BIODIVERSITAS
Volume 26, Number 8, August 2025
Pages: 3843-3854

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260816

Home-made and commercial eco-enzymes alter plankton communities and water chemistry in Rawa Pening Lake, Central Java, Indonesia

SUSANTI PUDJI HASTUTI, SUCAHYO, DESTI CHRISTIAN CAHYANINGRUM, RULLY ADI NUGROHO♥
Faculty of Biology, Universitas Kristen Satya Wacana. Jl. Diponegoro 52-60, Salatiga 50711, Central Java, Indonesia. Tel./fax.: +62-298-321212,
♥email: rully.nugroho@uksw.edu

Manuscript received: 4 December 2024. Revision accepted: 11 August 2025.

Abstract. *Hastuti SP, Sucahyo, Cahyaningrum DC, Nugroho RA. 2025. Home-made and commercial eco-enzymes alter plankton communities and water chemistry in Rawa Pening Lake, Central Java, Indonesia. Biodiversitas 26: 3843-3854.* Eco-enzymes are increasingly used to improve water quality by reducing organic pollutants and balancing nutrient levels in rivers and other aquatic systems. However, their typically low pH and high concentrations of organic matter may increase Biological Oxygen Demand (BOD) and acidify the water, potentially altering freshwater plankton species composition and increasing the risk of eutrophication in aquatic ecosystems. This study aimed to assess the effects of commercial and home-made eco-enzyme on freshwater plankton community structure and water quality parameters using a controlled aquaria design with four concentrations (2.5-20% v/v). Controls without eco-enzymes were included for comparison. Key parameters including nutrient levels, BOD, Total Dissolved Solids (TDS), pH, plankton diversity and abundance were monitored throughout the study. Plankton diversity across different eco-enzyme types and concentrations was compared by generating diversity profiles using Renyi's entropy values. Results showed that both types of eco-enzymes significantly altered water chemistry. BOD declined rapidly at 5% commercial and 2.5% (v/v) home-made eco-enzyme, while pH increased from 7.4 to 8.6 over time. Ammonia, nitrite, nitrate, and TDS levels rose with both eco-enzyme concentration and duration. At higher concentrations, commercial eco-enzymes promoted the dominance of Bacillariophyta and Cyanophyta, while home-made eco-enzymes favored Bacillariophyta and Chlorophyta. Although eco-enzymes stimulated microbial activity and organic matter decomposition, they also supported the growth of pollution-tolerant plankton species, potentially reducing overall biodiversity. Renyi entropy profiles revealed decreased richness and evenness, especially at higher concentrations. These results highlight that while eco-enzymes can contribute to water quality improvement, their ecological impacts, particularly on plankton communities, must be carefully considered. Further research is necessary to ensure their sustainable and responsible use in aquatic ecosystems.

Keywords: Abundance, freshwater, organic waste, plankton, Renyi diversity profile

INTRODUCTION

Eco-enzymes, also called garbage enzymes or bio-enzymes, are brown fermentation liquids derived from organic waste and molasses (Rungta et al. 2022; Benny et al. 2023). Traditionally produced from fruit and vegetable waste, they function similarly to regular enzymes by accelerating decomposition under favorable environmental conditions (Arun and Sivashanmugam 2017). Vegetable scraps, fruit peels, and viable fruit pulp are commonly used as raw materials (Pasalari et al. 2024). Due to the abundance of these wastes, eco-enzymes have become popular in daily activities and are regarded as multifunctional fluids. They serve as cleaning and sanitation agents, disinfectants, fertilizers, insecticides, and even water purifiers (Gu et al. 2021; Benny et al. 2023; Ihtiar et al. 2023; Vidalia et al. 2023). Their alcohol and acetic acid content contribute to many applications (Galintin et al. 2021), although research on their efficacy in various fields remains limited.

Eco-enzymes have gained attention as low-cost alternatives in environmental management, including wastewater treatment, landfill leachate mitigation, soil remediation, and composting (Pasalari et al. 2024). When combined with biochar, they help reduce nitrogen losses during composting (Jiang et al. 2024), and they regulate bacterial succession during sewage sludge processing (Jing et al. 2021). However, their composition indicates a substantial organic load, with high BOD and COD values (Galintin et al. 2021). In some cases, eco-enzymes neutralize pH and reduce ammonia, detergents, and coliform concentrations, though they can also elevate nitrate, phosphate, BOD, and COD levels in water (Tuhumury et al. 2024). Other studies found limited effects on COD removal (Yustiani et al. 2023). Field applications during cultural festivals demonstrated improved water pH and reduced solids, hardness, and nutrient concentrations (Kumar et al. 2019).

Beyond physicochemical impacts, eco-enzymes may influence aquatic microbial and planktonic communities (Glibert and Mitra 2022; Reinl et al. 2022; Costas-Selas et al. 2024). They contain organic acids, enzymes, and microbial consortia such as bacteria, yeasts, and fungi (Gu et al. 2021; Pasalari et al. 2024), which may restructure microbial succession, alter nutrient cycling, and shift food web interactions. Similar effects have been observed in other biotreatment interventions like nutrient additions or microbial inoculants (Borics et al. 2021; Groß et al. 2022). However, limited studies have addressed their ecological risks, especially concerning plankton biodiversity. Excess nutrient release from eco-enzymes could stimulate harmful algal blooms or eutrophication (Dondajewska et al. 2019).

Plankton play vital roles in energy transfer, nutrient cycling, and ecosystem functioning (Naselli-Flores and Padisák 2022; Gao et al. 2024). Changes in their abundance or diversity affect food web stability, productivity, and ecological resilience (Otero et al. 2020; Ramlee et al. 2022). Plankton diversity often signals long-term ecosystem health, while shifts in abundance indicate short-term disturbances such as nutrient enrichment or pollution (Tambaru et al. 2024). By regulating oxygen levels, nutrients, and water clarity, plankton are crucial for water quality maintenance (Enawgaw and Wagaw 2023; Zhang et al. 2024). Disturbances that alter plankton composition may thus degrade water quality and threaten aquatic biota (Hudson et al. 1999; Wang and Zhang 2020). Eco-enzymes, through enzymatic hydrolysis, can release nitrogen and phosphorus that stimulate plankton growth (Luo et al. 2017; Reinl et al. 2022), potentially altering abundance and community structure.

