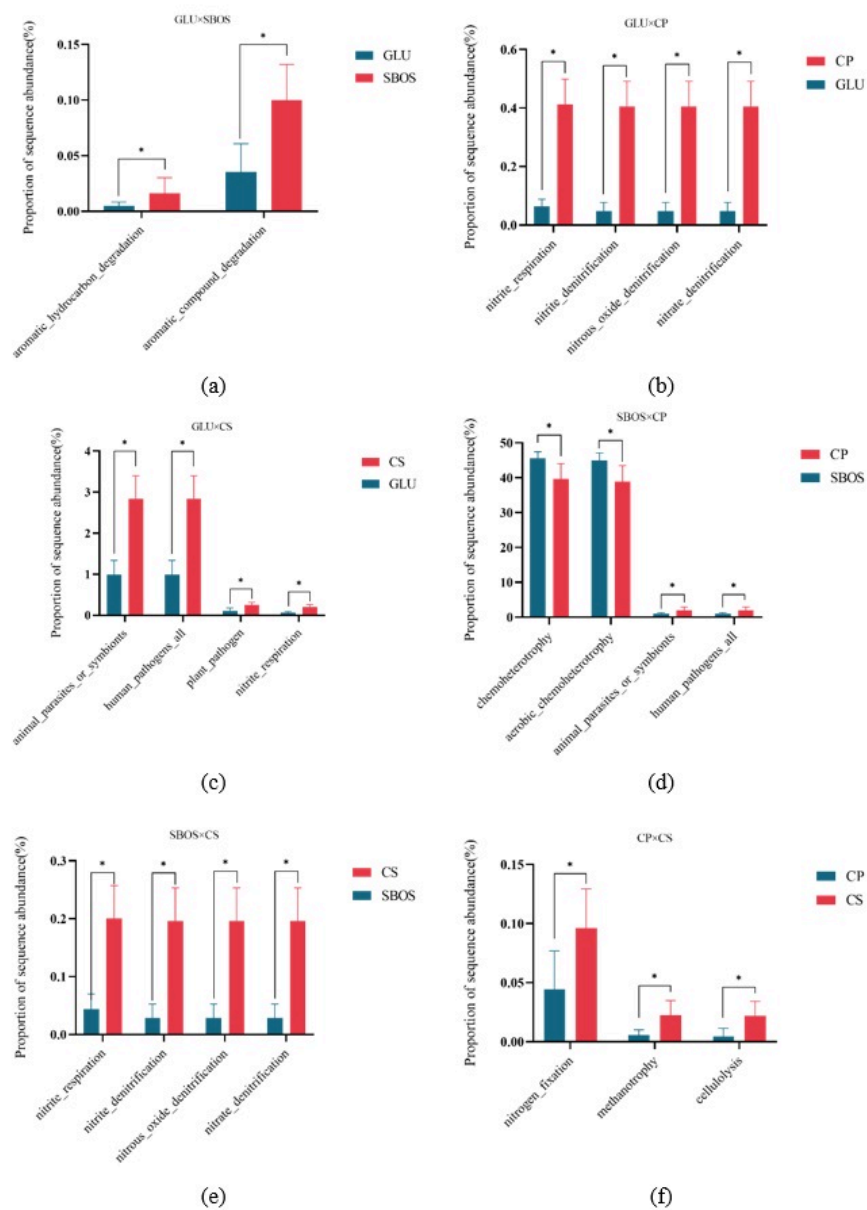
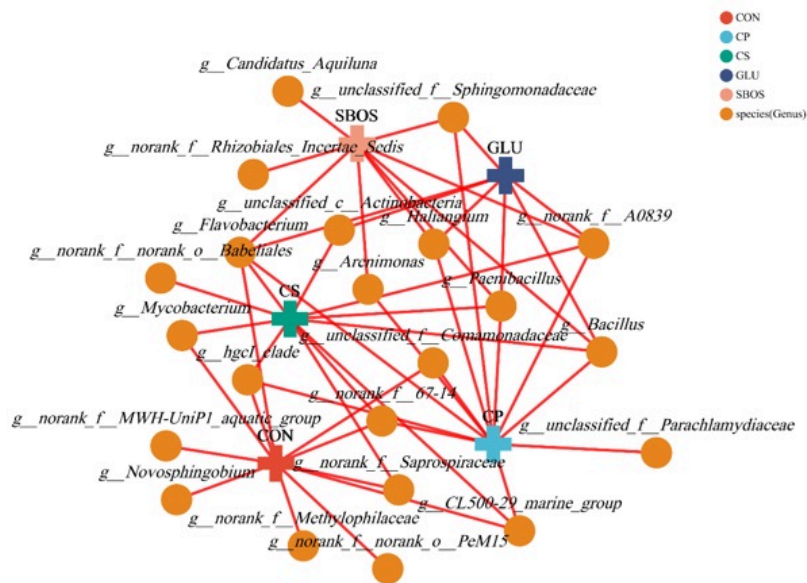


