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Microalgal Inoculation Modulates the Size-Dependent Assembly and Short-Term Stability of Eukaryotic Plankton Communities in Shrimp-Rearing Water

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Abstract

Microalgae-based regulation is increasingly recognized as an eco-friendly strategy for improving water quality and nutrient management in intensive aquaculture systems. Although its effects on bacterial communities have been extensively investigated, its ecological impacts on higher trophic levels—particularly eukaryotic plankton communities across different size fractions—remain poorly understood. In this study, two indigenous microalgae species, *Nannochloropsis oculata* and *Thalassiosira weissflogii*, were inoculated into shrimp rearing water to elucidate the dynamics and interactions among microalgae, nutrient factors, and eukaryotic plankton communities across the small-sized (0.22–3 µm) and large-sized (>3 µm) fractions. The results revealed significant differences in the composition and diversity of both plankton size fractions under different microalgae treatments. Partial least squares path modeling indicated that microalgae influenced plankton communities both directly and indirectly through nutrient-mediated pathways. According to the neutral community model, microalgae inoculation was associated with an increased contribution of deterministic processes to community assembly. Variance partitioning further revealed that the large-sized community was primarily governed by microalgae, whereas the small-sized community was mainly shaped by rearing time, indicating size-dependent assembly mechanisms. The average variation degree and coefficient of variation, combined with effect-size analyses, indicated that *N. oculata* inoculation was associated with higher short-term community stability, an effect most pronounced in the large-sized fraction. Overall, these findings demonstrate that microalgal inoculation modulates the structure, assembly processes, and short-term stability of eukaryotic plankton communities, providing new insights into size-dependent, microalgae-driven assembly mechanisms and their potential to stabilize plankton communities for sustainable aquaculture management.

Keywords: *Nannochloropsis oculata*; *Thalassiosira weissflogii*; eukaryotic plankton; community assembly; community stability; size-fractionated plankton

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1. Introduction

The aquaculture industry has experienced remarkable expansion and advancement in recent years. Increasing demand for aquatic products has driven the rapid development of intensive aqua-farming systems, characterized by higher yields and stocking densities [1]. However, intensive aquaculture systems are often accompanied by environmental deterioration and disease outbreaks, which can negatively affect both cultured organisms and surrounding ecosystems [2]. Therefore, maintaining a healthy rearing environment is crucial for achieving ecologically stable aquaculture development [3,4].

In aquatic ecosystems, including aquaculture farms, microalgae act as important primary producers contributing to nutrient cycling and energy flow [1,5]. These roles have driven increasing interest in microalgae-based management strategies [6,7]. Previous studies have shown that *Nannochloropsis oculata* can assimilate nutrients and inhibit pathogenic bacteria [8,9], while *Thalassiosira weissflogii* supplementation improves shrimp immunity and growth performance [10,11]. However, most existing studies, including our previous work [8–10], have primarily focused on nutrient dynamics and bacterial community responses, with limited consideration of higher trophic levels.

Eukaryotic plankton are key but understudied components of aquaculture ecosystems. They span a wide range of size fractions from pico- to micro-plankton [12,13], with strongly size-dependent ecological functions [14]: smaller taxa contribute to nutrient assimilation and primary production [15], whereas larger taxa participate in trophic interactions and energy transfer within microbial food webs [14]. Owing to their rapid turnover, they respond quickly to environmental change and serve as effective indicators of ecosystem dynamics [16]. However, their responses to microalgal inoculation remain insufficiently characterized, particularly regarding size-structured organization and trophic interactions [17,18].

In our previous studies, microalgal inoculation was shown to influence microbial community organization in shrimp-rearing systems [8–10]. However, the stability of plankton communities across trophic levels is increasingly recognized as a critical determinant of ecosystem functioning [19]. Disruptions in plankton community equilibrium may destabilize microbial networks and promote opportunistic microorganisms, thereby increasing disease risk [20,21]. Furthermore, the susceptibility of a community to invasion by opportunistic taxa is strongly determined by its internal structure and interaction strength, such that more stable, strongly interacting communities tend to be more resistant to invasion [22]. Moreover, recent studies suggest that community stability, rather than diversity metrics alone, better reflects ecosystem status, as unstable communities often exhibit greater temporal variability and are associated with poorer host performance [23]. From a community ecology perspective, ecosystem stability is closely linked to community assembly mechanisms, which are governed by the balance between deterministic processes (environmental selection and species interactions) and stochastic processes (ecological drift and dispersal) [24,25]. Deterministic assembly tends to promote more predictable and reproducible community structures, which may enhance ecosystem stability and resilience [26,27]. Given that more stable communities are generally considered less susceptible to opportunistic taxa, clarifying how microalgal inoculation shapes the assembly and stability of eukaryotic plankton communities—particularly across different size fractions—is of both ecological and practical importance, yet remains poorly understood.

In this study, two indigenous microalgal species, *N. oculata* and *T. weissflogii*, were inoculated into *Litopenaeus vannamei* rearing water to investigate the responses of eukaryotic plankton communities. Specifically, we aimed to (1) characterize the temporal dynamics of eukaryotic plankton across different size fractions, and (2) disentangle the relative contributions of microalgae, nutrients, and rearing time to community variation and determine how microalgal inoculation influences community assembly processes; and (3)

assess the effect of microalgal inoculation on the short-term temporal stability of eukaryotic plankton communities.

2. Materials and Methods

2.1. Microalgae Cultivation and Experimental Design

N. oculata and *T. weissflogii* were obtained from the Marine Biotechnology Laboratory of Ningbo University, China. *N. oculata* was cultured in NMB3 medium, and *T. weissflogii* in the same medium supplemented with Na₂SiO₃ (2 mg/L), at a salinity of 22‰.

The 10-day experiment was conducted at the production base of Ningbo University. Approximately 2000 shrimp (*L. vannamei*, ~5 cm) were acclimated for 10 days in eighteen 500 L polyethylene fiber tanks, after which healthy individuals were selected. The rearing water was pretreated by sedimentation (10 days) and sequential filtration through 100 µm and 1 µm nylon mesh to remove particles and native microalgae. Three treatments with three replicates each were established using nine 500 L tanks: a control group without microalgal inoculation (C), a group inoculated with *N. oculata* (N), and a group inoculated with *T. weissflogii* (T). Each tank held 450 L of filtered rearing water and was stocked with 150 shrimp (~330 individuals m⁻³). Group N received 4.5 L of *N. oculata* suspension (~3 × 10⁵ cells/mL) and Group T received 4.5 L of *T. weissflogii* suspension (~8 × 10³ cells/mL), giving an initial microalgal biomass of about 25 mg/L in each inoculated tank. Background conditions were maintained stable throughout the experiment (temperature ~26 °C, salinity 22‰, pH ~8.2, and dissolved oxygen > 5 mg/L via continuous aeration). Shrimp were fed a commercial diet twice daily (08:00 and 20:00) until satiation, and no water exchange was performed.