Given their dual role as biotreatment agents and possible ecological stressors, it is necessary to assess eco-enzymes holistically, not only through physicochemical parameters but also through biological indicators. Plankton communities offer an integrative measure of ecological integrity and water quality. Biological monitoring is often more reliable for long-term assessment compared to highly dynamic chemical indicators (Garg et al. 2022).

Therefore, this study aimed to evaluate the effects of commercial and home-made eco-enzymes on freshwater plankton abundance and diversity, and to relate these changes with water quality variations. By employing plankton as bioindicators, this research seeks to provide insights into the ecological implications of eco-enzyme application and its potential role in biodiversity-friendly water purification strategies

MATERIALS AND METHODS

Water samples and eco-enzymes

Water samples containing plankton communities were collected from Rawa Pening Lake in the limnetic zone. The eco-enzymes used were commercial eco-enzyme and home-made eco-enzyme. The commercial eco-enzyme (BIOS eco-enzyme) was bought from a local market. The composition of the home-made eco-enzymes was molasses, organic waste (pineapple peel, banana peel, orange peel, potato peel, and mustard greens), and water in a ratio of 1:3:10 (v/w) as described by Arun and Sivashanmugam (2017). Organic waste was composed of equal proportions. The mixture was then placed in an airtight plastic container and fermented for 3 months at room temperatures between 25-28°C. The properties of commercial eco-enzyme included pH: 2.66, TDS: 1723 mg/L, ash content: 0.26%, lipid content: 0.28%, protein content: 3.17%, while the home-made eco-enzyme included pH: 2.59, TDS: 2257 mg/L, ash content: 1.04%, lipid content: 0.07%, protein content: 4.97%. Both eco-enzymes produced a dark yellow color and fermented aroma.

Plankton and water quality analysis

This study followed a factorial Completely Randomized Design (CRD) experimental design, involving two factors in this research, namely the type of eco-enzymes (with two levels: commercial eco-enzyme (C) and home-made eco-enzyme (H)) and the concentration of eco-enzymes (with five levels: 0, 2.5, 5, 10, and 20% v/v). The eco-enzyme concentrations were prepared by mixing different volumes of stock solutions with water from Rawa Pening Lake to achieve the desired concentration. The experiment had three replicates per treatment group. The experiment was carried out in a 4 L aquarium containing 2 L of water from Rawa Pening Lake for 1 month without aeration, nutritional input, or water changes. Observations were conducted weekly, at week 1, 2 and 3 of the experiment (T1, T2, T3, respectively). Parameters observed included water quality indicators, namely pH, temperature, nitrate, ammonia, Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD), and phosphate concentrations, and plankton cells (genera and number of individuals in each genus). Water parameters were measured according to American Public Health Association (APHA) Standard Methods for the Examination of Water and Wastewater (APHA 2017). Plankton were sampled using nets with a mesh size of 90 mm. For plankton cells identification and quantification, 30 mL of a 5% (w/v) Lugol’s solution was added into 10 mL of a water sample at a time. Plankton cells were identified to the genus level using a microscope (Olympus CX23) according to the relevant references (Bellinger and Sigee 2010; Streble and Krauter 2010).

Data analysis

Prior to conducting the Analysis of Variance (ANOVA), the assumptions of normality and homogeneity of variances were tested using the Shapiro-Wilk test and Levene’s test, respectively. A two-way ANOVA at the 0.05 significance level was used to examine the effects of two factors on environmental variables followed by Tukey test for comparing multiple treatments if needed. To compare plankton diversity results among eco-enzyme types and concentrations, we produced diversity profiles using Renyi's entropy values, which allows us to evaluate diversity between datasets using several diversity indices simultaneously (Chao and Jost 2015; Roswell et al. 2021). Renyi profile values at the corresponding scales of 0, 1, 2, and ∞ are associated with species richness S, the Shannon diversity index H, the Simpson concentration index D^{-1}, and the Berger-Parker dominance index d^{-1} (Roswell et al. 2021; Wani et al. 2022). All statistical analysis and graphical presentation of data were done using PAST statistical software.

RESULTS AND DISCUSSION

The physicochemical characteristics of each treated water containing freshwater plankton communities are shown in Figure 1. During the study, the average ammonia, nitrite and nitrate concentrations increased throughout increasing experimental period and concentration of both eco-enzymes, but there was no significant difference in phosphate concentration between both treatments. This figure also showed that BOD concentration decreased rapidly by commercial eco-enzyme concentration of 5% (v/v) and home-made eco-enzyme of 2.5% (v/v). No significant difference was found between eco-enzyme concentrations in water pH, but water pH increased from 7.4 after one week to 8.6 by week three of the experiment ($p<0.05$). There was no significant difference between eco-enzyme concentrations and experimental period in water temperature (Figure 1).

The data presented in Figure 1 provide an overview of the physicochemical changes in treated water containing freshwater plankton communities throughout the experiment. These changes, which include variations in nutrient concentrations, Biological Oxygen Demand (BOD), pH, temperature, and Total Dissolved Solids (TDS), offer insights into the environmental impacts of eco-enzyme treatments on aquatic ecosystems. The concentrations of ammonia, nitrite, and nitrate increased with both the experimental period and the concentration of eco-enzyme treatments. These findings suggest that eco-enzyme treatments may have stimulated microbial activity, leading to elevated levels of nitrogen compounds in the water. Ammonia and nitrite are typically intermediates in the nitrogen cycle, while nitrate is the final product of nitrification (Ni et al. 2023). The rise in these nutrients may reflect enhanced microbial activity driven by eco-enzymes, potentially due to their role in breaking down organic matter, which releases nitrogen compounds into the water. While these nutrients can promote plankton growth, but excessive concentrations can also contribute to eutrophication, leading to harmful algal blooms (Akinnawo 2023).

