Advancing Aquaculture Sustainability : A Comprehensive Review of Biofloc Technology Trends, Innovative Research Approaches, and Future Prospects

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https://doi.org/10.1111/raq.12970

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Reviews in Aquaculture

REVIEWS IN Aquaculture

REVIEW

Advancing Aquaculture Sustainability: A Comprehensive Review of Biofloc Technology Trends, Innovative Research Approaches, and Future Prospects

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Received: 1 April 2024 | **Revised:** 15 July 2024 | **Accepted:** 19 August 2024

Keywords: bibliometric | biofloc technology system | growth performance | microbiome | network analysis | probiotic

ABSTRACT

Biofloc technology (BFT), initially adapted for shrimp farming in the 1970s, represents a sophisticated ecosystem of microorganisms designed to enhance aquaculture productivity and sustainability. Despite its established history, research into BFT is surprisingly still at an early stage globally. This review conducted a bibliometric analysis of 612 articles from major aquaculture journals spanning 2008–2023 to systematically explore the development, trends, and focal points of BFT research. The analysis revealed that the bulk of significant contributions originates from Brazil and China, and highlighting areas of interest can be categorized into four hotspots, such as (1) efficient nitrogen transformation, (2) biofloc microbiology, (3) biofloc's immunostimulant properties, and (4) the evaluation of research methodologies. At the end, the microecology concept was introduced, and the cross-discipline methods were promoted in the aquaculture field. Notably, much of the BFT research is still at an exploratory phase, with numerous functional bacteria unidentified and optimization strategies for BFT underdeveloped. These gaps present opportunities for enhancing aquaculture through improvements in wastewater management, product quality, safety, and yield. Furthermore, the review notes a growing trend in applying microbiome research and microecological analysis in aquaculture, with high-throughput sequencing data increasingly used to understand microbial interactions and nitrogen transformation within bioflocs. This direction promises to unlock further insights into the complex microbial ecosystems of bioflocs and their applications in sustainable aquaculture.

1 | Introduction

Recirculating aquaculture systems (RAS) and biofloc technology (BFT) are lauded for their sustainability and minimal water exchange, making them environmentally friendly models of aquaculture [1–5]. RAS use varied biofilters to biologically process nitrogen (N) and phosphorus (P) in wastewater, allowing for its reuse. Here, the primary water-treating microbes attach to biofilter fillers [1, 6]. Conversely, BFT systems, devoid of fillers, maintain their microbes in suspension within the water, relying on added organic carbon to stimulate floc growth for pollutant treatment [1, 6, 7].

The sustainable potential of BFT has been acknowledged since the 1970s [8]. Substantial research to refine and apply BFT commenced in the early 1990s and continues to demonstrate

https://doi.org/10.1111/raq.12970

promising advancements [6]. This evolution reflects a growing commitment to ecological aquaculture practices, where efficiency dovetails with environmental stewardship. The innovations in BFT especially highlight a trend towards systems that support aquaculture productivity while concurrently enhancing water quality and ecosystem health [9]. These endeavors underscore the potential for BFT to significantly impact sustainable food production, aligning with global efforts to balance human needs with environmental conservation.

Research categorizes the water treatment capabilities of BFT aquaculture systems into three main processes: heterotrophic assimilation, autotrophic nitrification, and heterotrophic denitrification for nitrogen and phosphorus removal [3, 4, 6, 10–15]. These biochemical reactions are facilitated by microorganisms (MOs), which play a pivotal role in such systems [16–18]. The employment of MOs in aquaculture has broadened over the past decade, serving as environmental biomarkers, effluent bioremediations, probiotics, and direct food sources for the cultured aquatic species [18, 19]. Despite this increase in the utilization of MOs, the specific bacterial species and their roles within culture systems are largely unidentified. It is estimated that up to 99% of bacterial species in various environments are unculturable, rendering their diversity elusive to even fundamental scientific investigation [20].

In addition, The BFT microecosystem comprises diverse nitrogen transformation pathways and enigmatic microbial groups with undetermined roles [21]. Variations in biofloc volume directly impact denitrification stability, where a decline in microbial diversity dampens the system's resilience to shocks. Wagg et al. [22] demonstrated that different soil sieves lead to a loss of microbial diversity and function, revealing the influence of particle size and volume on the interaction network of key nitrogen and phosphorus-transforming bacteria and fungi. Similarly, floc density and size within BFT systems correlate with denitrification efficiency [23, 24]. Biochemical and function-taxon network analyses indicate that the *Bacillus* genus primarily governs nitrogen assimilation, whereas the Proteobacteria phylum leads nitrification and denitrification activities [25]. This insight has prompted the creation of a bipartite topological network linking microbial taxonomy and function within aerobic denitrification systems, aiming to map these relationships. In addition, metagenomic, metatranscriptomic studies, and microbial co-occurrence network analysis have unearthed pivotal archaea in the marine sediment carbon cycle, designated as Thermoprofundales (MBG-D archaea) [26]. Employing ecological network analysis to microsystem studies is projected to reveal undisclosed microbial interactions, classifications, and metabolic pathways.

Wei, Liao, and Wang [27] investigated varying microbial compositions stemming from different carbohydrate applications. Concurrently, Liu et al. [28] reported that a spectrum of bacteria, including denitrifying bacteria (DNB), denitrifying phosphorus accumulating organisms (DNPAOs), phosphorus accumulating organisms (PAOs), glycogen accumulating organisms (GAOs), ammonia-oxidizing bacteria (AOB), and nitrite-oxidizing bacteria (NOB), coexist within BFT systems. These findings enrich our understanding of microbial dynamics and highlight potential targets for optimizing BFT efficacy.

The concept of “small-world” networks, introduced by Watts and Strogatz [29], highlights the disproportionate interconnections within complex systems. Such networks are crucial for elucidating the functional mechanisms of microecosystems. While previous research has not considered BFTs in their totality as ecosystems, our study draws inspiration from comprehensive ecological research, including investigations into seawater sediment [26], forest soil ecosystems [29], and activated sludge [30], all of which have utilized microbiome network analysis. Additionally, our prior research has shed light on the bacterial co-occurrence disparities between freshwater and seawater denitrification systems through network analysis [31]. Network analysis is instrumental in examining the intricate interactions between bacterial communities or gene functions and the resultant ecosystem services. Hence, it is a key approach in exploring bacterial “dark matter” [25, 32] and in characterizing the veiled microbial ecology [33].

Firstly, this review employs bibliometric analysis to distill key findings from BFT-related research conducted between 2008 and 2023, aiming to illuminate the technology's evolution, pivotal trends, and the pressing need for a transition towards more scientifically informed optimization strategies. Secondly, as the understanding of biofloc microbiomes deepens, future research directions are poised to unlock the intricate microbial interactions fundamental to BFT's efficacy, heralding a new era of precision and sustainability in aquaculture practices.

2 | Methods

2.1 | Data Collection

The Web of Science (WoS; https://www.webofscience.com/wos) was consulted for the present bibliometric analysis of literature pertaining to BFT in aquaculture. WoS is an internationally recognized database containing stable, comprehensive data [34]. BFT-related articles were searched in the WoS core. Pertinent papers published between 2008 and 2023 were search using the formula “TS=((“biofloc” OR biofloc) AND (“water quality*” OR nitrogen OR carbon OR carbohydrate OR C:N OR C/N OR autotrophic OR heterotrophic OR aquaculture OR ammonia)).” Major journals in the aquaculture field were selected including “*Aquaculture*, *Reviews in Aquaculture*, *Aquaculture Engineering*, *Aquaculture International*, *Aquaculture Nutrition*, *Aquaculture Reports*, *Aquaculture Research*, *Fish & Shellfish Immunology*, *Journal of the World Aquaculture Society* and *North American Journal of Aquaculture*.” Certain irrelevant research articles were deleted. BFT-related articles were also found in environmental journals such as *Bioresource Technology* and *Science of the Total Environment* and so on. A total of 612 publications from 2008 to 2023 met the selection criteria. Article content, keywords, publication year, and other parameters were comprehensively examined.

2.2 | Bibliometric Analysis

To conduct the bibliometric analysis, we utilized VOSviewer in combination with the R package bibliometrix. These tools are commonly used in social network analyses [35, 36]. Network visualization allowed us to identify key topics and issues related to BFT, as well as the authors and research institutions most active in this field.

For this review, data from the Web of Knowledge (as mentioned in Section 2.1) was exported in TXT format. This document was then imported into VOSviewer to visualize author keywords and co-authorship patterns. Additionally, the data was imported into Biblioshiny to visualize information on different countries and organizations involved in BFT research.

3 | Evolution and Impact of Biofloc Technology Research

From 2008 to 2023, research was actively conducted on BFT and numerous reports were published on the subject (Figure 1). Prior to 2013, there were fewer than 10 BFT-related papers per year. The three leading journals publishing BFT-associated articles were *Aquaculture*, *Aquaculture Research*, and *Aquaculture International* and they accounted for 35% (214), 22% (130), and 14% (83) of all BFT articles, respectively (Figure 2).

3.1 | Historical Overview and Publication Trends

Highly cited articles are listed in Table 1 and all of them addressed inorganic nitrogen dynamics and Pacific white shrimp or tilapia health in aquatic ecosystems. El-Sayed [43] also found that over 550 documents published on the application of BFT in aquaculture, most of them appeared during 2010–2020. Over 40% of these publications were dedicated to shrimp aquaculture, with white shrimp (*Litopenaeus vannamei*) receiving most of the attention. More than 200 papers were published on raising this species in biofloc systems. Research and development on rearing shrimps in biofloc systems are also still escalating at an outstanding rate.

3.2 | Influential Organizations, Research Groups, and Researchers

The first three organizations are shown in Figure 3, they are in Brazil and China. Collaborations among authors are shown in Figure 4, and the countries are shown in Figure 5. Authors that published over five articles are shown and isolated nodes are ignored. The top three most prolific authors are Wasielesky, W (yellow frame) of the Universidade Federal do Rio Grande, Brazil (48 articles) followed by Guozhi Luo (red frame) of Shanghai Ocean University, Shanghai, China (25 articles) and Seiffert WQ of the Universidade Federal de Santa Catarina Brazil (22 articles). A significant portion of the relevant papers have been published in Brazil, where *L. vannamei* is widely cultivated. This organism was the principal research object in many BFT-related reports. The Songming Zhu group (blue frame) includes the corresponding author of the present review and contributed to certain BFT-related studies on *L. vannamei*.