2.2. Sample Collection and Physicochemical Analyses

Water samples were collected on days 1, 4, 7, and 10 after inoculation. For eukaryotic plankton analysis, samples were sequentially filtered through 3 µm and 0.22 µm polycarbonate membranes (47 mm diameter; Millipore, Burlington, MA, USA) to collect large-sized (>3 µm) and small-sized (0.22–3 µm) communities, respectively. The membranes were transferred into 1.5 mL sterile tubes and stored at –80 °C. Concentrations of NH₄⁺, NO₂[–], NO₃[–], and PO₄^{3–} were determined by standard spectrophotometric methods (NH₄⁺ by the indophenol blue method, NO₂[–] by the diazo-coupling method, NO₃[–] by the zinc-cadmium reduction method, and PO₄^{3–} by the molybdenum blue method) [10], and microalgal biomass was estimated based on cell density determined by microscopic counting [10].

2.3. DNA Extraction, Sequencing, and Bioinformatics Analysis

Eukaryotic microbial DNA was obtained from the same samples as used in our previous study [10]. PCR amplification of the 18S rRNA gene was performed using primers 3NDF (5'-GCGTAATTCCAGCTCCAA-3') and V4_euk_R2 (5'-AATCCRAGAATTTACCTCCAA-3') [28]. The reaction system and amplification procedures followed our previous study [10], except for an annealing temperature of 55 °C. Library construction and Illumina sequencing were performed by Guangdong Magigene Biotechnology Co., Ltd. (Guangzhou, China). Raw reads were denoised using the UNOISE3 pipeline in USEARCH (version 11) to generate zero-radius operational taxonomic units (ZOTUs) [29]. Taxonomic assignment was conducted against the SILVA 18S database (release 123) with a 99% similarity threshold and the Ribosomal Database Project (RDP) classifier [30].

2.4. Statistical Analysis

All analyses were performed in R (version 4.2.1), after removing ZOTUs assigned to *N. oculata* and *T. weissflogii* to focus on the responses of other eukaryotic plankton. Alpha-diversity indices were calculated using the vegan package. Beta-diversity was assessed by principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity, with group differences tested by PERMANOVA and ANOSIM (999 permutations; vegan). Canonical correspondence analysis (CCA) and partial Mantel tests (Spearman, 999 permutations) were used to relate community structure to environmental variables, and partial least squares path modeling (PLS-PM) was performed using the plsmpm package to disentangle direct and indirect effects [10]. The neutral community model was applied to assess the contribution of stochastic processes to community assembly [31,32]. Community stability was evaluated using two complementary metrics. The average variation degree (AVD), which inversely reflects community stability, was calculated by standardizing the abundance of each ZOTU across all samples and averaging the absolute deviations per sample [33]. The coefficient of variation (CV) of community composition across the four sampling time points was calculated for each replicate. Both metrics were compared among treatments within each size fraction. Variance partitioning analysis (VPA) was performed for each size fraction to quantify the relative contributions of microalgae, nutrients, and rearing time, using the varpart function in the vegan package on Hellinger-transformed community data [34]. Differences among treatments in AVD and CV were tested by one-way ANOVA followed by Tukey's HSD test, and effect sizes were quantified using eta-squared (η^2) and Cohen's *d*.

3. Results

3.1. Variation in Microalgal Biomass and Nutrient Factors

Detailed data on nutrient concentrations and microalgal biomass have been reported in our previous study [10]. Briefly, nutrient concentrations exhibited clear temporal variation during the experimental period but limited differences among treatments, with inorganic nitrogen and phosphorus concentrations increasing toward the later stages. The biomass of *N. oculata* and *T. weissflogii* increased during the initial phase and remained relatively stable thereafter. Background environmental parameters, including temperature (~26 °C), salinity (22‰), pH (~8.2), and dissolved oxygen (>5 mg/L), remained stable throughout the experiment, with no notable differences among treatments.

3.2. Effects of Different Microalgal Treatments on the Diversity of Eukaryotic Plankton Communities

A total of 2236 ZOTUs were obtained from 72 sequenced samples. Alpha diversity indices showed variation among treatments, with generally higher diversity observed in Groups C and T compared to Group N (Figure 1). Principal coordinate analysis (PCoA) revealed clear separation in community composition among treatments, which was supported by analysis of similarity (ANOSIM) ($R = 0.267$, $p = 0.001$) (Figure 2a). Permutational multivariate analysis of variance (PERMANOVA) further indicated that microalgal treatment explained the largest proportion of community variation, followed by rearing time and their interaction (Table 1). PCoA and constrained PCoA (CPCoA) confirmed that microalgal treatments significantly influenced both small-sized (0.22–3 µm) and large-sized (>3 µm) eukaryotic plankton communities (Figure 2a–c).