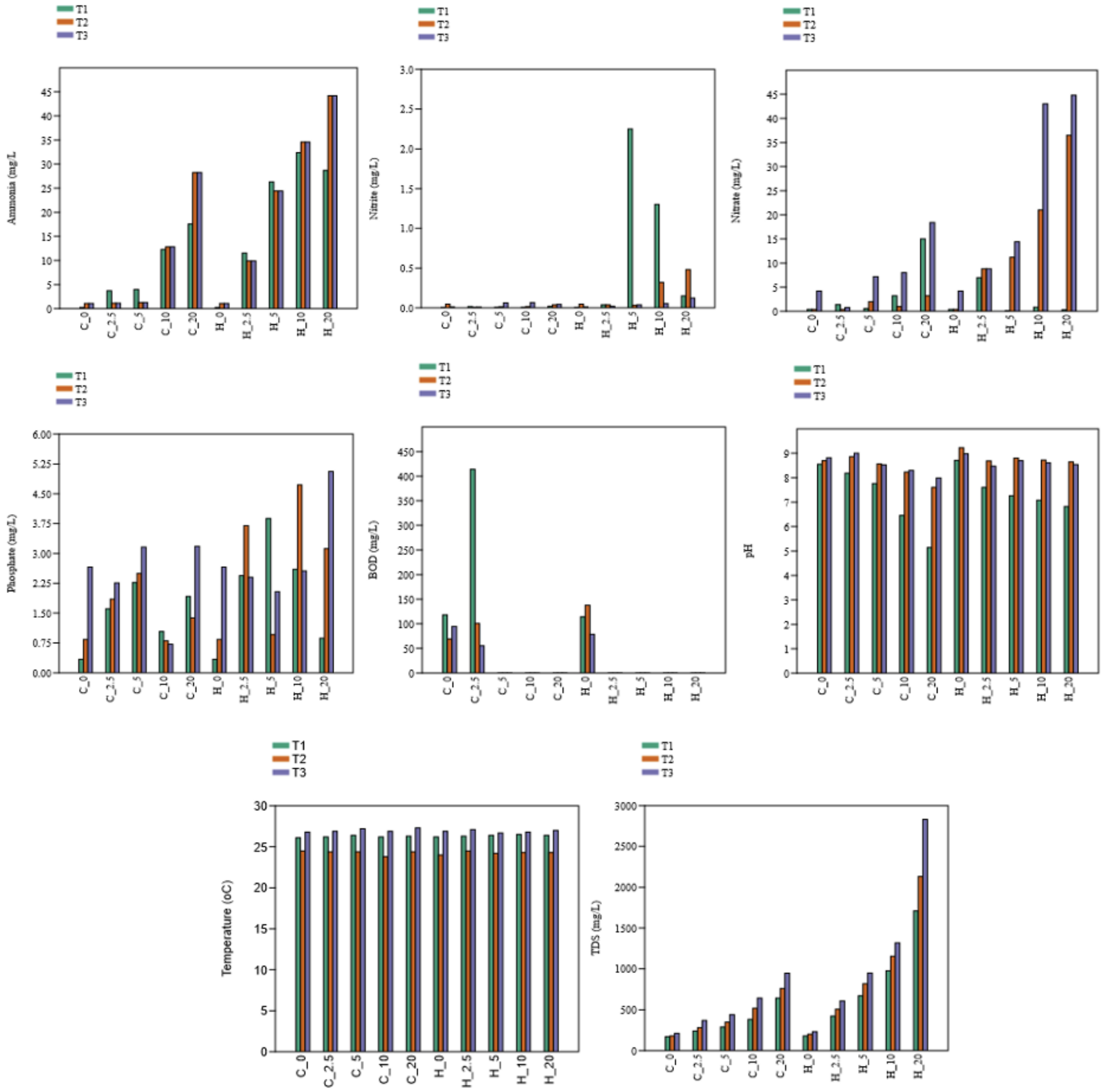

Figure 1. Variation in water quality parameters (ammonia, nitrite, nitrate, phosphate, BOD, pH, temperature, and TDS). T1, T2, and T3 represent observations taken after 1, 2, and 3 weeks of experiment, respectively. C_0, C_2.5, C_5, C_10, and C_20 represent commercial eco-enzyme at concentrations of 0 to 20% (v/v), respectively. H_0, H_2.5, H_5, H_10, and H_20 represent home-made eco-enzyme at concentrations of 0 to 20% (v/v), respectively

Interestingly, no significant difference was observed in phosphate concentrations throughout the experimental period or across eco-enzyme concentrations. This may indicate that the eco-enzyme treatments had little effect on phosphate dynamics in the water, possibly due to the limited phosphate availability in the system or the eco-enzymes did not influence phosphate mobilization and uptake by plankton and other organisms (Akinnawo 2023). As phosphate is typically a limiting nutrient for primary production, its stability suggests that other factors, such as nitrogen availability, may have played a more dominant role in driving plankton dynamics in this study.

The rapid decrease in BOD with increasing concentrations of both commercial and home-made eco-enzymes suggests that these treatments effectively enhanced the breakdown of organic matter in the water. BOD is a measure of the amount of oxygen required by microorganisms to decompose organic substances in water. A decrease in BOD indicates that the eco-enzyme treatments accelerated this decomposition process, likely due to the action of the enzymes in breaking down organic pollutants. This finding aligns with studies that have shown eco-enzyme treatments can help improve water quality by reducing organic load (Das et al. 2024).

The observed reduction in BOD at a concentration of 5% (v/v) for the commercial eco-enzyme and 2.5% (v/v) for the home-made eco-enzyme suggests that the home-made variant may have been more effective at lower concentrations. This could be attributed to differences in the formulation, enzymatic activity, or microbial communities in the home-made eco-enzyme compared to the commercial product. However, while a reduction in BOD indicates improved water quality in terms of organic matter breakdown, it is critical to monitor other parameters, such as nutrient concentrations, to ensure that the overall health of the ecosystem is not compromised.

Moreover, the gradual increase in pH from 7.4 at the start of the experiment to 8.6 after three weeks, regardless of the eco-enzyme concentration, suggests a slight alkalinization of the water over time. The increase in pH may be associated with the microbial activity induced by eco-enzyme treatments, as the breakdown of organic matter can release alkaline byproducts, leading to an increase in water pH (Pinheiro et al. 2021). Alkaline pH levels, particularly above 8, can influence plankton communities, favoring species that tolerate higher pH levels, while stressing others (Wear et al. 2021). The observed increase in pH may reflect a shift in the plankton community toward that are better adapted to more alkaline conditions. Despite this shift, there was no significant difference in pH across different eco-enzyme concentrations, suggesting that the pH change may not have been directly influenced by the concentration of the eco-enzyme treatments. Rather, it could be a general effect of organic matter breakdown and microbial metabolism, which is common in many freshwater systems undergoing eutrophication or nutrient loading (Neijnens et al. 2023).