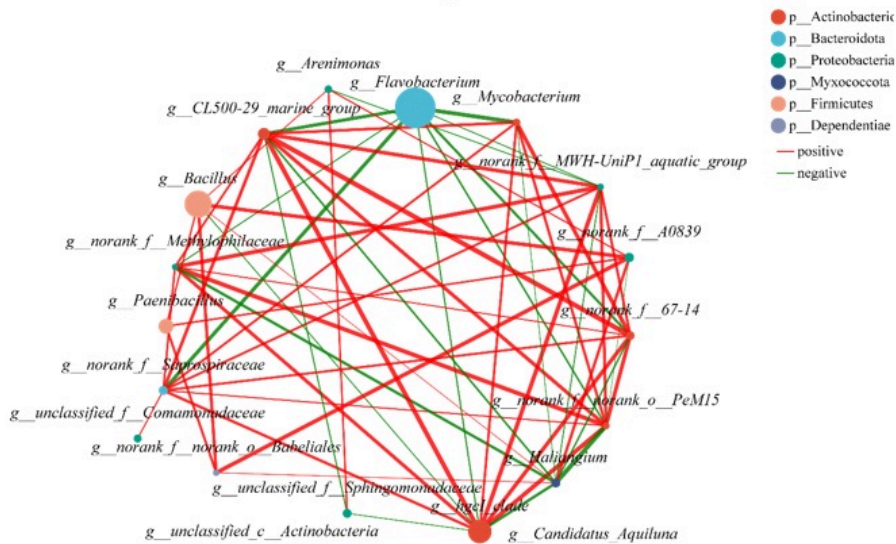
Figure 10

The most abundant functional differences in bacterial communities in pairwise comparison

Note: CON: control group without additives; GLU: glucose added; SBOS: 97.5% glucose and 2.5% soybean oligosaccharide added; CP: glucose and *Chlorella pyrenoidosa* added; CS: 97.5% glucose, 2.5% soybean oligosaccharide, and *Chlorella pyrenoidosa* added.



(a)



(b)

Figure 11

Bacterial Community Correlation Network. (a) The collinear network between bacteria in different groups (b): Interactions between bacteria and bacteria in all experimental groups and the red line represents a positive impact, the green line represents a negative impact

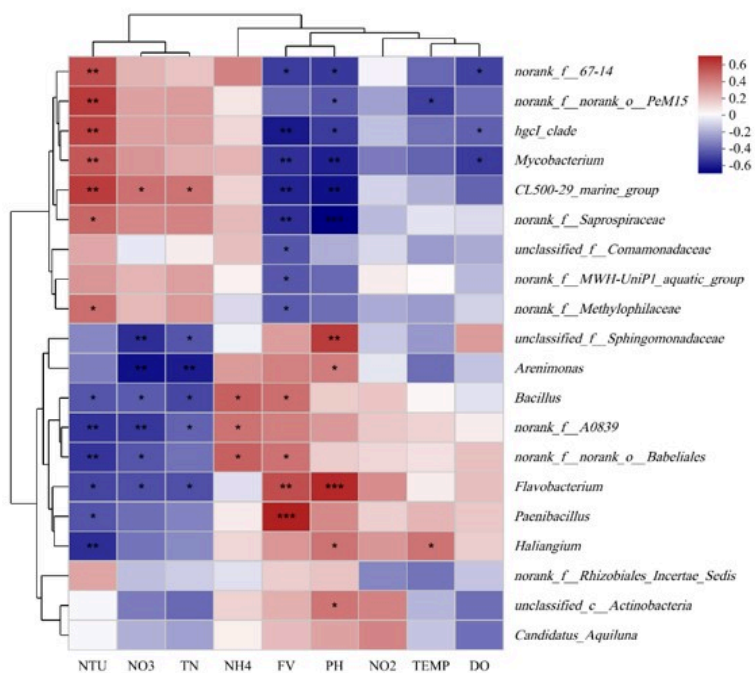


Figure 12

Heatmap of correlation between environmental factors and bacterial community

Supplementary Files

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