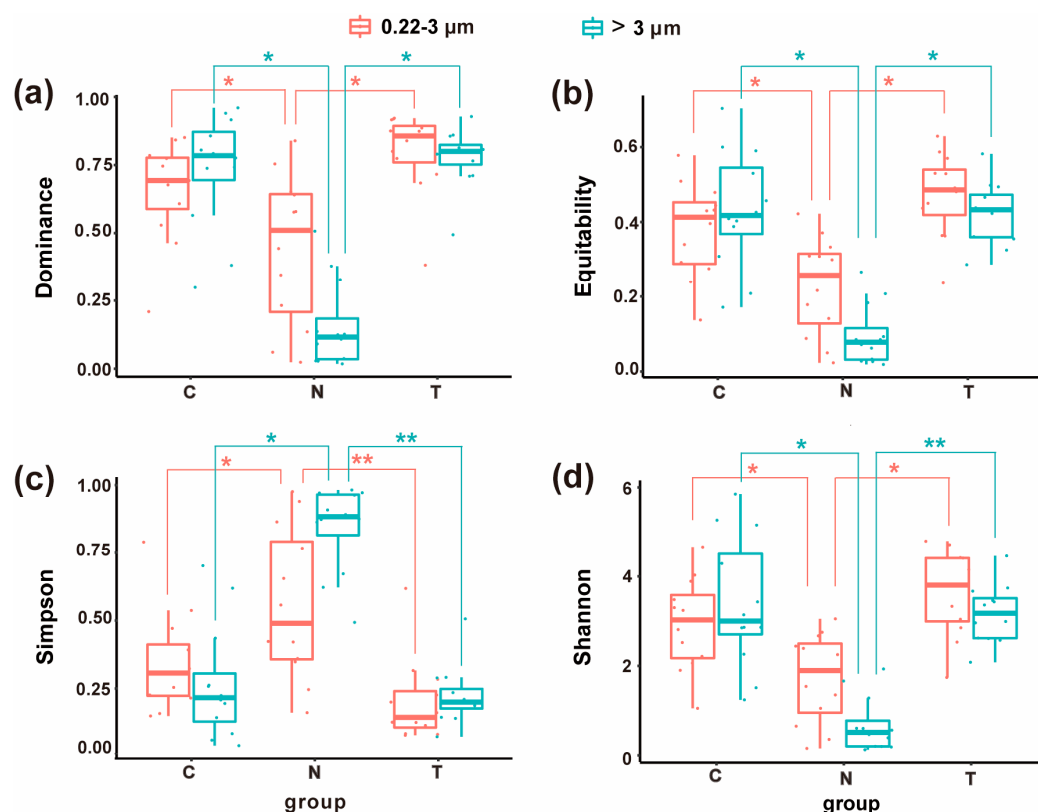


Figure 1. Variation in the α -diversity indices of eukaryotic plankton communities among the three treatments (C, N, and T). (a) Dominance; (b) Equitability; (c) Simpson; (d) Shannon. Asterisks indicate statistically significant pairwise differences among treatments (* $p < 0.05$, ** $p < 0.01$; one-way ANOVA followed by least significant difference (LSD) tests). Red and blue boxes represent the small-sized (0.22–3 μm) and large-sized (>3 μm) eukaryotic plankton fractions, respectively.

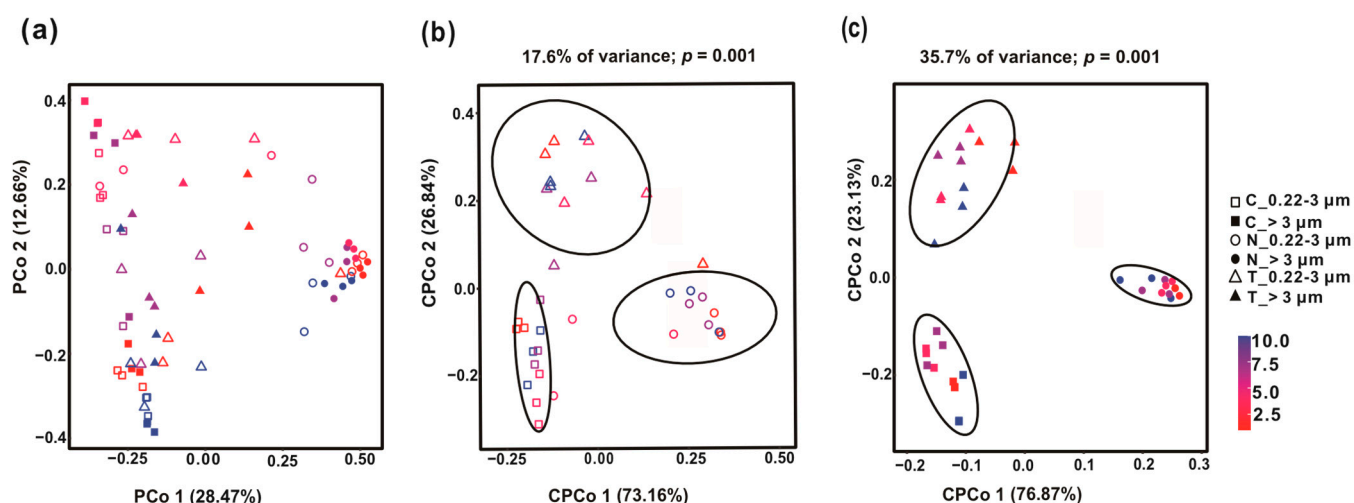


Figure 2. Dynamic changes in the structure of eukaryotic plankton communities. (a) PCoA based on Bray–Curtis dissimilarities of total communities; (b) CPCoA of small-sized (0.22–3 μm) communities; (c) CPCoA of large-sized (>3 μm) communities. Ellipses indicate 95% confidence intervals.

Table 1. Quantitative evaluation of the effects of rearing time and different microalgal treatments on the structural variation in community based on PERMANOVA.

Factors	Total Community	>3 μm	0.22–3 μm
Treatment	0.274 ***	0.443 ***	0.220 **
Rearing time	0.066 ***	0.079 ***	0.103 ***
Interaction	0.050 **	0.079 **	0.066
Size fraction	0.421 **	—	—

Note: R^2 reflects changes in the contribution of individual variables and interactions between variables to the structure of the community. With false discovery rate (FDR)-adjusted p values ** $p < 0.01$, *** $p < 0.001$.

3.3. Relationship Between Representative Genera and Nutrient Factors Under Different Microalgal Treatments

Representative taxa varied among microalgal treatments, with each treatment group characterized by a distinct set of taxa, indicating treatment-specific community responses to microalgal inoculation (Figure 3a,b). Spearman correlation analysis showed that most representative taxa were significantly associated with at least one environmental variable, including nutrient concentrations and microalgal biomass, with the exception of *Uronema* (Figure 3c). Among these, *Aspidisca*, *Sorodiplophrys*, and *Wobblia* exhibited relatively strong associations with nutrient variables and microalgal biomass, whereas other genera demonstrated weaker correlations. Overall, these results suggested that microalgal treatments may selectively influence subsets of eukaryotic plankton communities through their interactions with environmental factors.