No significant difference was observed in water temperature across the experimental period or between eco-enzyme concentrations. This suggests that the eco-enzyme treatments did not significantly affect the thermal properties of the water, and any temperature changes likely resulted from natural fluctuation effects. Although temperature is an important factor influencing microbial and planktonic activity, it remained stable in this study.

Conversely, TDS values increased with both the concentration of eco-enzymes and the experimental period. TDS is a measure of the total concentration of dissolved ions in water, including salts, minerals, and other inorganic substances. The increase in TDS could be a result of the addition of eco-enzymes themselves, which may contain dissolved ions, as well as the breakdown of organic matter in the water, which releases dissolved organic compounds and nutrients (Arun and Sivashanmugam 2015; Leong et al. 2023). High TDS values can affect water quality, particularly in terms of salinity and ionic composition, which in turn may impact plankton and other aquatic organisms (Costa et al. 2024).

Figure 2 shows the abundance of the freshwater plankton community during the experiment. A total of 32 genera were identified in treated samples (Figure 3). These genera were distributed in 4 phyla and 21 families. The data indicate that both eco-enzymes caused significant changes in the pattern of freshwater plankton abundance. However, there was no significant difference in the pattern of freshwater plankton abundance during the experiment. Prior to treatment, the plankton community was dominated by the members of Heterokontophyta (*Aulacoseira*, *Synedra*, *Fragilaria*, *Nitzchia*), followed by Desmidiaceae (*Micrasterias*), Sphaerocystidaceae (*Sphaerocystis*), Fragilariaceae (*Fragilaria*), and Selenastraceae (*Monoraphidium*) (Figure 4), indicating a moderately eutrophic freshwater ecosystem with stable light and mixing conditions (Zhikharev et al. 2023). The plankton community exhibited distinct compositional shifts depending on eco-enzyme type, concentration, and incubation period (Figure 4). In commercial eco-enzyme treatments, *Cylindrospermopsis* dominated at concentration of 10% (v/v) after 1 week, while *Gloeocapsa* became dominant at concentration of 2.5% (v/v) after 2 weeks. After 3 weeks, the concentration of 2.5% (v/v) was dominated by *Planktothrix*, *Scenedesmus*, and *Sphaerocystis*. In the home-made eco-enzyme treatments, the concentration of 2.5% (v/v) was dominated by *Chlorella* and *Eudorina* after 2 weeks, shifting to *Chlorella* alone after 3 weeks. At concentration of 5% (v/v), *Planktothrix* dominated after 3 weeks, whereas at concentration of 20% (v/v), dominance returned to *Chlorella* and *Eudorina*. In contrast, all other treatments across both eco-enzyme types and time points were consistently dominated by *Aulacoseira* and *Synedra*, indicating their broader tolerance or resilience under varying treatment conditions. However, *Synedra* was also no longer dominant at lower concentrations of both eco-enzymes after 3 weeks, indicating a marked decline in its relative abundance over time.

Figure 2. The pattern of freshwater plankton abundance. C and H represent commercial and home-made eco-enzyme treatments, respectively. T1, T2, and T3 represent observations taken after 1, 2 and 3 weeks of experiment, respectively. 0, 2.5, 5, 10, and 20 represent eco-enzymes concentrations of 0 to 20% (v/v), respectively