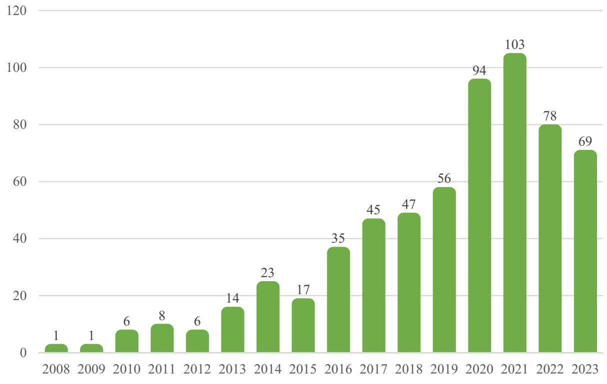


FIGURE 1 | BFT-related articles published between 2008 and 2023.

3.3 | Current Issues and Trends in BFT Research

Co-occurrence network analyses predict major topics of public interest as well as research development in those fields. We curated 612 articles that included English author key words (Figure 6). We conducted statistical analyses on the author key words and most frequently encountered "biofloc" (159 instances) followed by "Pacific white shrimp" (154 instances) and "water quality" (152 instances). Other frequently appearing key words related to biofloc included "immune response," "different carbon sources" (42 instances), and "zero exchange" (29 instances). Carbon source and carbon: nitrogen ratio (C/N) are important regulatory factors that also appeared as key words.

The author keywords were classified into four clusters represented by different colors. The first cluster (red nodes) included aquaculture systems, reared species, biofloc, carbohydrate addition, zero-water exchange, and Pacific white shrimp. These terms pertain to the exploration of cultured species and the feasibility of aquaculture production. The second cluster (green nodes) indicated a strong co-occurrence between antioxidants and digestive enzymes, highlighting that these issues are of high concern in BFT research, focusing on the effects of the biofloc system on cultured animals. The third cluster (blue nodes) included "activated sludge," "bacteria," "microbial community," and other related terms, demonstrating that the microbial community is also a significant topic in BFT research, particularly regarding nitrogen transformation. However, microbiome studies have only been applied to BFT research since 2016 (according to the literature). The fourth cluster (yellow nodes) mainly focused on aquatic nitrogen dynamics in BFT, indicating that researchers are concerned with nitrogen cycling in the water.

These clusters reveal the multifaceted nature of BFT research, addressing various aspects from practical aquaculture operations to the intricate microbial interactions within the system. By identifying these key topics, the study underscores the importance of integrating different scientific disciplines to enhance the understanding and optimization of BFT.

4 | Key Findings From the Bibliometric Analysis

4.1 | Nitrogen Dynamics

In biofloc, bacterial nitrifiers and denitrifiers transform inorganic nitrogen into nitrogen gas. Nitrification and denitrification require different substrates and reaction conditions.

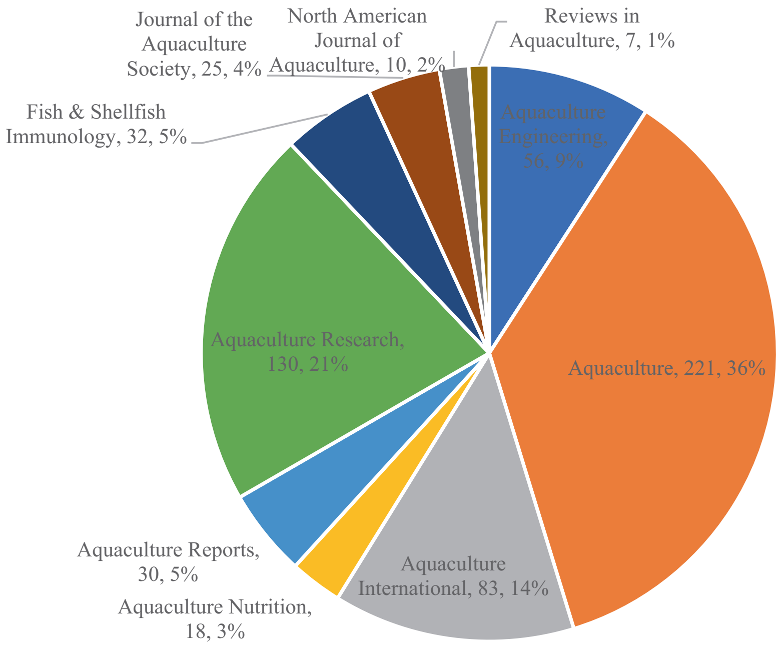


FIGURE 2 | Journals publishing BFT-related articles between 2008 and 2023.

Nitrification converts ammonia into nitrate. The latter is relatively less toxic to aquaculture animals than the former. The two basic reactions in nitrification are described below.

Nitrosomonas converts ammonia nitrogen to nitrite nitrogen (NO_2-N) and then *Nitrobacter* transforms NO_2-N into nitrate nitrogen (NO_3-N). Nitrite bacteria include *Nitromonas*, *Nitrospirillum*, and so on. Nitrate bacteria include *Bacillus*, *Spirillum*, and *Coccus*. Nitrite and nitrate bacteria are chemoautotrophic. They use CO_2, CO_3^{2-}, and HCO_3^- as carbon sources and obtain energy through redox reactions with NH_3, NH_4^+, or NO_2^-. The entire nitrification reaction must proceed under aerobic conditions and oxygen is the terminal electron acceptor. The reactions are as follows:

$$NH_4^+ + 3/2O_2 \rightarrow NO_2^- + H_2O + 2H^+ \tag{1}$$

$$NO_2^- + 1/2O_2 \rightarrow NO_3^- \tag{2}$$

Denitrification reduces NO_3-N or NO_2-N to N_2. DNB are chemo energetic heterotrophs and anoxic MOs. Denitrification must proceed under anoxic conditions. DNB require an organic carbon source as an electron donor and use the oxygen in NO_3-N for anoxic respiration. The reaction is as follows:

$$5C(\text{carbohydrate}) + 2H_2O + 4NO_3^- \rightarrow 2N_2 + 4OH^- + 5CO_2 \tag{3}$$

Overall, NO_3-N reduction to N_2 occurs through four consecutive steps:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \tag{4}$$

Biological denitrification is a seemingly contradictory process. Nitrification requires nitrifying bacteria, aged sludge, and aerobic conditions. By contrast, denitrification requires DNB, relatively new sludge, and anoxic conditions [44]. Moreover, they require and utilize different organic carbon sources. In environments abundant in carbon sources, heterotrophic bacteria compete for both oxygen and nutrients and create conditions not conducive to nitrification. DNB require organic carbon sources as electron donors to complete denitrification. Therefore, the divergent physiological and biochemical mechanisms of bacterial nitrifiers and denitrifiers create various combinations of biological denitrification systems. BFT is a complex autotrophic and heterotrophic sludge system wherein various biochemical reactions occur within the same area. It is difficult to stabilize all of these reactions in a single zone. If nitrification occurs in situ, an additional carbon source will be required as the denitrification electron donor.

The BFT system resolves the aforementioned paradox through simultaneous nitrification and denitrification (SND). Many studies have empirically validated that SND does, in fact, occur in the BFT system [5, 28, 45]. Avnimelech and Kochba [46] studied nitrogen-15 (^{15}N) absorption and excretion by tilapia in a BFT system. The biofloc suspension, containing approximately 200 mg/L suspended solids, was enriched with ^{15}N via labeled ammonium salt and starch. This process immobilized ammonium within the bioflocs. For 14 days, tilapia exclusively consumed bioflocs in their tanks. They monitored the nitrogen species in the water and the ^{15}N enrichment in both the suspended matter and the fish muscle. The study delineated the underlying biological processes, including organic nitrogen absorption by the fish, nitrogen excretion, microbial biofloc decomposition, and biofloc regeneration from nitrogen released by the fish. Daily nitrogen retention in the fish averaged 240 mg N/kg, translating to a daily protein intake of about 1.6 g/kg, or roughly 25% of typical feed protein provision. The excretion rate was nearly double the retention, with a total nitrogen intake

TABLE 1 | The top cited paper from 2008 to 2023.

Title	Journal	TC_{2021}	Year	Country rank 1st	References
Biofloc technology in aquaculture: Beneficial effects and future challenges	Aquaculture	569	2012	Belgium	[37]
Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating C/N ratio in feed	Aquaculture	159	2012	China	[38]
Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (*Oreochromis niloticus*)	Aquaculture	130	2015	China	[39]
Effects of C/N ratio on biofloc development, water quality, and performance of *Litopenaeus vannamei* juveniles in a biofloc-based, high-density, zero-exchange, outdoor tank system	Aquaculture	108	2016	China	[40]
The effect of different carbon sources on water quality, microbial community and structure of biofloc systems	Aquaculture	55	2018	China	[41]
The impact of stocking density and dietary carbon sources on the growth, oxidative status and stress markers of Nile tilapia (*Oreochromis niloticus*) reared under biofloc conditions	Aquaculture reports	33	2020	Egypt	[42]

Note: TC_{2021} refers to the number of citations in 2021 in the Web of Science.