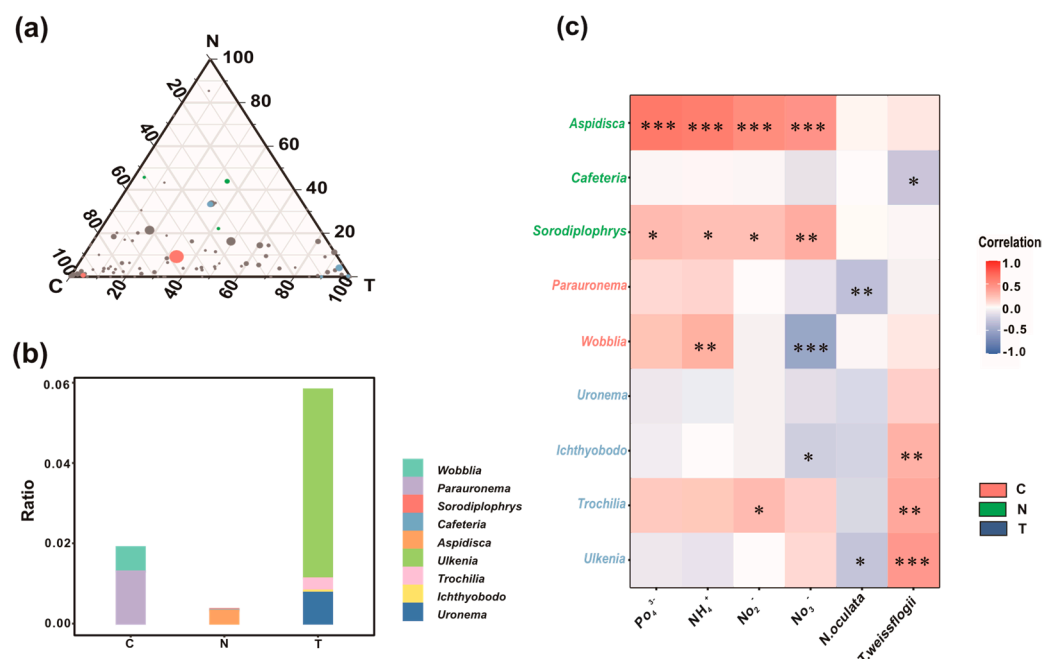


Figure 3. Relationship between representative genera and nutrient factors. (a) Ternary plots illustrating differential zero-radius operational taxonomic units (ZOTUs) for each treatment group (C, N, T), showing enrichment patterns relative to the other two groups. Each circle represents an individual ZOTU, with size indicating relative abundance (RA). Circle position corresponds to the contribution of each compartment to RA. Enriched ZOTUs were determined using a Bayes moderated t -test (FDR-corrected, $p < 0.05$). Circle colors indicate the treatment group in which each ZOTU was enriched (green, Group N; red, Group C; blue, Group T; grey, not significantly enriched). (b) Ratio of representative genera to total genera within each treatment group. (c) Heatmap displaying Spear-

man's correlation between environmental variables and representative genera. Genus names are colored by the treatment group in which they were representative (red, C; green, N; blue, T; see the C/N/T legend). The heatmap color scale represents Spearman's correlation coefficient (red, positive; blue, negative). Asterisks denote significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.4. Potential Effects of Environmental Factors on Eukaryotic Plankton Communities

Microalgae and nutrient variables were associated with variation in eukaryotic plankton community structure through potential direct and indirect pathways (Figure 4a). Partial least squares path modeling (PLS-PM) suggested that microalgae may influence community composition through nutrient variables, particularly inorganic nitrogen and phosphorus (Figure 4a). In addition, the model indicated interactions between small-sized (0.22–3 μm) and large-sized (>3 μm) eukaryotic plankton communities, suggesting potential coupling between different size fractions. The Mantel test revealed significant correlations between environmental variables and total and large-sized communities, whereas no significant association was detected for the small-sized community (Table 2). Consistently, canonical correspondence analysis (CCA) showed that nutrient variables were associated with variation in community composition (Figure 4b–d), supporting their role as important drivers of eukaryotic plankton structure.

Table 2. Mantel test for the relationships between environmental variables and eukaryotic plankton communities.

Community	r	p Value
Total Community	0.1563	0.008
>3 μm Community	0.1953	0.007
0.22–3 μm Community	0.1044	0.097

3.5. Assembly Processes of Eukaryotic Plankton Communities Under Different Microalgal Treatments

The neutral community model effectively described the relationship between ZOTU occurrence frequency and relative abundance across treatments. The model showed moderate fits ($R^2 = 0.321, 0.298$, and 0.338 for Groups C, N, and T, respectively; Figure 5a–c), indicating that stochastic processes contributed to community assembly to varying extents. The estimated Nm value (the product of metacommunity size and migration rate) was higher in Group N (Nm = 1119) than in Groups T (Nm = 229) and C (Nm = 228) (Figure 5a–c), suggesting potential differences in dispersal-related processes among treatments. In addition, the proportion of neutrally distributed taxa varied across treatments (Figure 5d), with Group C showing a higher proportion than Groups N and T, indicating a relatively stronger contribution of stochastic processes in the control condition. These results suggest that microalgal inoculation, particularly with *N. oculata*, was associated with a trend toward an increased relative importance of deterministic assembly processes.

3.6. Size-Dependent Drivers and Short-Term Stability of Eukaryotic Plankton Communities

Variance partitioning analysis (VPA) was performed to quantify the relative contributions of microalgae, nutrients, and rearing time to community variation in each size fraction (Figure 6a,b). A clear size-dependent pattern was observed. In the large-sized (>3 μm) fraction, microalgae made the largest independent contribution (pure adjusted $R^2 \approx 24.8\%$), exceeding that of rearing time ($\approx 7.8\%$). In contrast, in the small-sized (0.22–3 μm) fraction, rearing time was the dominant driver ($\approx 16.5\%$), whereas the independent contribution of microalgae was smaller ($\approx 8.3\%$). In both fractions, the independent contribution

of nutrients was negligible ($\approx 0\%$), with nutrient effects largely shared with rearing time (10.1–10.8%).