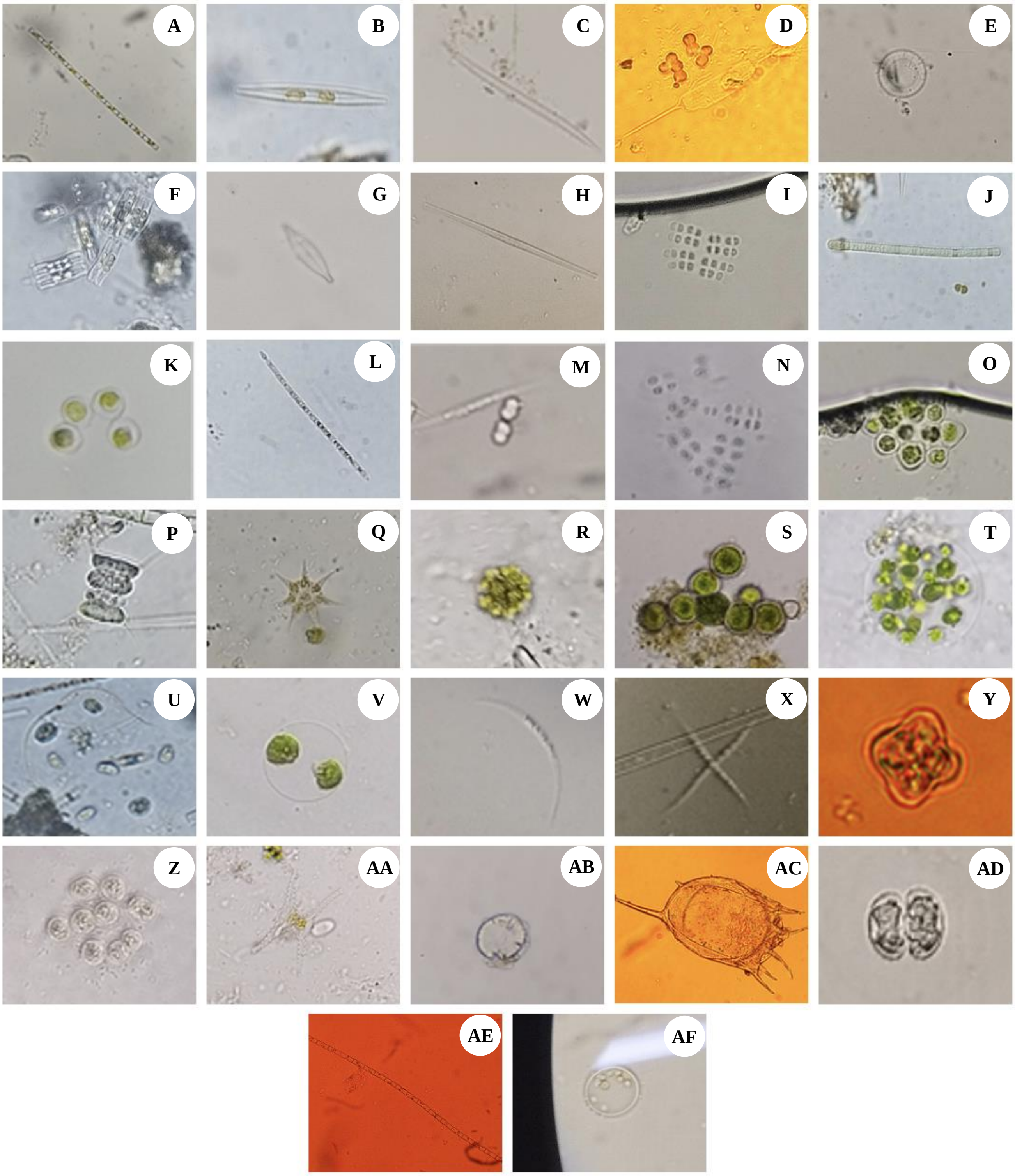

Figure 3. The plankton genera detected in treated samples (without scale). A. *Aulacoseira*, B. *Synedra*, C. *Nitzchia*, D. *Urosolenia*, E. *Cyclotella*, F. *Achnanthidium*, G. *Navicula*, H. *Fragilaria*, I. *Merismopedia*, J. *Planktothrix*, K. *Gleocapsa*, L. *Cylindrospermopsis*, M. *Eucapsis*, N. *Chroococcus*, O. *Coelastrum*, P. *Scenedesmus*, Q. *Monactinus*, R. *Sphaerocystis*, S. *Chlorella*, T. *Eudorina*, U. *Oocystis*, V. *Asterococcus*, W. *Monoraphidium*, X. *Ankristodesmus*, Y. *Tetraëdron*, Z. *Gonium*, AA. *Straurastrum*, AB. *Micrasterias*, AC. *Keratella*, AD. *Cosmarium*, AE. *Leptocylindrus*, AF. *Volvox*

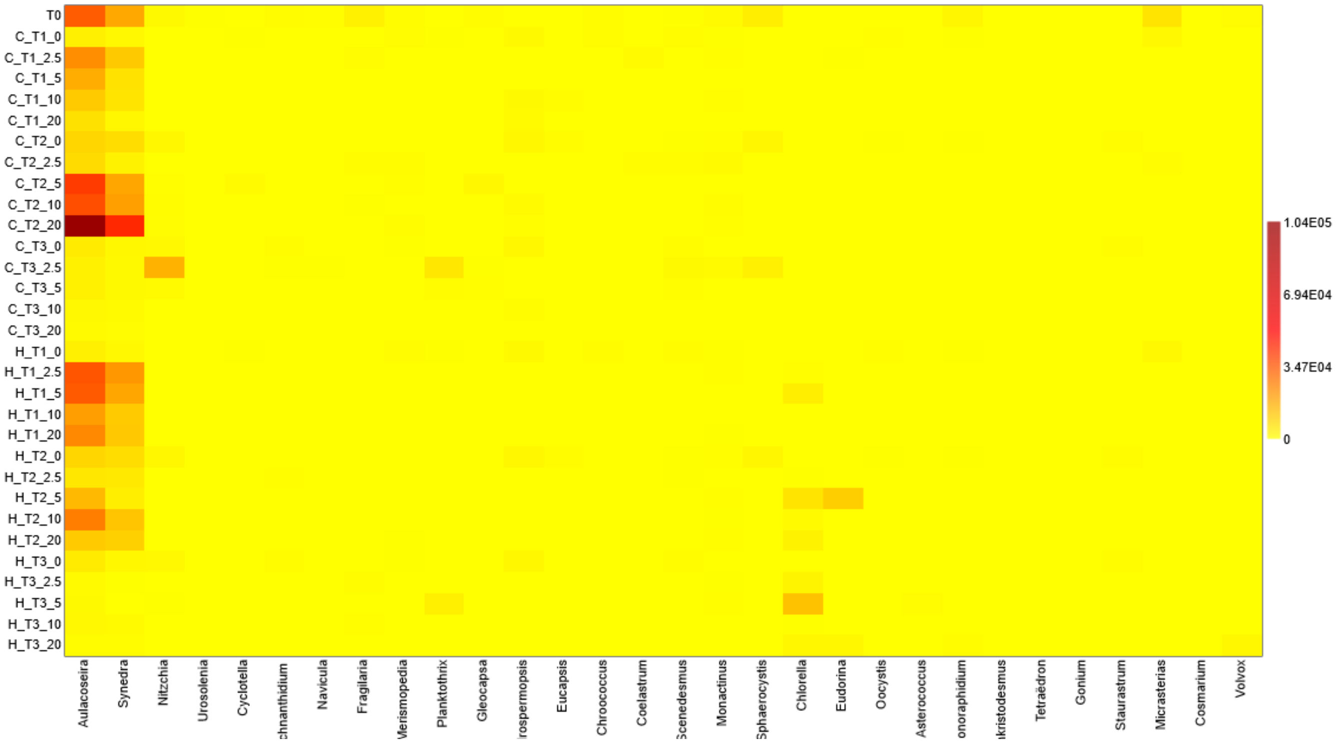

Figure 4. Heat map with dominant plankton abundance. T0 represents observation before the treatment. C and H represent commercial and home-made eco-enzyme treatments, respectively. T1, T2, and T3 represent observations taken after 1, 2 and 3 weeks of experiment, respectively. 0, 2.5, 5, 10, and 20 represent eco-enzymes concentrations of 0 to 20% (v/v), respectively

The application of both commercial and home-made eco-enzymes resulted in notable shifts in freshwater plankton community composition, with effects depending on concentration and exposure time. At concentration of 2.5-5% (v/v), bloom-forming cyanobacteria such as *Cylindrospermopsis*, *Gloeocapsa*, and *Planktothrix* became dominant, suggesting bloom stimulation likely due to increased nutrient availability or altered microbial interactions. In contrast, concentration of 20% (v/v) appeared to suppress cyanobacteria and instead favored green algae such as *Chlorella* and *Eudorina*, indicating potential bloom control under elevated eco-enzyme concentrations. These results align with previous findings that eco-remediation treatments can shift communities away from harmful bloom-formers toward less problematic taxa (Harris et al. 2024; Zhu et al. 2024).