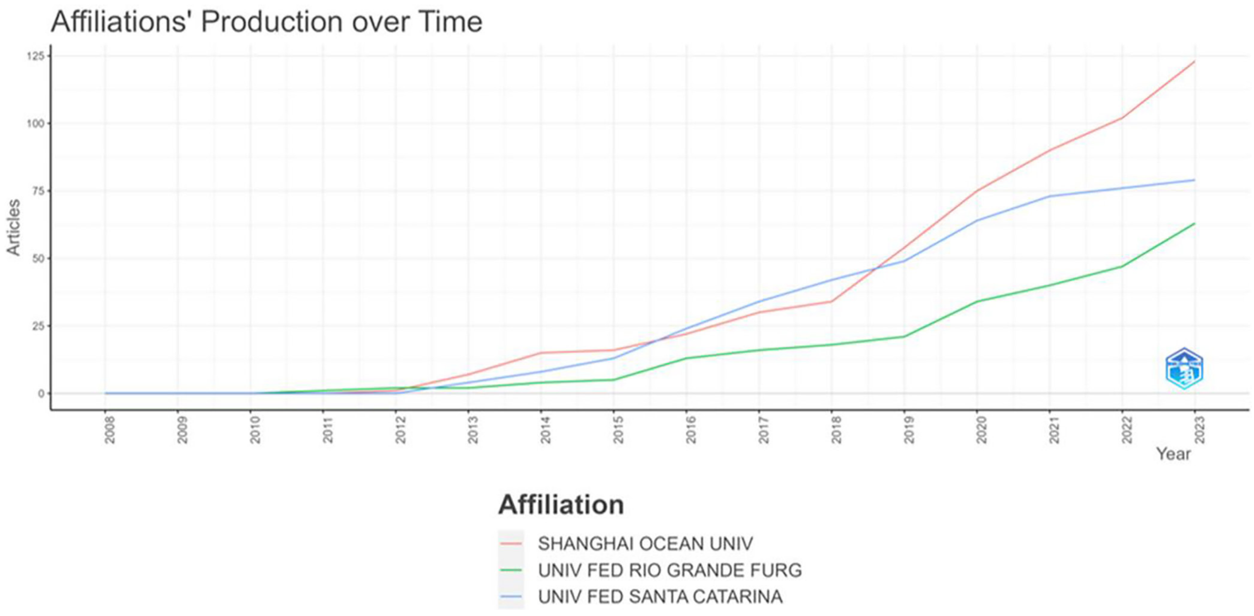


FIGURE 3 | Numbers of published BFT-related articles by organization between 2008 and 2023.

of approximately 700 mg N/kg daily. The bioflocs' estimated residence time was 8 h, indicating the system's rapid turnover. These insights have enhanced methods for assessing nitrogen flows in BFT systems.

4.2 | Microorganisms in BFT Systems

Microbial mechanisms and regulation determine the nitrogen removal efficacy of BFT [21]. Aquaculture wastewater with a low

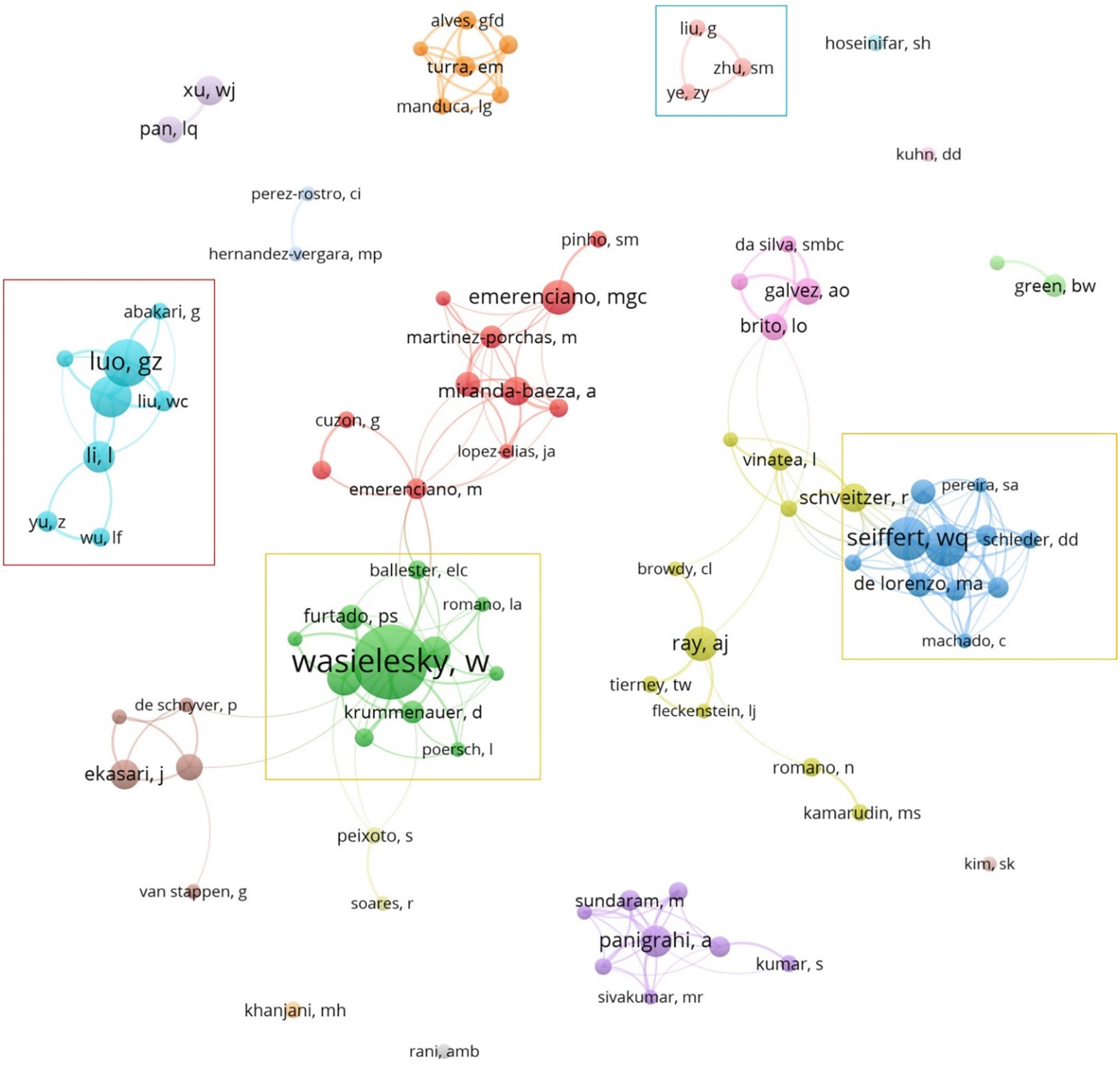


FIGURE 4 | Publication prolificacy of authors and their collaborations. The yellow frame represents authors from Brazil, the red frame indicates the second largest group of contributors from China, and the green frame highlights the group led by the author of this review.

carbon: nitrogen ratio (C/N) could provide a carbon source and conditions amenable to the proliferation of heterotrophic bacteria. In this manner, the nitrogen content in the water would be utilized and reduced and the aquaculture wastewater could comply with national standards and be safely reused or discharged. Liu et al. [47] reported that when the carbon source was inadequate, autotrophic nitrification predominated. This type of aquaculture is called a "nitrification BFT system." Ferreira et al. [48, 49] also found that in the "nitrification BFT system," water quality can be maintained more stable than in the "heterotrophic BFT system," and both systems were suitable for shrimp rearing. It can conserve the carbon source and easily reach the required dissolved oxygen (DO) level. In nitrification BFT systems, however, nitrate accumulates. Initially, BFT aquaculture systems are heterotrophic and rapidly remove inorganic nitrogen and mature the system. After the "nitrification BFT system" is established, it may be operated with reduced carbon source input or even without carbohydrate addition for several days [50].

The recent advent of sequencing technology has facilitated the study of the biofloc microbiome. This approach disclosed numerous changes and identified novel organisms in the bacterial community of the biofloc. Chen et al. [51] found that *Bacillus* predominated and account for 81%, 82%, and 75% of all bacterial genera in the <50-µm, >50-µm, and unsieved groups, respectively. Copetti et al. [52] reported that the biofloc concentration is a critical ecological strategy in the microbial control of BFT systems. The total suspended solids (TSS) 500 mg/L group had a lower microbial carrying capacity (CC) than the TSS 700 mg/L group. Fast-growing opportunistic R-strategists are favored when intercellular space and resources are abundant. Under these conditions, they strongly compete and against slow-growing K-strategists. Deng et al. [41] stated that Alphaproteobacteria abound in response to the addition of plant carbohydrates whereas Betaproteobacteria prevail without this supplementation. Deng et al. [53] indicated that nitrogen loss was greater in biofloc-based recirculating aquaculture systems