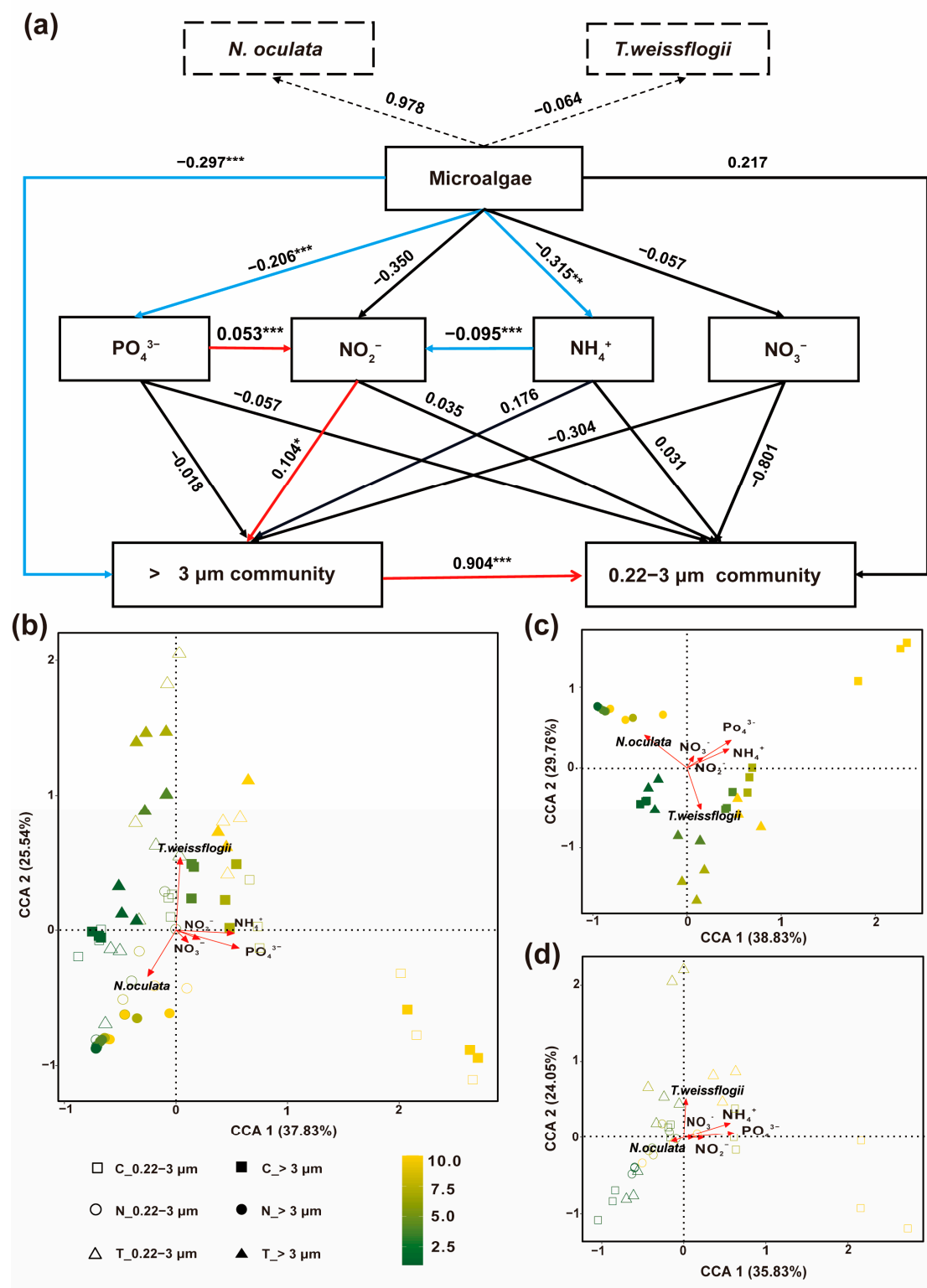


Figure 4. Integrated analysis of the effects of microalgae and nutrient variables on eukaryotic plankton communities. (a) PLS-PM showing direct and indirect relationships among microalgae, nutrient variables, and plankton communities. Each box represents an observed (measured) or latent variable (e.g., microalgae). Dashed lines link the latent variable (Microalgae) to its measured indicators (*N. oculata* and *T. weissflogii*). Red and blue arrows indicate positive and negative relationships, respectively, whereas black lines denote nonsignificant associations. Standardized path coefficients

and corresponding FDR-adjusted p -values are shown on the arrows (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). (b–d) CCA illustrating the relationships between environmental variables and community composition.