Diatoms such as *Aulacoseira* and *Synedra* dominated prior to treatment and in several low-concentration treatments, reflecting mesotrophic conditions and stable environmental parameters (Bitting et al. 2023; Luong et al. 2024). However, the decline of *Synedra* after prolonged exposure, even at low doses, suggests sensitivity to eco-enzyme-induced changes. Overall, the observed community shifts demonstrate that eco-enzyme treatments can either stimulate or suppress blooms depending on their application regime, with higher concentrations showing greater potential to support improved water quality through cyanobacterial suppression and promotion of green algae and diatom assemblages.

The overall changes in plankton abundance due to eco-enzyme treatments highlight the influence of these additives on aquatic ecosystems. The fact that both eco-enzyme treatments led to significant changes in the plankton community indicates that eco-enzymes can alter the structure of microbial communities, with potential ecological implications. Changes in plankton community composition are known to affect ecosystem processes, such as nutrient cycling and primary production, due to the central role of plankton in freshwater food webs (Clementson et al. 2022).

However, the absence of significant differences in the overall abundance of plankton during the experiment suggests that the eco-enzyme treatments may not have led to a drastic reduction in the plankton population or that these communities were able to adapt to the treatment conditions over time. A similar lack of significant differences in plankton abundance after eco-enzyme treatment was observed in other studies, which highlighted shifts in community structure without a clear impact on overall abundance (Trommer et al. 2020).

Furthermore, at the highest concentration of the commercial eco-enzyme, the plankton community was dominated by species from the phylum Heterokontophyta, including *Aulacoseira*, *Synedra*, and *Nitzschia*, as well as from Cyanophyta, particularly *Cylindrospermopsis*. These genera are known for their resilience to environmental changes, especially under conditions of organic pollution (Pratiwi et al. 2024). The dominance of *Cylindrospermopsis*, a known cyanobacterium, could indicate a stress-induced shift in the community, as cyanobacteria typically thrive in nutrient-rich environments characterized by organic pollution (Paerl and Otten 2013). This suggests that the commercial eco-enzyme may have altered the nutrient dynamics in the water, promoting the growth of more pollution-tolerant species.

In contrast, at the highest concentration of the home-made eco-enzyme, the plankton communities were dominated by Heterokontophyta species (*Aulacoseira*, *Synedra*) and members of Chlorophyta such as *Chlorella*, *Eudorina*, and *Monoraphidium*. Chlorophytes are commonly found in nutrient-rich environments, and their increased abundance suggests that the home-made eco-enzyme might have provided a more favorable environment for these genera. Like Cyanophyta, Chlorophytes are generally tolerant of organic pollution, although they often dominate in less eutrophic conditions (Akinnawo 2023).

The dominance of genera known for their tolerance to organic pollution, such as *Aulacoseira*, *Synedra*, *Cylindrospermopsis*, *Chlorella*, *Eudorina*, and *Monoraphidium*, raises the question about the interaction between eco-enzyme treatments and plankton species to shape their community structure. Many of these taxa are well-adapted to nutrient rich, organically loaded environments, often caused by pollution or anthropogenic activities. This suggests that the eco-enzyme treatments, whether commercial or home-made, might have influenced the nutrient dynamics in the water, creating an environment that favor the growth of these pollution-tolerant species.

It is important to note that while some genera like *Cylindrospermopsis* are considered indicators of eutrophication and organic pollution (Paerl and Otten 2013), others like *Chlorella* and *Monoraphidium* are frequently used in bioassays to evaluate the impact of organic pollutants in freshwater systems (Pratiwi et al. 2024). This suggests that the eco-enzyme treatments could be indirectly fostering conditions that promote the proliferation of these genera, which may affect the ecological balance and long-term sustainability of the freshwater ecosystem.

The dominance of these pollution-tolerant genera could signal a shift in the overall health of the freshwater ecosystem. If eco-enzyme treatments consistently favor the growth of genera that are indicative of organic pollution, this could be a cause for concern, especially in systems already stressed by nutrient loading or pollution. The presence of such genera may lead to alterations in the food web, affecting higher trophic levels and potentially reducing overall ecosystem resilience (Zhu et al. 2020). On the other hand, if these treatments are designed to remediate organic pollution and promote the growth of species that are better suited to polluted environments, they could offer a novel approach to mitigating eutrophication and other anthropogenic stressors in freshwater systems.

Early eutrophication signals which are commonly observed in conditions of elevated trophic state are consistent with the observed shift towards pollution-tolerant taxa, such as *Cylindrospermopsis* (Cyanophyta) and *Chlorella* (Chlorophyta) at high eco-enzyme concentrations. According to studies, Cyanobacteria and eukaryotic algae's dominance causes plankton communities' compositional dissimilarity to decline with higher Trophic State Index (TSI) values (Yang et al. 2012; Li et al. 2022). This in turn compromises trophic cascades and ecosystem stability. Furthermore, spatial surveys in hydrologically variable systems show that changes in algal diversity are used by total TSI which integrates chlorophyll-a, total nitrogen, total phosphorus, and chemical oxygen demand to track transitions from mesotrophic to highly eutrophic states (Meng et al. 2020). As a result, the predominance of pollution-tolerant genera in our study might indicate early eutrophication due to an increase in local TSI. Monitoring these community changes in addition to traditional TSI metrics may improve early eutrophic condition detection and allow for more proactive aquatic health management.

Figure 5 shows the diversity of profiles of freshwater plankton genera using Renyi's entropy values for each treatment combination. The figure reveals that genera richness (alpha: 0) which measures the total number of genera present (Roswell et al. 2021; Wani et al. 2022) decreased significantly with increasing both commercial and home-made eco-enzymes concentrations. This decrease implies that greater concentrations of eco-enzymes might selectively inhibit particular genera or alter habitat which would lower the complexity of the community as a whole (Koffel et al. 2022).