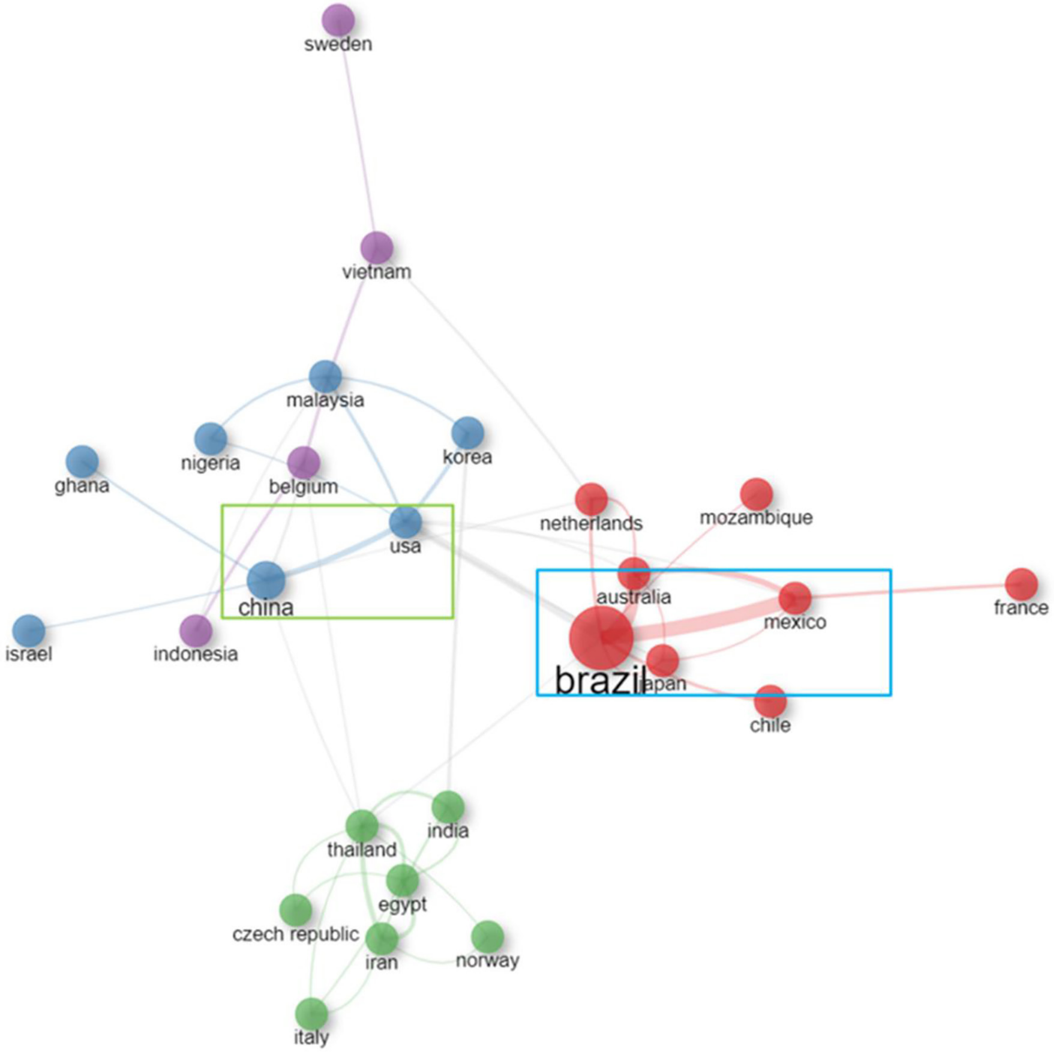


FIGURE 5 | Frequency of collaboration among countries on BFT-related research. The size of the nodes represents the number of publications, while the thickness of the edges indicates the frequency of collaboration. The two frames highlight the two largest groups of collaborating countries.

(BRAS) than flow-through systems. In the former, aerobic denitrification-related *napA*, *nirK*, and *nosZ* were upregulated.

Fatimah et al. [54] showed that vanillin acts as a quorum-sensing (QS) biofloc inhibitor. Vanillin (0.30 mg/L) significantly reduced microbial aggregation, particle size, and protein content relative to the control. Biofloc consumption by African catfish doubled in the presence of QS signal molecules and enhanced ammonia removal. Wei et al. [55]; Wei, Wang, and Liao [56] detected *QS* in larger bioflocs. Researchers have paid increasing attention to the roles of MOs in BFT. Recent articles reporting on biofloc MOs and microbiomes are listed in Table 2. Most of studies represented in the table above have predominantly focused on the microbial composition within bioflocs, with scant attention paid to their interactions. This gap in research forms the crux of the author's subsequent discussion (Section 4.7). Liu et al. [45] posits that conducting co-analyses of biofloc microbiomes and environmental factors could significantly advance our understanding of the BFT mechanism. To date, prior investigations have largely concentrated on cataloging the relative abundances of MOs present in the BFT system. Moving forward, it is imperative to explore the complex web of interactions among these microbial communities and how they are influenced by or influence their surrounding environment. Such an approach could unveil new insights into the dynamics of biofloc systems.

MOs are essential to any aquaculture system, including BFT systems. These diverse organisms perform various roles crucial to the system's efficiency. Common MOs in BFT systems include photoautotrophic organisms (e.g., microalgae), chemoautotrophic organisms (e.g., nitrifying bacteria), and heterotrophic organisms, including fungi, ciliates, protozoans, and zooplankton (e.g., rotifers, copepods, and nematodes) [65]. Biofloc organisms (BFOs) help maintain water quality by metabolizing fecal matter and unconsumed food, reducing harmful nitrogenous compounds like ammonia and nitrite [16, 17]. This process is crucial for controlling total ammonia nitrogen (TAN) levels in the environment. Studies show that greater diversity in BFOs enhances waste mineralization, protein utilization, and pathogen control, thereby improving overall system health [66]. In BFT systems, BFOs provide essential nutrients, including proteins, lipids, and vitamins, to farmed species, promoting growth, and reducing the need for these compounds in commercial feeds [16, 17]. This

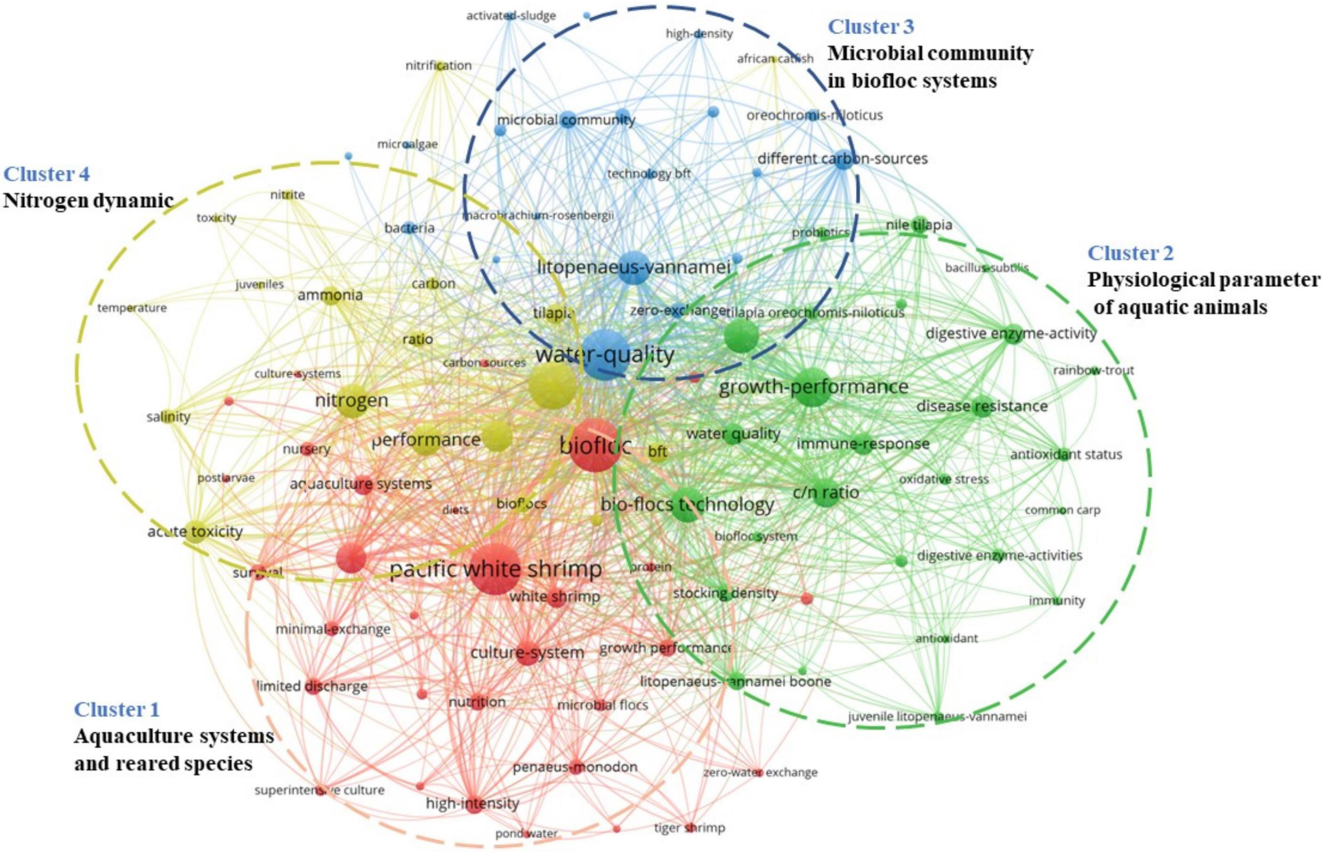


FIGURE 6 | Keywords used in 612 BFT-related papers, the network of keywords, the size of the nodes means number of occurrences, the different colors mean different categories.

capability optimizes resource utilization and reduces the environmental footprint of aquaculture operations [66]. BFOs also contribute to pathogen competition and create probiotic properties within the BFT system [67]. Heterotrophic bacteria, such as *Bacillus* spp., play a significant role by competing with pathogenic bacteria, thereby reducing disease outbreaks and enhancing the health of cultured species [6].

Several factors, including salinity, carbon source type, C/N ratio, aeration, light, stocking density, and TSS, influence the quality, density, and diversity of BFOs [68, 69]. For instance, different carbon sources promote different microbial community compositions. Simple carbon sources like starch and molasses lead to higher densities of heterotrophic bacteria, while complex sources result in a more balanced microbial community [41]. Additionally, maintaining appropriate DO levels through adequate aeration is crucial for supporting the metabolic activities of both heterotrophic and chemoautotrophic bacteria [70, 71]. Recent advancements in sequencing technology have enabled detailed studies of the biofloc microbiome, revealing numerous changes and identifying novel organisms [72]. Understanding these microbial communities' interactions and their responses to environmental changes is essential for optimizing BFT systems. Future research should focus on exploring the complex web of interactions among microbial communities and their influence on or by their environment [45]. This approach can provide new insights into the dynamics of biofloc systems, ultimately enhancing their efficiency, and sustainability.

4.3 | In Situ Immunostimulant Function of Biofloc

Biofloc systems improve disease resistance in aquaculture animals, including fishes and shrimps [73, 74]. Table 3 shows the physiological and immunological properties improved by biofloc. Antibiotic overuse has increased drug resistance and decreased drug efficacy in many bacteria. By contrast, biofloc systems supplemented with triglycerides helped artemia combat *Vibrio harveyi*. Biofloc competitively displaced the pathogenic bacterial population and markedly improved larval survival [73]. Poly-β-hydroxybutyric acid (PHB) protects various cultured organisms and biofloc contains 0.5%–18% PHB [90]. Biofloc PHB is a short-chain fatty acid (SCFA) that serves as a carbon source for intestinal flora, creates an acidic intestinal microenvironment, and inhibits pathogen proliferation [90, 91]. Adding 3% PHB to feed could alter the intestinal microflora structure and promote the growth of beneficial gut bacteria in *Penaeus vannamei* [92]. Haridas et al. [93] observed that biofloc enhances digestive enzymes like amylase, lipase, and protease, as well as serum protein, albumin, nitro blue tetrazolium (NBT), and myeloperoxidase activity in the early stages of grey mullet (*Mugil cephalus*) development. Additionally, growth rates, body crude protein, and lipid content were found to be higher.