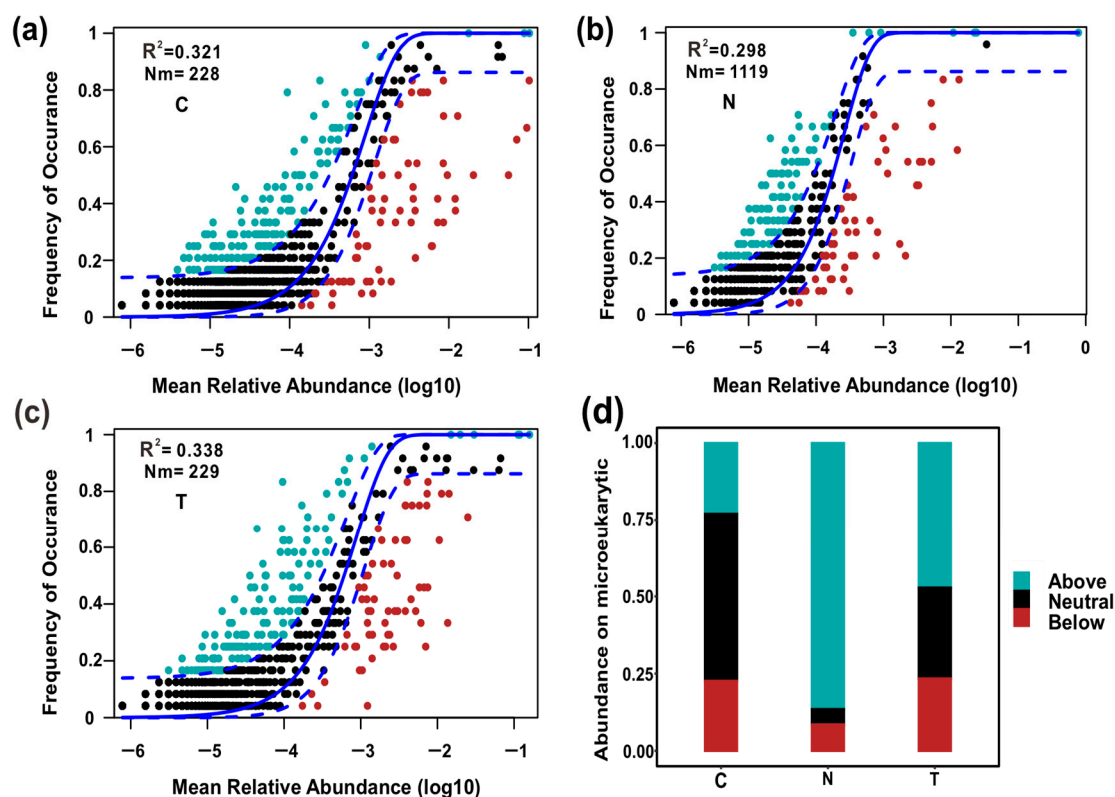


Figure 5. Assembly processes of eukaryotic plankton communities under different microalgal treatments. (a–c) Fit of the neutral community model for Groups C, N, and T, respectively, showing the relationship between the occurrence frequency of ZOTUs and their mean relative abundance; solid blue lines indicate the best fit, and dashed lines represent the 95% confidence intervals. R^2 denotes the goodness of fit, and Nm represents the product of metacommunity size and migration rate. ZOTUs occurring more frequently than predicted (above), as predicted (neutral), and less frequently than predicted (below) are shown in different colors. (d) Relative proportions of above-prediction, neutral, and below-prediction ZOTUs in each treatment.

Community stability was evaluated using two complementary metrics, the average variation degree (AVD) and the coefficient of variation (CV), both of which inversely reflect short-term temporal stability (Figure 6c–f). Group N consistently exhibited the lowest values of both metrics across size fractions. AVD was significantly lower in Group N than in Group C in both the large-sized and small-sized fractions (Tukey HSD, $p < 0.05$; Figure 6c,d). CV showed a consistent pattern, with the difference being significant in the large-sized fraction ($p = 0.019$) but not in the small-sized fraction ($p = 0.66$; Figure 6e,f). The corresponding effect sizes for all pairwise comparisons are summarized in Table 3, which shows moderate-to-large effects for the comparisons involving Group N, particularly in the large-sized fraction. Together, these results indicate that *N. oculata* inoculation was associated with reduced short-term community variability in both size fractions, with the effect being most pronounced in the large-sized fraction.

Table 3. Effect sizes for treatment differences in community stability metrics across size fractions.

Metric	Size Fraction	η^2	d (N vs. C)	d (N vs. T)	d (T vs. C)
AVD	Large ($>3 \mu\text{m}$)	0.26	−1.21	−1.39	−0.63

	Small (0.22–3 μm)	0.22	−1.18	−1.09	−0.30
CV	Large (>3 μm)	0.74	−2.95	−1.48	−2.62
	Small (0.22–3 μm)	0.13	−0.70	−0.46	−0.33

Note: AVD, average variation degree; CV, coefficient of variation; η^2 , eta-squared (overall effect size of treatment); d, Cohen's d. Negative d values indicate higher short-term stability in the first-listed group.

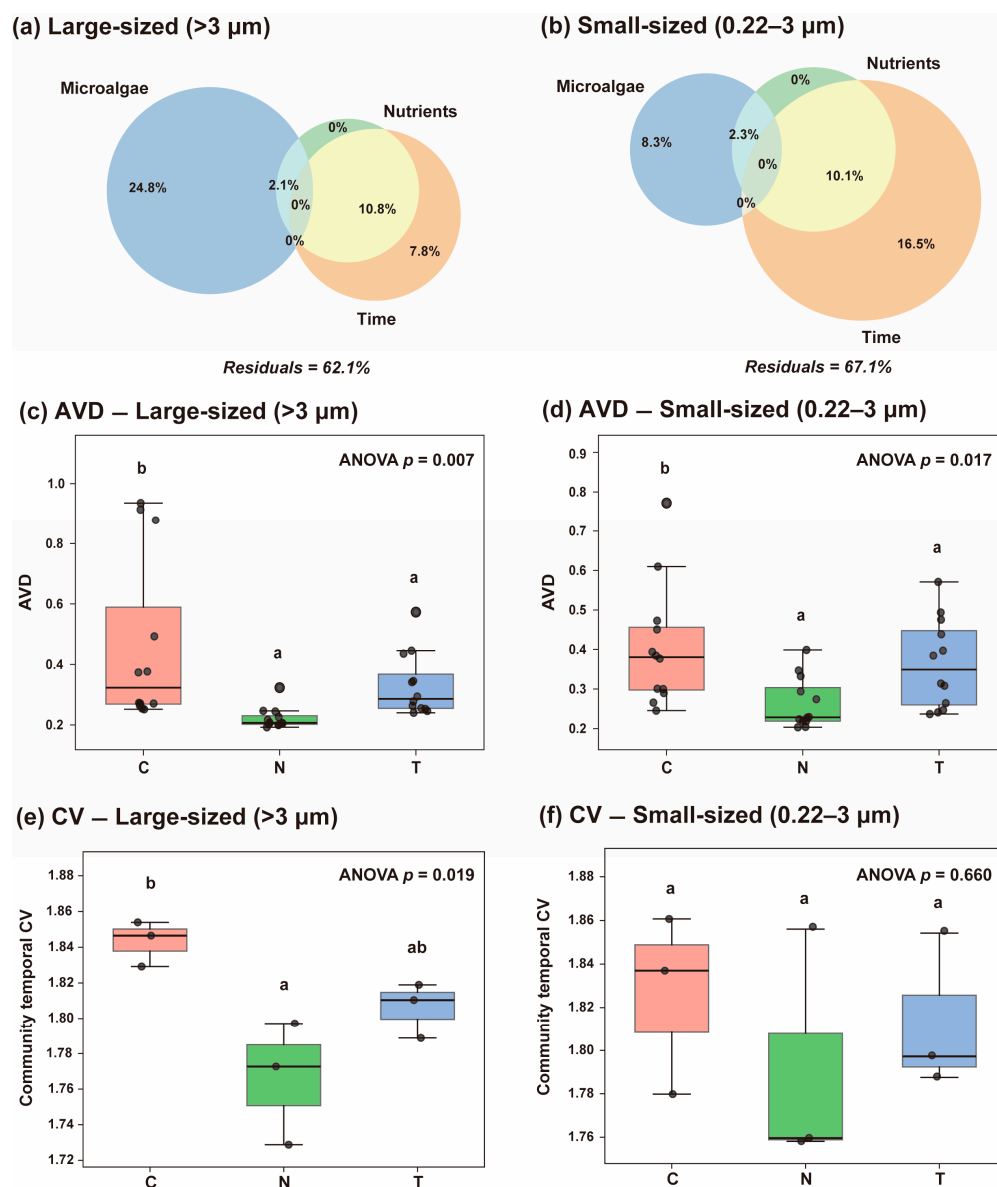


Figure 6. Drivers and short-term stability of eukaryotic plankton communities across size fractions. (a,b) Variance partitioning analysis (VPA) of community variation (adjusted R^2) into the independent and shared contributions of microalgae, nutrients, and rearing time in the (a) large-sized (>3 μm) and (b) small-sized (0.22–3 μm) fractions. (c,d) Average variation degree (AVD) and (e,f) coefficient of variation (CV) of community composition among treatments in the (c,e) large-sized and (d,f) small-sized fractions; lower values indicate lower temporal variability and thus higher short-term stability. Different lowercase letters denote significant differences among treatments (Tukey HSD, $p < 0.05$), and ANOVA p values are shown in each panel.