The evenness and diversity of the community are reflected in the Shannon diversity index (alpha: 1) which takes into consideration both number of genera and their relative abundances. Higher values show a more balanced community, while lower values imply a small number of genera are dominant (Roswell et al. 2021; Wani et al. 2022). This study found that Shannon diversity was highest in the control samples and decreased as eco-enzyme concentrations increased. This suggests that eco-enzyme treatments resulted in a less equitable distribution of genera, possibly favoring taxa that can withstand pollution (Dilys 2019). Eco-enzyme concentrations also led to a decrease in the Simpson concentration index (alpha: 2) which highlights the dominance of the most abundant genera (Roswell et al. 2021; Wani et al. 2022). This decrease suggests that the dominance of certain taxa became more noticeable as concentrations increased, further diminishing the overall evenness of the community. These trends point to an ecological shift that favors a subset of taxa that can survive in the changed environment (Varshini and Gayathri 2023).

The most dominant genus in the community is directly measured by the Berger-Parker dominance index (alpha: ∞). High values suggest that one genus predominates in the community, frequently at the expense of diversity (Roswell et al. 2021; Wani et al. 2022). The results of this study showed that the commercial eco-enzyme treatments were most dominant in the control samples, suggesting that the natural community composition was healthy. On the other hand, home-made eco-enzyme treatments initially displayed low dominance, which increased after a week, indicating transient changes in favor of genera more adapted to the altered environment. This trend demonstrates the capacity of some genera to adapt to stress and the possibility that eco-enzymes may cause imbalances in community (Hou et al. 2023).

Every diversity index offers a different perspective on the stability and structure of communities. Overall ecosystem potential is captured by genera richness, evenness and resilience are revealed by the Shannon and Simpson indices, and dominance trends are identified by Berger-Parker Index. When taken as a whole, these metrics show how eco-enzyme treatments, especially at higher concentrations, may change plankton communities by favoring a few hardy taxa which could upset ecological balances and lower biodiversity (Philippot et al. 2021).

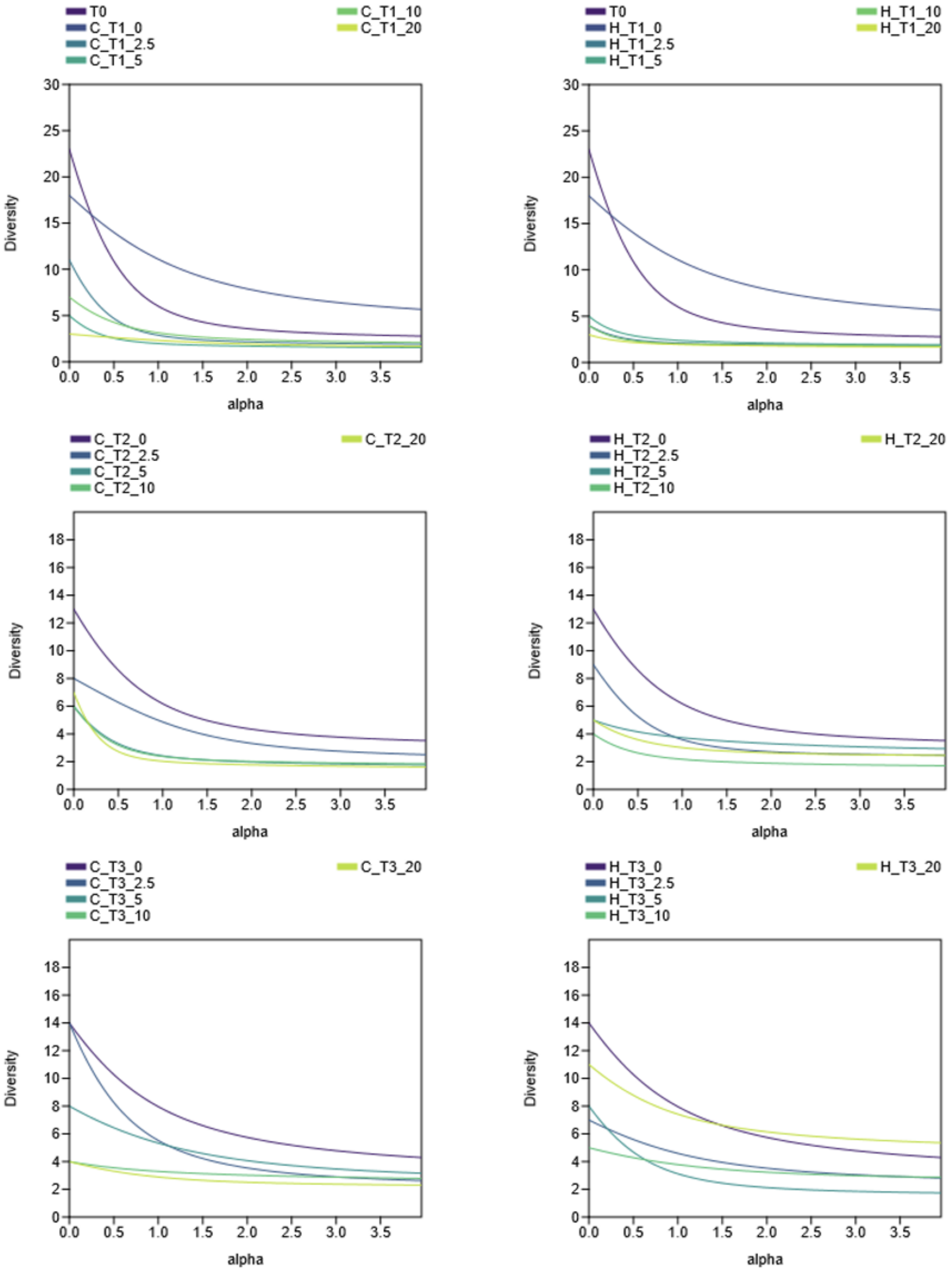

Figure 5. Freshwater plankton genera diversity profiles are described by Renyi's entropy values for each treatment combination. C and H represent commercial and home-made eco-enzyme, respectively. T1, T2, and T3 represent observations taken after 1, 2 and 3 weeks of experiment, respectively. 0, 2.5, 5, 10, and 20 represent eco-enzymes concentrations of 0 to 20% (v/v), respectively

Because nutrient enrichment usually favors fast-growing, pollution-tolerant taxa over sensitive species, previous research has demonstrated that decreased diversity and evenness in phytoplankton communities are early indicators of eutrophication (Koffel et al. 2022; Hou et al. 2023). For example, a study conducted in Tai Lake found a significant relationship between cyanobacteria community dominance and TSI values which reflects organic pollution and nutrient loading (Morris et al. 2020). In tropical and temperate aquatic systems, higher TSI values have also been associated with decreased species richness and evenness, especially when nitrogen and phosphorus levels are high (Zhu et al. 2020).