The probiotics in biofloc inhibit the growth of pathogenic MOs by improving the intestinal microflora balance, increasing digestive enzyme activity, and enhancing host immunity

TABLE 2 | Application of high-throughput sequencing technology in biofloc technology in recent years in the journal “*Aquaculture*.”

High-throughput sequencing technology/Other microbial technology	Dominant bacteria	Results	References
16S rRNA	Alphaproteobacteria	The biofloc system can reduce ammonia levels, enhance bacterial diversity in the water, and promote the growth of ammonia-oxidizing bacteria	[41]
16S rDNA	Proteobacteria	The bacterial composition of biofloc varies significantly with different carbohydrate additions. *Candida tropicalis* HH8 and *Pseudomonas stutzeri* LZX301 can enhance nitrogen removal and accelerate biofloc formation	[57]
16S rRNA	Planctomycetes, Proteobacteria, Bacteroidetes, and Firmicutes	This study provides the first insight into the functions of bioflocs in aquaculture systems, suggesting that nitrogenous and carbonaceous compounds can be simultaneously processed through diverse pathways by the bacteria thriving in bioflocs	[58]
16S rRNA	Proteobacteria	Increased aerobic denitrification may be a significant factor contributing to the higher gaseous nitrogen loss in biofloc reactors	[53]
DGGE		Shrimp-fish polyculture in the BFT system enhances animal production and has a synergistic effect on water quality	[59]
16S rRNA	Rhodobacteraceae and flavobacteriaceae	The larger particles of bioflocs impact the shrimp gut microbiota, potentially enhancing shrimp growth	[60]
16S rRNA	Bacteroidetes	The denitrifying bacteria genus *Paracoccus* was present in systems provided with light and carbohydrates, while harmful bacteria, such as *Leucothrix*, were identified in systems with added carbohydrates but without light. *Paracoccus* showed a positive correlation with NO_2-N, NO_3-N, and TOC. In contrast, *Leucothrix* was positively correlated with NO_3-N and negatively correlated with TAN	[61]
16S rRNA	Chloroflexi	Microbial communities in the reaction zones were more diverse than those in the culture tanks, buffer tank, and settling zones of the biofloc reactor. Denitrifiers, nitrifying bacteria, and key functional microbial populations for phosphorus removal were all present in the reaction zones at higher levels compared to other zones	[47]
16S rRNA	Proteobacteria	The difference in species composition is likely related to biological flocculation, where microorganisms spontaneously form large, dense, and rapidly settling aggregates. This also indicates that settling tanks can reduce the abundance of microorganisms, favoring planktonic bacteria or microorganisms associated with small and open flocs	[62]

(Continues)

TABLE 2 | (Continued)

High-throughput sequencing technology/Other microbial technology	Dominant bacteria	Results	References
16S rRNA	Alphaproteobacteria	Based on the distinct relationships between size fractions and prokaryotic communities, a framework model was proposed to elucidate the microbe-mediated biofloc formation process. This model encompasses the successive stages of "Finding-Proliferating-Regulating-Agglomerating" (FPRA)	[55]
16S rRNA	Proteobacteria and Bacteroidetes	Different carbohydrates can significantly influence the microbial community composition, the structure of biofloc, and the number of pathogens	[56]
16S rRNA	Rhodobacteraceae	The abundance of various bacterial families was observed in different BFT treatments. These results confirm that using rice bran fermented with a consortium of probiotic bacteria provides beneficial effects on shrimp culture	[63]
16S rRNA	Proteobacteria	The alpha-diversity of bacteria was higher in the outdoor BFT system, which also produced more shrimp and maintained more stable water quality compared to the indoor BFT system	[64]

[94]. Aguilera-Rivera et al. [95] indicated that probiotics aid in maintaining homeostasis and thwarting the emergence of opportunistic pathogens. Specifically, the presence of *Bacillus* in biofloc was found to boost survival rates, enhance immune function, and bolster disease resistance in Pacific white shrimp. Prior studies demonstrated that certain MOs and their cellular components or metabolites function as probiotics and immunostimulants in aquatic animals. They enhanced innate immunity, antioxidant capacity, and pathogen resistance in aquaculture animals [96, 97]. Xu and Pan [98] reported that certain probiotic bacteria activated the blood cells and phagocytes and improved the antioxidant capacity in Pacific white shrimp. The genes encoding phenol oxidase (*propo1* and *propo2*), serine protease (*SP1*), phenol oxidase plus sex kinase (*ppae1*), and serine protease (*masquerade-like*) were all significantly upregulated in the presence of certain probiotic bacteria and their functions are directly or indirectly related to host immunity [99]. Haridas et al. [100] reported enhanced immune responses in a biofloc system, noting increased respiratory burst, serum lysozyme, and myeloperoxidase activity, along with higher survival rates following exposure to the virulent strain of *Aeromonas hydrophila*. Similarly, Kheti et al. [101] observed that the expression of immune-related genes, including *IL-1β*, *IFN-γ*, *TNF-α*, *C3*, *iNOS*, and *IL-10*, significantly increased after administering a diet containing 4% biofloc.

Strengthening the innate immunity of shrimp enables them to resist bacterial infection. Immune-enhancing substances include bacteria and their products, carbon compounds, nutritional factors, animal extracts, cytokines, lectins, plant extracts, and synthetic drugs such as levamisole. The seedling stage of *L. vannamei* is susceptible to numerous viral and bacterial pathogens and its premature death from zoothamnium, mucus, and acute hepatopancreatic necrosis disease (AHPND/EMNS) results in vast economic losses. Various drug treatments are available for these infections but they are expensive and have limited efficacy. The prebiotic function of biological flocs can effectively remediate these disorders. *L. vannamei* seedlings fail usually because of poor water quality control. Such conditions undermine the pathogen resistance and overall health of the seedlings. In contrast, BFT minimizes direct seedling contact with external water resources and the prebiotic efficacy of its biofloc strengthens larval immunity.

4.4 | Aquaculture Animal Growth and Yield in BFT Systems

Numerous studies have reported varied effects of BFT on aquaculture animal performance, ranging from positive to neutral to negative outcomes [102]. Mahanand, Moulick, and Rao [103] found that the optimal growth parameters for rohu, such as net yield, specific growth rate, protein efficiency ratio, and feed conversion ratio, were achieved with a feed mixture of 50% standard fish feed and 50% wet floc, indicating the nutritional suitability of biofloc for rohu.

Izel-Silva et al. [104] observed that cannibalism in Brycon amazonicus larviculture was reduced in biofloc systems with TSS concentrations between 200 and 350 mg/L, which increased productivity. However, Pellegrin et al. [105] reported that TSS

TABLE 3 | The changing parameters of the aquatic animals reared in the biofloc systems.

Aquatic animals	Experiment treatments	The improved physiological or immunological parameters/gene expression	Challenge test/Poison attack experiment/negative environment stress	References
Nile tilapia (*Oreochromis niloticus*)	Pineapple peel powder and *Lactobacillus plantarum* CR1T5 individual or mixed addition in the feed	Increased serum LYS, ACH50, and PI activities	*Streptococcus agalactiae* challenge. The mixed group had a highest survival (73.08%) compare to 46.15% and 50% in the other group	[75]
Indian white shrimp (*Penaeus indicus*)	Feeded varying protein levels (25%, 30%, and 35%) in a biofloc-based rearing system	Increased PO activity, protein, and inhibition of SOD; decreased LYS activity; increased BGBP, hemocyanin gene, crustin, ProPO, SOD, peroxinectin genes		[76]
Olive flounders (*Paralichthys olivaceus*)	Glucose based biofloc and seawater conditions		*Streptococcus iniae* challenge, decreased hemoglobin and hematocrit, calcium, increased glucose, GOT, and GPT. And decreased acetylcholinesterase activity	[77]
Rhynchocypris lagowski Dybowski	Control and glucose based C/N 15, C/N 20, and C/N 25 biofloc systems		Cu exposure. Decreased *NF-κBp65*, *TNF-α*, *IL-1β* and *IL-8*, increased *IL-10* and *TGF-β* in C/N 15, C/N 20 and C/N 25 groups. Increased LYS, AKP, ACP, C3, C4, IgM, and NOS in C/N 20. Decreased *Keap1* and increased Nrf2, Maf, HO-1, CAT, CuZn-SOD, GCLC, and GPX in C/N 20. Increased SOD, T-AOC, CAT, and GR activity, decreased MDA in C/N 20 group. Decreased TLR4, MyD88, phosphorylated NF-κB, and phosphorylated I-κBα in C/N 20 group. survival: 62% (control), 71% (C/N 15), 83% (C/N 20), and 77% (C/N 25)	[78]
Litopenaeus vannamei	Molasses based biofloc system, different treatments of C/N 12 and C/N 16 group with 70% or 100% feeding level	Increased SOD activity in 100% feeding level and C/N in 16 group, higher T-AOC in 70% feeding level and C/N12 group, higher antibacterial activity, and bacteriolytic activity in C/N16 group. Decreased THC in 70% feeding level and C/N12 group		[79]

(Continues)

TABLE 3 | (Continued)