4. Discussion

4.1. Microalgae-Driven Restructuring of Eukaryotic Plankton Communities

Microalgal metabolism can substantially modify the chemical conditions of aquaculture water, particularly through nutrient assimilation and organic matter transformation [35]. In the present study, microalgal inoculation altered nutrient dynamics in shrimp-rearing water, as reported previously [10]. Elevated nutrient concentrations observed in the later stage were likely associated with the zero water-exchange regime combined with relatively high stocking density, which may have promoted the accumulation and decomposition of organic matter [36,37]. Although shrimp survival did not differ significantly among treatments during the experimental period, elevated nutrient levels may have imposed a certain degree of physiological stress on the cultured shrimp. Because the primary objective of this study was to investigate the assembly processes and stability of eukaryotic plankton communities, additional physiological indicators of shrimp health were not evaluated; nevertheless, beneficial microalgae have been shown to mitigate eutrophication and improve shrimp survival in aquaculture systems [9,10].

Beyond their influence on chemical conditions, microalgae can also modify the biological environment through ecological interactions with surrounding microorganisms [1]. In the present study, inoculation with different microalgal species altered the composition and structure of eukaryotic plankton communities in shrimp-rearing water (Figure 2), suggesting that microalgae may influence plankton communities both directly, through ecological interactions such as resource competition and trophic relationships, and indirectly through modifications of nutrient availability and organic matter dynamics (Figure 4) [38].

Differences in the diversity and composition of eukaryotic plankton communities were observed among treatments and between size fractions. In particular, the small-sized eukaryotic plankton community tended to exhibit higher α -diversity than the large-sized fraction in microalgae-inoculated groups (Figure 1), and the two size fractions showed distinct response patterns under microalgal inoculation (Figure 2). These contrasting responses may reflect size-dependent ecological strategies. Smaller taxa generally exhibit higher metabolic and growth rates due to allometric scaling relationships between cell size and physiological processes, enabling them to respond more rapidly to environmental fluctuations and changes in resource availability [39]. In contrast, larger taxa generally possess slower growth rates and greater resource demands, making them more dependent on stable environmental conditions and more susceptible to fluctuations in nutrient availability and microbial competition [40]. This size-dependent asymmetry is consistent with the companion bacterial study of the same rearing system [10], in which the larger, particle-attached fraction acted as the primary source of community variation that was subsequently propagated to the smaller, free-living fraction. The convergence of this directional pattern across both bacterial and eukaryotic domains indicates that the larger size fraction functions as the active responder to microalgal inoculation, whereas the smaller fraction tracks these changes more passively—providing an ecological basis for the size-dependent partitioning subsequently resolved by the variance partitioning analysis (Section 4.2).

Distinct representative genera were also associated with different microalgal treatments (Figure 3a,b), suggesting species-specific ecological interactions between inoculated algae and plankton communities. Several representative taxa showed significant associations with nutrient variables and microalgal biomass (Figure 3c), consistent with the role of microbial taxa in aquaculture systems in the decomposition of organic matter and in nitrogen assimilation and recycling [41,42]. Notably, *Wobblia*, identified in this study, has been reported to participate in nitrogen-related transformations, which may help ex-

plain its observed correlations with nitrogen variables. Moreover, the enrichment of *Aspidisca* in the *N. oculata* treatment and of *Uronema* in the *T. weissflogii* treatment may reflect species-specific interactions; ciliated protozoa such as *Aspidisca* can regulate bacterial populations and contribute to nutrient recycling [42], while the high nutritional quality of diatoms like *T. weissflogii* may favor associated grazer taxa, contributing to the observed differences in community composition among treatments.

Importantly, these results suggest that microalgal inoculation may influence interactions among different components of the planktonic community. The PLS-PM analysis revealed a strong positive association from the large-sized ($>3\ \mu\text{m}$) to the small-sized ($0.22\text{--}3\ \mu\text{m}$) eukaryotic plankton community, indicating a directional linkage between size fractions (Figure 4a). This pattern suggests that the dynamics of small-sized communities may be closely coupled with changes in larger plankton components, potentially through indirect ecological interactions or shared responses to microalgal-driven resource dynamics rather than direct trophic relationships [1,43]. Size-structured plankton communities are often interconnected through ecological and biogeochemical processes across size classes [44]. By altering the availability and composition of primary producers, microalgae may consequently influence community organization across multiple trophic levels.

Overall, microalgal inoculation appears to restructure eukaryotic plankton communities through a combination of nutrient-mediated effects, microbial interactions, and size-structured linkages. Compared with our previous work focusing on bacterial communities [27], the present study highlights the importance of multi-trophic interactions in shaping community assembly in aquaculture systems.

4.2. Microalgal Inoculation Modulates the Assembly Processes and Short-Term Stability

Community stability is a fundamental property of aquatic ecosystems and is closely linked to temporal variability in community composition and community assembly processes [24,45]. In the present study, short-term stability was assessed using the average variation degree (AVD) and the coefficient of variation (CV), both of which inversely reflect temporal fluctuations and have been applied as proxies for microbial community stability [27,46]. Group N exhibited the lowest AVD and CV values across size fractions, indicating a higher level of short-term stability under *N. oculata* inoculation (Figure 6c–f). This observation is consistent with previous studies reporting that microalgae-associated systems can contribute to stabilizing microbial communities through the regulation of environmental conditions and biotic interactions [47,48].