Aquatic animals	Experiment treatments	The improved physiological or immunological parameters/ gene expression	Challenge test/Poison attack experiment/negative environment stress	References
Gibel carp (*Carassius auratus gibelio*)	PHB feeded (treatment) or not (control)	Increased genes gabT, sdhA, hsp70, RAF1, JAK, PIK3R1 and mTOR in PHB-feeding group. PHB-feeding changed the microbial structure but not diversity, and significantly increased beneficial bacteria such as *Bacillus* sp. Also investigate the histological structure of forgut	*CyHV-2* infection. The PHB-feeding group had a higher survival	[80]
Indian white shrimp (*Penaeus indicus*)	Blend of carbohydrate based biofloc sytstem with *Bacillus* spp. addition or without *Bacillus* spp. Addition and control (clear water) group	Increased THC in BFT system. Increased phagocytic activity, SGH, GH, and PI in probiotic addition group; increased immunological gene expression transglutaminase genes and PPAE genes in BFT system with probiotic addition		[81]
Olive flounder (*Paralichthys olivaceus*)	Biofloc system and seawater		Hematological parameters: increased hemoglobin and hematocrit in BFT system, antioxidant responses: increased SOD, catalase, and glutathione-S-transferase activities in BFT system, decreased glutathione in BFT system. Immunological parameters: increased LYS activity, decreased AChE activity. Stress indicator: increased cortisol and HSP 70. higher survial in BFT system	[3, 4]
Penaeid monodon	Feed with different content biofloc (0%–100%)	Increased survival rates, the levels of immune parameters (PO, phagocytic activity, and THC), and expression of immune genes (proPO-I, PEN-4, and dicer) were significantly higher in bioflocs fed shrimp	*Vibrio parahaemolyticus* challenge. Higher survival in biofloc feeding group. Also the hepatopancreatic tubule were similar to the normal one	[82]
Pacific white shrimp (*Litopenaeus vannamei*)	Eight different carbohydrates based biofloc systems and control system (clear water system)	Immune parameters: increased THC and ProPO activity, upregulated proPO cascade (PX, BGBP), and antioxidant defense systems (SOD, MnSOD, CAT) gene expression.	*Vibrio parahaemolyticus* challenge. Higher survival in biofloc systems.	[83]

(Continues)

TABLE 3 | (Continued)

Aquatic animals	Experiment treatments	The improved physiological or immunological parameters/gene expression	Challenge test/Poison attack experiment/negative environment stress	References
Genetically improved farmed tilapia (GIFT)	Biofloc system group and biofloc as feed	Increased immunological (serum protein, RBT, and myeloperoxidase) and antioxidant indicators (glucose, SOD) and catalase were found to be significantly higher in in situ biofloc system	*Aeromonas hydrophila* challenge. Lower degree levels of infection in biofloc system	[84]
Common carp (*Cyprinus carpio*)	Clear water system and BFT systems with two stocking density	Increased amylase and protease activities, increased serum CAT, SOD, and GPx activities, and MDA content; decreased serum globulin, increased serum LYS, ACH50, and ALP activities in biofloc system, decreased serum cortisol and glucose levels and ALT and AST activities in bifloc system	Acute crowding stress. Biofloc showed anti-stress effect	[85]
Gibel carp (*Carassius auratus gibelio*)	Clear water system and different TSS concentration biofloc systems	Upregulated MPO, KRT 8, DUSP 1, IL-11, ITLN, PNP 5α, and clys in biofloc systems	Cyprinid herpesvirus 2 (*CyHV-2*) challenge. The higher survival was observed in biofloc systems	[86]
Shrimp (*Litopenaeus vannamei*)	Clear water system and biofloc system with different C/N ratio	Increased four immune-related genes RAN, SP, prophenoloxidase crustin	*Vibrio harveyi* challenge. The higher survival was observed in biofloc systems	[87]
Tilapia (*Oreochromis niloticus*)	Clear water system and biofloc system with different stocking density	Increased lipase, trypsin, and amylase activities, increased LYS, C3, and glutathione, increased cortisol, 5-hydroxytryptamine, and glucose concentrations. decreased ALT, AST, and ALP in biofloc systems	*Vibrio harveyi* challenge. The higher survival was observed in biofloc systems	[11, 88]
Sea cucumber *Apostichopus japonicus* (Selenka)	Different content of biofloc feeded	Increased trypsin, lipase and amylase activities; increased glucose, cortisol and glycogen levels; increased SOD, T-NOS, LYS, ACP and AKP activities, upregulated Hsp90, Hsp70, p105, Rel, NOS, and LYS	*Vibrio splendidus* challenge. The higher survival was observed in biofloc systems	[89]

Abbreviations: ACH: alternative complement activity, AChE: acetylcholinesterase, ACP: acid phosphatase, ACP: acid phosphatase, AKP: alkaline phosphatase, AKP: alkaline phosphatase, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate transaminase, BGBP: beta-1,3-glucan binding protein, C3: complement C3, C4: Complement C4, CAT: catalase, CAT: catalyze, clys: c-type lysozyme, DUSP 1: dual specificity phosphatase 1, GH: granular hemocyte, GOT: glutamic oxalate transaminase, GPT: glutamic pyruvate transaminase, GPx: glutathione peroxidase, GR: glutathione peroxidase, IgM: immunoglobulin M, IL-11: interleukin-11, ITLN: intelectin, KRT 8: keratin 8, LYS: lysozyme, MDA: malondialdehyde, MPO: myeloid-specific- peroxidase, NOS: nitric oxide synthase, PI: phagocytic index, PI: phagocytosis, PNP 5α: purine nucleoside phosphorylase 5α, PO: phenol oxidase, PPAE: Prophenoloxidase activating enzyme, ProPO: prophenoloxidase, ProPO: prophenoloxidase, RAN: ras-related nuclear, RBT: Respiratory burst test, SGH: semi granular hemocytes, SOD: superoxide dismutase, SP: serine proteinase, T-AOC: total antioxidant capacity, THC: total hemocyte count, T-NOS: total nitric oxide synthase.

TABLE 4 | The growth performance after the biofloc system rearing.

Species	Carbon source	Initial individual body weight	Growth performance in BFT system	Days	References
Litopenaeus vannamei	Sucrose	4.34 ± 0.64 g	Improved 36.07%	28	[116]
Litopenaeus vannamei	Corn starch	Post-larvae of day 15	Improved 15.27%	42	[117]
Litopenaeus vannamei	Combination (wheat flour, rice flour, and Ragi flour)	1.12 ± 0.07 g	Improved 144%	30	[118]
Litopenaeus vannamei	Tapioca powder	Post-larvae of day 15	Improved 15.28%	42	[119]
Litopenaeus vannamei	Molasses	0.016 ± 0.007 g	Improved 175%	35	[120]
Litopenaeus vannamei	Rice brain with a commercial probiotic	34.6 ± 0.9 g	Improved 16.96%	28	[63]
Litopenaeus vannamei	Rice bran	0.38 g	Improved 15.56%	56	[121]
Litopenaeus vannamei		0.561 ± 0.040 g	Improved 6.58%	35	[122]
Litopenaeus vannamei	Combination (molasses, rice bran and wheat flour)	0.374 g	Improved 68.45%	28	[123]
Litopenaeus vannamei	Sugarcane molasses	7.0 ± 1.37 g	Improved 3.31%	60	[124]
Penaeus vannamei	Molasses	Post-larvae of day 3	Improved 107.98%	30	[125]
Penaeus indicus	Combination (molasses, wheat flour, rice, corn, refined wheat, flour and flour of finger millet, and gram)	0.12 g	Improved 33.33%		[81]
Litopenaeus vannamei	Molasses	2.56 ± 0.33 g	Improved 1.02%		[16, 17]
Litopenaeus vannamei	Multigrain flour	1.10 ± 0.07 g	Improved 93.79%	120	[126]

in biofloc systems could hinder the growth of pacu (*Piaractus mesopotamicus*). Schveitzer et al. [106] also emphasized the importance of managing TSS in biofloc systems.

Contrary to some positive reports, Promthale et al. [82] showed that biofloc did not significantly enhance the growth performance of *L. vannamei* compared to those raised in clear water. Pellegrin et al. [107] indicated that pacu (*Piaractus mesopotamicus*) could be reared at a density of 600 fish/m^3 without compromising health, though with reduced final weight.

Rajkumar et al. [108] demonstrated that incorporating wheat flour into BFT significantly boosted biofloc production, leading to improved water quality and increased *L. vannamei* production. Brandão et al. [109] found that biofloc inoculum in denitrified seawater promoted the productivity of *L. vannamei*. Ahmad et al. [110] reported that in situ biofloc generated with tapioca enhanced growth and boosted non-specific immune responses.

Santos et al. [111] showed that adding a fermented product to biofloc systems, creating a "symbiotic system," improved the growth performance of *L. vannamei*. de Oliveira Ramiro et al. [112] utilized a mix of nano and microbubbles for aeration in biofloc systems, which improved water quality control and *P. vannamei* production. Wang et al. [113] found that adding 10% biofloc to the feed of *Carassius auratus* promoted growth performance and liver antioxidation capabilities, as evidenced by increased levels of t-superoxide dismutase (T-SOD), glutathione S-transferase (GST), catalase (CAT), and malondialdehyde (MDA).

Poli, Schveitzer, and Nuner [114] noted that South American catfish (*Rhamdia quelen*) could thrive in biofloc systems with TSS concentrations up to 1000 mg/L, although the best growth was seen in tanks with a higher percentage of volatile suspended solids. Zhao et al. [115] found that a higher carbon/nitrogen (C/N) ratio, particularly in the C/N = 23 group, enhanced production performance and feed utilization in mirror carp (*Cyprinus carpio specularis*) and bighead carp (*Hypophthalmichthys nobilis*), along with improved water quality.

Other studies also suggested improved growth performance following biofloc feeding or rearing in biofloc systems (see Table 4).