Notably, Group N displayed relatively lower species diversity than the other treatments (Figure 1), suggesting that higher diversity does not necessarily translate into greater stability in this system. This observation supports the view that biodiversity–stability relationships in microbial communities are context-dependent and may be mediated by species interactions and community assembly processes rather than diversity alone [24,49].

Neutral community model analysis revealed moderate model fits across treatments (Figure 5a–c), indicating that stochastic processes such as ecological drift and dispersal contributed to community assembly to varying extents [31]. The reduced proportion of neutrally distributed taxa in the microalgae-inoculated groups (Figure 5d) suggests an increased relative importance of deterministic assembly processes compared with the control. This shift may be associated with microalgae-induced environmental filtering and selective biotic interactions, as phytoplankton are known to influence the structure of associated microbial communities [50,51]. The markedly higher N_m value in the *N. oculata* treatment further indicates reduced dispersal limitation and stronger connectivity with the metacommunity [52]; because well-mixed communities are less subject to ecological drift, this pattern is consistent with, rather than contrary to, the reduced stochasticity in-

ferred above, and likely reflects microalgae-driven changes in resource availability and habitat conditions [53].

To further disentangle the drivers of community variation, variance partitioning analysis was performed for each size fraction (Figure 6a,b), revealing a clear size-dependent pattern: the large-sized fraction was primarily governed by microalgae (pure adjusted $R^2 \approx 24.8\%$), whereas the small-sized fraction was mainly shaped by rearing time ($\approx 16.5\%$). This divergence is consistent with size-dependent ecological strategies and with the size-plasticity hypothesis, which posits that larger taxa—owing to their slower growth and greater resource demands—respond more directly to microalgae-mediated resource and habitat changes, whereas smaller, fast-turnover taxa are more strongly influenced by temporal succession [54]. The independent contribution of nutrients was negligible in both fractions, with nutrient effects largely shared with rearing time, indicating that nutrients acted as a temporally mediated rather than an independent driver—consistent with the indirect, nutrient-mediated pathways revealed by the PLS-PM analysis (Figure 4a). Although the AVD index indicated greater stability under *N. oculata* inoculation in both fractions, the CV-based stabilizing effect was statistically significant only in the large-sized fraction—the same fraction primarily governed by microalgae—pointing to an internally consistent, size-dependent mechanism whereby microalgae most strongly shape and stabilize the larger plankton community. It should also be noted that a substantial proportion of the community variation remained unexplained (residuals $\approx 62\%$ and 67% in the large- and small-sized fractions, respectively; Figure 6a,b), indicating that additional unmeasured factors—such as organic carbon, biotic interactions (e.g., predation, competition, and viral lysis), and dispersal-related stochasticity—likely also contributed to community assembly. The measured drivers should therefore be interpreted as important but partial, rather than exhaustive, determinants of community variation.

Taken together, these findings suggest that microalgal inoculation, particularly with *N. oculata*, was associated with a greater relative importance of deterministic processes and reduced short-term community variability, most evidently in the large-sized fraction (Figures 5 and 6). Ecologically, communities with greater stability and a stronger deterministic structure are more resistant to the invasion and proliferation of opportunistic, potentially pathogenic taxa [19,21,22]. This inference is reinforced by the companion bacterial study of the same rearing system [10], in which the direct influence of microalgae was likewise concentrated in the $>3\ \mu\text{m}$, particle-attached fraction—mirroring the size-dependent pattern reported here—and microalgal inoculation specifically enriched Rhodobacteraceae/Roseobacter-clade taxa (e.g., *Celeribacter*, *Ponticoccus*, and *Sulfitobacter* under *N. oculata*; *Marivita* under *T. weissflogii*), a group widely regarded as beneficial and antagonistic toward opportunistic pathogens such as *Vibrio* in aquaculture [55]. The concordant steering of both eukaryotic and bacterial communities toward more stable, symbiont-enriched configurations indicates that *N. oculata* inoculation actively reshapes the rearing microbiome into a state less permissive to opportunistic taxa. Although neither study directly quantified pathogenic taxa, this convergent, cross-domain evidence elevates the proposed disease-prevention role of microalgae from a general expectation to a mechanistically grounded inference. Thus, beyond its established role in improving water quality, microalgal inoculation—particularly with *N. oculata*—may represent a potential ecological strategy for stabilizing plankton community structure and lowering disease risk in aquaculture systems [55]. We note, however, that these patterns were observed over a short-term (10-day) experiment, and the differences in neutral-model fits among treatments were modest ($R^2 = 0.321, 0.298$, and 0.338 for Groups C, N, and T, respectively); the observed shifts should therefore be interpreted as trends rather than definitive changes. In addition, organic carbon was not measured in this study; given the zero-water-exchange regime, future work incorporating organic carbon dynamics, together with

longer-term observations, would help determine whether these stabilizing effects persist and translate into sustained reductions in disease risk. Moreover, the AVD and CV indices used here capture short-term compositional variability and do not necessarily reflect long-term ecological stability or ecosystem resilience, which additionally encompass resistance to and recovery from disturbance; the stability reported here should therefore be understood specifically as short-term temporal stability rather than stability in the broader ecological sense.

5. Conclusions

This study demonstrates that microalgal inoculation modulates the assembly and short-term stability of eukaryotic plankton communities in shrimp-rearing water in a size-dependent manner. Integrating neutral community modeling, variance partitioning, and complementary stability metrics (AVD and CV), we found that microalgal inoculation—particularly with *N. oculata*—increased the relative importance of deterministic assembly and reduced short-term variability, predominantly in the large-sized fraction, which was primarily governed by microalgae, whereas the small-sized fraction was mainly shaped by rearing time and nutrients acted only as a temporally mediated driver. Together with the concordant patterns in the companion bacterial study of the same system, these results indicate that *N. oculata* inoculation reshapes the rearing microbiome toward a more stable, symbiont-enriched configuration that is inherently more resistant to opportunistic taxa. Microalgal inoculation therefore represents a promising ecological strategy that may extend beyond water-quality improvement toward lowering disease risk in aquaculture, an inference that remains to be confirmed by longer-term studies with direct pathogen monitoring. From an ecological perspective, these findings clarify how microalgal inoculation shapes the size-dependent assembly and short-term stability of eukaryotic plankton communities. From an applied perspective, however, whether this enhanced stability translates into measurable improvements in shrimp health, productivity, or disease resistance was not evaluated here and remains to be established; the practical benefits for aquaculture should therefore be regarded as a promising hypothesis requiring direct validation rather than a demonstrated outcome.

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