4.5 | Floc Size and Water Treatment Efficacy

Few studies have investigated floc particle size. Nevertheless, this property is crucial to biological floc performance. Biofloc denitrification is related to floc particle size. Wagg et al. [22] passed forest soil through various screens and observed that microbial diversity and denitrification function significantly varied with soil particle diameter. Soil microbial diversity and gene function tended to decline with soil particle size. The earliest work on floc particle size was reported by Ekasari et al. [127], the authors comparatively analyzed the growth of cultured animals fed biofloc consisting of particles differing in both size and nutritional composition. Shrimp (*L. vannamei*), red tilapia (*O. niloticus*), and mussels (*Perna viridis*) consumed biofloc of all particle sizes. However, nutritional composition and animal nitrogen retention markedly varied with floc size. Liu et al. [5] demonstrated that most amino acids could meet these nutritional requirements. Different carbohydrate polymers promote the formation of specialized flocs. Methionine and lysine were deficient in biofloc derived from molasses, glucose, and longan seed powder carbon sources [5, 127, 128]. However, it remains to be determined whether all bioflocs are deficient in these amino acids.

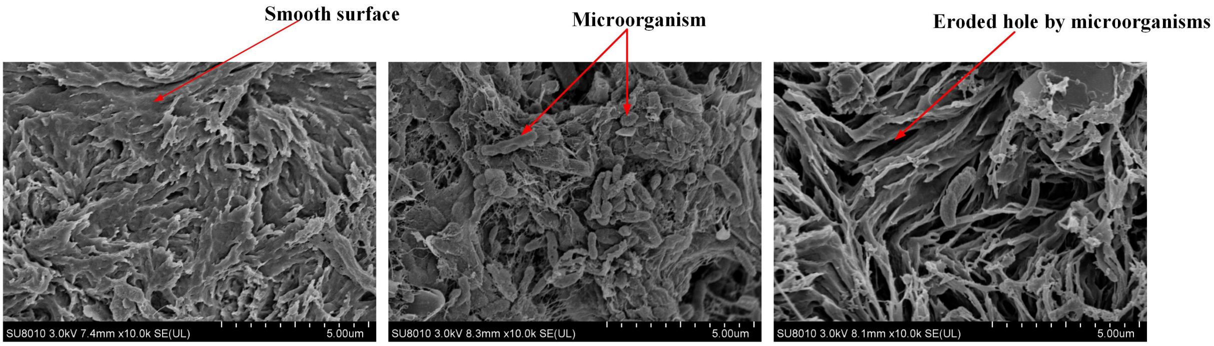
Chen et al. [51] suggested under optimal C/N, variations in floc size should not affect the crude protein content, ammonia removal efficiency, or inorganic nitrogen accumulation in a BFT reactor. Nevertheless, they could influence the abundance of the dominant bacterial phyla. Souza et al. [129] reported that neither floc size nor bacterial taxon abundance affects nitrification in *L. vannamei* BFT system. Nevertheless, floc integrity might affect nitrifying bacteria distribution and interactions. Wagg et al. [22] mentioned that floc filtration could alter the physicochemical properties of functional chemical compounds. Wei et al. [55] and Wei, Wang, and Liao [56] performed microbiome analyses to establish the influences of floc particle sizes on microbial distribution and applied network analyses to determine the interactions among MOs in *L. vannamei* BFT system.

4.6 | Enhancing BFT Efficiency Through Solid-Phase Carbohydrates

Prior research investigated the impact of C/N on changes in the inorganic nitrogen content of BFT systems [88, 130]. Shi et al. [131] used computational fluid dynamics (CFD) to simulate multiphase flow and optimize BFT systems. Water quality improved after a biofloc reactor and a RAS system were combined [28].

Luo et al. [132] used poly-β-hydroxybutyric acid (PHB) and polycaprolactone (PCL) as solid carbon sources in a biofloc system and reported that after tilapia were cultured in it for 120 days, the ammonia and nitrite concentrations were stabilized at 2.0 and 0.5 mg/L, respectively. Solid-phase carbohydrate addition controls water quality, optimizes carbon addition, and reduces labor. In practice, however, biodegradable polymers (BDPs), such as PHBV (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)), PBS (poly(butylene succinate)), and PCL, are seldom applied as solid-phase carbon sources in BFT systems. As innovative aquaculture models emerge, BFT will be combined with them and economic and ecological benefits could be realized.

The type and abundance of the carbon source are vital to biofloc formation. The carbon source provides electrons for heterotrophic MOs. Various carbon sources have different effects on ammonia removal efficiency. More precisely, it is the C/N of the aquaculture water body that determines this property. The efficiency of carbon source utilization directly influences the immediate aquatic C/N level [5]. BDPs such as PHBV, PCL, and PBS are widely used in sewage treatment denitrification reactors. Chu and Wang [133] used PCL as a biofilm carrier and a carbon source for SND. In a 90 days aerobic SND test, 1.27 g PCL was required to degrade each gram of inorganic nitrogen and the total ammonia + nitrate removal rate was 74.6%. In a batch experiment, the total inorganic nitrogen removal rates were 69.9% and 98% under aerobic and anaerobic conditions, respectively. By contrast, total inorganic nitrogen removal was only 52% in the presence of glucose as the carbon source. Figure 7a–c shows abundant MOs attached to the surface of PHBV. The erosion of this material indicated that it was both a bacterial carrier and a carbon source. In previous experiments, a BFT system based on PHBV and PBS achieved high ammonia removal efficiency, and papers related to solid-phase carbon source BFT systems rearing tilapia (*O. niloticus*) have been published [5, 134]. Hence, PHBV is feasible and stable as a solid carbon source in BFT systems. This approach reduces the amount of carbon source required and can stably control the aquatic C/N. Solid-phase carbon sources can release the carbon according to the bacterial demand in the water body. On the other hand, ordinary soluble carbon sources can only be supplied at certain stages and may either be inadequate or excessive. Thus, solid-phase carbon sources may create conditions conducive to high-efficiency nitrogen conversion. Solid carbon sources are efficient and sustainable in wastewater denitrification and have excellent development potential



Initial stage (first 30 days)

Middle stage (30–60 days)

End stage (after 120 days)

FIGURE 7 | SEM images of PHBV (poly(3-hydroxybutyrate-co-3-hydroxyvalerate)) and microorganisms on biofilm.

[135, 136]. However, little is known about their roles in nitrogen conversion in BFT systems.

4.7 | Pioneering Research Techniques and Network Analysis in BFT

BFT systems are characterized by complex microbial collections and functional clusters. In nitrogen transformation, various MOs catalyze the complex interaction among different biochemical reactions. The main nitrogen forms in aquaculture water range from nitric acid (+5 valence) to ammonia (−3 valence). The BFT system occurs primarily in aerobic environments but may also exist in hypoxic and anaerobic microenvironments. The various changes that occur in the microbial colonies of BFT systems are dynamic and complex and might include both anaerobic and aerobic biochemical reactions such as nitrification, denitrification, and nitrogen assimilation [45, 53, 137]. For nitrogen transformation to proceed in BFT systems, there must be mutual restriction, competition, and coordination among the MOs and their metabolic pathways. MOs mutually adapt to other bacterial populations and cooperate with them through molecular signals [138]. However, little is known about BFT as a microecosystem.

In BFT systems, there is relatively high nitrate accumulation and fluctuating ammonia levels especially under low C/N conditions in the *O. niloticus* BFT system [88]. Therefore, BFT systems dynamically change nitrification. Nitrate accumulation might actually be commonplace in BFT systems [50, 139]. Dauda et al. [140] reported that even when $C/N > 20$, the nitrate content in the BFT water body was $191.67\,\mathrm{mg\,L^{-1}}$ and exceeded those measured at $C/N = 10$ or 15. Nevertheless, Avnimelech [141] maintained that under high C/N, nitrogen assimilation and heterotrophic bacteria prevailed in BFT water and no inorganic nitrogen accumulated. Therefore, nitrogen transformation mechanisms are complex in BFT systems and remain to be elucidated. For these reasons, it is difficult to suppress nitrification based on currently available information. In contrast, nitrate can be effectively controlled. Liu et al. [28] designed an ex situ carbon addition reactor that could rapidly switch the BFT from "nitrification mode" to "assimilation mode," thereby, lowering carbon source costs and effectively controlling nitrate accumulation. In a 43 days assay, nitrate accumulated in the range of only $6.69–7.18\,\mathrm{mg\,L^{-1}}$. Moreover, the aerobic denitrifiers *Pseudomonas* spp., *Geobacter* spp., *Bacillus* spp., *Flavobacterium* spp. and others were detected. These findings provide a reference for microbial species to deploy in future BFT research. The biodegradable plastics PHBV and PBS were used as the solid carbon sources in the preliminary BFT test and the ammonia removal efficiency was >90% in the *O. niloticus* BFT system [5, 134]. SND was achieved and the C/N was relatively stable. The main bacterial phyla in the biofloc were Proteobacteria, Bacteroides, and Firmicutes. The same microbial taxa were identified in BFT [55, 56, 64]. The relative abundances of these phyla change and they are the main bacterial nitrifiers and denitrifiers. Numerous studies still use the 16S amplicon method to detect these bacteria. Though prior investigations discussed the microbial composition of BFT, few have examined the interactions among bacterial taxa or their impact on nitrogen transformation in BFT microecosystems.

Microbiome research has been extensively and comprehensively applied in gut and environmental ecology. This method analyzes the relationships among MOs, host health status, and target ecosystem homeostasis with a view towards understanding and regulating the target ecosystem. Nevertheless, the BFT microbiome has seldom been explored [142]. Co-occurrence network analyses were conducted on large-scale ecosystems such as the activated sludge in global sewage treatment plants and the bacterial assemblages in the forest soils of Eastern China [30, 143]. It was concluded that microbial community composition varied with geographical pattern. In BFT, bacterial co-occurrence widely differs with carbon source [45] and varies with floc particle size [55, 56]. The foregoing studies indirectly indicated that the BFT system is under the influence of various factors and is comparable to an ecosystem under different spatiotemporal conditions influencing the characteristics of bacterial community co-occurrence. The "small-world" network proposed by Watts and Strogatz [29] and the scale-free network suggested by Barabasi and Albert [144] are consistent with the bacterial interactions within the microecosystem and reflect the uneven distribution of complex ecosystems, such as activated sludge [30], seawater [26, 145, 146], freshwater [147, 148], sediment, and soil [149]. Hence, network analyses are feasible for multi-omics studies of the BFT systems.

Other nitrogen transformation metabolic pathways besides nitrification, denitrification, and nitrogen assimilation might also occur in BFT. Microbial "dark matter" may be present [32]. These are functional but unknown microbial clusters that cannot be cultured and form complex BFT microecosystems. Certain researchers clustered soil genetic correlation networks by functional modules and constructed functional subnetworks. Unknown gene functions in the microflora were predicted and verified according to the topological structure between gene functions [150]. This method is feasible for the prediction of unknown microbial functions in BFT systems. Certain authors constructed a function tax bipartite network to analyze coupling relationships among biochemical reactions and microflora classifications. They found that *Bacillus* spp. dominate nitrogen assimilation while Proteobacteria dominate nitrification and denitrification in the soil [25].

Activated sludge, soil ecosystems, and BFT microecosystems have similar microbial functions and mechanisms and are dominated by bacteria varying in fitness and with different niches. Network analyses can classify the bacterial communities in a BFT system according to the biochemical reactions they perform in nitrogen transformation. This information could guide the targeted regulation of certain nitrogen transformations within the microecosystem. Network analysis revealed that *Pseudomonas aeruginosa* ZM03 cooperates with *Arthrobacter nicotianae* ZM05, which degrades soil pollutants. Therefore, ZM03 was enriched, cultured, and added to ZM05 to enhance the capacity of the latter to degrade xenobiotics in the soil [151].

Metagenomic, metatranscriptomic, and co-occurrence network analyses of microflora disclosed important heretofore unknown archaea in the carbon cycle of marine sediments that were designated Thermoprofundales (MBG-D archaea) [26]. This discovery suggests the existence of other unknown and unclassified

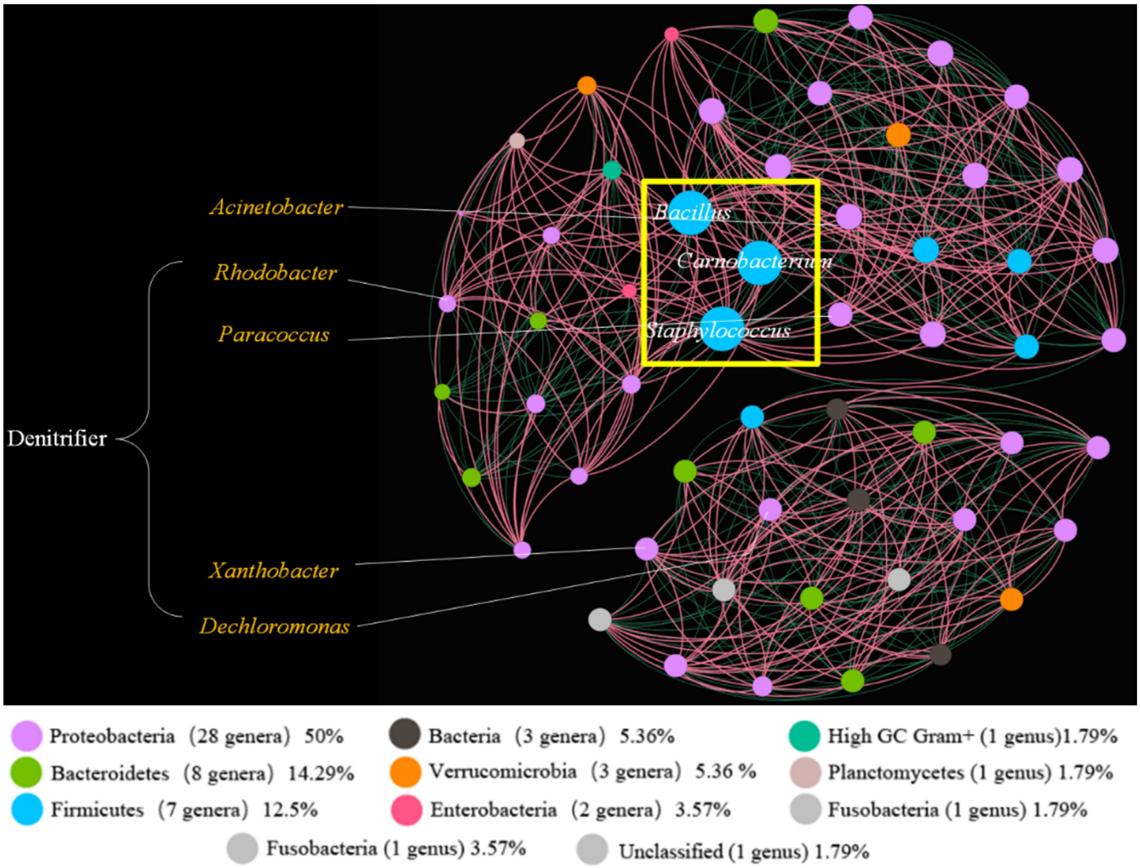


FIGURE 8 | Network analysis of the 56 genera in biofloc system. Different colors represent different phyla, illustrating the relationships and interactions among the genera within their respective phyla.

functional bacteria constituting the microbial "dark matter" in BFT systems. Liu et al. [45], based on network analysis (the network was established by using R and python program, then visualized by Gephi, the python script to calculate the co-occurrence rate between different phylum), the bacteria that play a role in the BFT system are identified as *Bacillus*, *Carnobacterium*, and *Staphylococcus* (Figure 8), which belong to Firmicutes, a typical probiotic, including many common nitrifying and DNB. In the network topology, it is found that some bacteria, although small in abundance, are indispensable companion bacteria, such as *Ruminococcus* (Figure 8). This kind of bacteria is called "satellite taxon" flora and plays a certain role in the ecosystem [152]. Although indispensable, the function of the accompanying bacteria provides a preliminary reference for the next biofloc study.

Deng et al. [153] observed nonrandom microbial assembly patterns and different microbial niches and fitness in biofilm and biofloc. They observed general characteristics and trends among the bacteria within the same domain (such as soil or freshwater) and across domains (such as the ecological linkages among marine bacteria, archaea, and protists). Yuan et al. [154] reported that the development and succession of bacterial nitrifiers were correlated with the observed process changes in a moving bed biofilm reaction (MBBR). However, little is known about the bacterial interactions within the aquatic environment of BFT systems. BFT is an artificial aquatic ecosystem with over 50 microbial genera [153] and a biomass in the range of 2–15 g/L [141]. Collaboration among core bacterial species accounts for high-efficiency nitrification in wastewater treatment [30]. Future research should use network analyses and other modalities to elucidate the mechanisms underlying the changes that occur in the inorganic nitrogen dynamics and microbial diversity of BFT systems.

5 | Challenges and Future Directions

A critical challenge in BFT lies in deciphering the complex interactions within the microbial community. Understanding how turbulence affects floc characteristics—such as size and microbial composition—and the processes of nitrification and denitrification is essential. The role of solid carbohydrates in BFT systems, though underexplored, offers a promising avenue for enhancing biofloc efficiency and warrants further investigation. Floc size is a key determinant of both aquatic animal health and the effectiveness of nitrogen transformation, highlighting the need for detailed studies in this area. Additionally, leveraging high-throughput sequencing data requires proficiency in programming languages like Python and R, which are crucial for the in-depth analysis of microbial communities. This expertise will enable the adaptive optimization of BFT systems, improving nitrogen metabolism and overall efficiency. Incorporating microbiome research into BFT studies can provide new insights, particularly through the integration of microalgae. Their photosynthetic activity not only supplements oxygen and carbon sources for nitrifiers and denitrifiers but also enhances nitrogen removal efficiency. Given their ability to thrive under both natural and artificial light conditions, microalgae can be a valuable component of BFT systems.

Future research should prioritize the examination of water quality indices and employ advanced molecular techniques, such as 16S rRNA amplicons, metagenomics, and metatranscriptomics. These interdisciplinary methods will elucidate the microbial networks and their functional genes within BFT systems, shedding light on nitrogen transformation mechanisms. Optimizing BFT-based aquaculture through such investigations will contribute to sustainable practices that conserve energy, minimize resource use, and align with carbon neutrality goals. Moreover, insights from upcoming microbiome studies are expected to clarify nitrogen cycling within complex algae-bacteria-archaea ecosystems, paving the way for more efficient and environmentally friendly aquaculture solutions.

6 | Conclusion

In this comprehensive review, we have delved into the expanding realm of BFT, highlighting its critical role in promoting sustainable aquaculture. Through detailed bibliometric analysis, the study traces BFT's development, key research domains, and groundbreaking methods. It emphasizes the importance of analyzing microbial behaviors, enhancing floc qualities, and adopting innovative feeding strategies to boost efficiency. Despite significant progress, mastering microbial ecosystem complexities and refining operational parameters for wider application remain challenges. Incorporating advanced genomic techniques and network analyses could reveal the intricate biofloc interactions, contributing to more resilient and productive aquaculture systems. Moreover, leveraging data analysis as an experimental extension can deepen our understanding of underlying mechanisms. As we face urgent demands for sustainable food sources, BFT exemplifies the innovative spirit necessary to address global food security while maintaining environmental integrity. Continued interdisciplinary research are vital to unlocking BFT's full potential for the sustainable intensification of aquaculture.

Author Contributions

Gang Liu: writing – review and editing, writing – original draft. **Marc Verdegem:** conceptualization. **Zhangying Ye:** methodology, conceptualization. **Jian Zhao:** data curation. **Jinxing Xiao:** conceptualization, visualization, writing – review and editing. **Xingguo Liu:** supervision, formal analysis. **Qinlang Liang:** conceptualization, visualization, supervision, project administration. **Kun Xiang:** project administration. **Songming Zhu:** writing – review and editing, project administration.

Acknowledgments

This work was supported by the National Key R&D Program of China (Grant No. 2022YFD2001705), Key Program of Science and Technology of Zhejiang Province (Grant No. 2023C02050), Key Laboratory of Healthy Freshwater Aquaculture, Zhejiang Institute of Freshwater Fisheries (Grant No. ZJK202110); Key Laboratory of Environment Controlled Aquaculture, Ministry of Education (Grant No. 2021-MOEKLECA-KF-09).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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