The effects of the commercial and home-made eco-enzyme treatments are noteworthy. While the commercial eco-enzyme consistently reduced biodiversity, the home-made eco-enzyme showed a more complex pattern, with initial increases in dominance followed by a subsequent shift. This difference could be attributes to variations in the composition of the eco-enzymes, such as the presence of certain enzymes, nutrients, or microbial communities that influence interactions with the plankton. Studies comparing the effects of commercial and home-made products on aquatic ecosystems have reported similar discrepancies, suggesting that home-made formulations may be less standardized and could lead to more variable ecological outcomes (Low et al. 2021).

The findings from Figure 5 emphasize the necessity of taking into account the ecological effects of using eco-enzymes especially when concentrations are high. These treatments might make water quality metrics better, but they might also create conditions that favor pollution-tolerant genera, which could lead to early eutrophication. Using TSI and diversity indices together gives a full picture of how eco-enzyme treatments affect freshwater ecosystems.

In conclusion, the results of this study demonstrate that eco-enzyme treatments, both commercial and home-made, significantly impact the physicochemical properties of freshwater ecosystems, including nutrient dynamics, BOD, pH, temperature, and TDS. While ammonia, nitrite, and nitrate concentrations increased with eco-enzyme concentrations, suggesting enhanced microbial activity and nutrient cycling, phosphate levels remained stable, indicating minimal effect on phosphate dynamics. The rapid decrease in BOD, especially at higher concentrations, underscores the effectiveness of eco-enzyme treatments in promoting organic matter breakdown. However, the shifts in plankton community composition towards pollution-tolerant genera, such as *Cylindrospermopsis*, *Chlorella*, *Aulacoseira*, and *Synedra* suggest potential for eco-enzyme inadvertently favor species associated with eutrophication, particularly at higher concentrations. Additionally, both genera richness and diversity decreased with increasing eco-enzyme concentrations, particularly in the commercial eco-enzyme treatments, highlighting the potential risk of biodiversity loss and ecological imbalance.

These findings indicate that although eco-enzymes enhance specific water quality metrics, their use could undermine ecosystem integrity by diminishing biodiversity and fostering eutrophic conditions, especially at high concentrations. We recommend careful management of eco-enzyme use in ecological water treatment, including controlled dosing strategies and routine monitoring of physicochemical parameters and biological indicators, such as TSI and community diversity indices. The differences observed between commercial and home-made formulations highlight the necessity of standardizing eco-enzyme composition to mitigate unpredictable ecological impacts.

Future research should examine the long-term and seasonal impacts of eco-enzyme application on trophic dynamics, functional groups, and higher trophic levels within freshwater ecosystems. Integrating eco-enzymes into nature-based water treatment frameworks necessitates the establishment of clear ecological guidelines, biodiversity safeguards, and public education to promote sustainable and adaptive freshwater management.

ACKNOWLEDGEMENTS

The authors would like to thank Indonesia, especially *Direktorat Riset, Teknologi dan Pengabdian kepada Masyarakat, Direktorat Jenderal Pendidikan Tinggi, Riset, dan Teknologi Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi*, for supporting this research through *Hibah Penelitian* Reguler No. 108/E5/PG.02.00.PL/2024.

REFERENCES

Akinnawo SO. 2023. Eutrophication: Causes, consequences, physical, chemical and biological techniques for mitigation strategies. Environ Chall 12: 100733. DOI: 10.1016/j.envc.2023.100733.

American Public Health Association (APHA). 2017. Standard Methods for the Examination of Water and Wastewater 23rd Edition. American Public Health Association, Washington DC.

Arun C, Sivashanmugam P. 2015. Solubilization of waste-activated sludge using a garbage enzyme produced from different pre-consumer organic waste. RCS Adv 5: 51421-51427. DOI: 10.1039/C5RA07959D.

Arun C, Sivashanmugam P. 2017. Study on optimization of process parameters for enhancing the multi-hydrolytic enzyme activity in garbage enzyme produced from preconsumer organic waste. Bioresour Technol 226: 200-210. DOI: 10.1016/j.biortech.2016.12.029.

Bellinger EG, Sigee DC. 2010. Freshwater Algae: Identification and Use as Bioindicators. John Wiley and Sons, West Sussex. DOI: 10.1002/9780470689554.

Benny N, Shams R, Dash KK, Pandey VK, Bashir O. 2023. Recent trends in utilization of citrus fruits in production of eco-enzyme. J Agric Food Res 13: 100657. DOI: 10.1016/j.jafr.2023.100657.

Bitting M, Johanson EN, Haberyan KA, Horn SP. 2023. Response of diatom communities to climate and human disturbance: A 4200-year record from Costa Rica. Holocene 33: 1534-1546. DOI: 10.1177/09596836231197775.

Borics G, Abonyi A, Salmaso N, Ptacnik R. 2021. Freshwater phytoplankton diversity: Models, drivers and implications for ecosystem properties. Hydrobiologia 848: 53-75. DOI: 10.1007/s10750-020-04332-9.

Chao A, Jost L. 2015. Estimating diversity and entropy profiles via discovery rates of new species. Methods Ecol Evol 6: 873-882. DOI: 10.1111/2041-210X.12349.

Clementson L, Erikson R, Willis A. 2022. Advances in Phytoplankton Ecology: Applications of Emerging Technologies. Elsevier, Amsterdam, Oxford.

Costa MRA, Cardoso MML, Selmeczy GB, Padisák J, Becker V. 2024. Phytoplankton functional responses induced by extreme hydrological events in a tropical reservoir. Hydrobiologia 851: 849-867. DOI: 10.1007/s10750-023-05241-3.

Costas-Selas C, Martínez-García S, Delgadillo-Nuño E, Justel-Díez M, Fuentes-Lema A, Fernández E, Teira E. 2024. Linking the impact of bacteria on phytoplankton growth with microbial community composition and co-occurrence patterns. Mar Environ Res 193: 106262. DOI: 10.1016/j.marenvres.2023.106262.

Das SC, Khan O, Khadem AH, Rahman MA, Bedoura S, Uddin MA, Islam MS. 2024. Evaluating the biocatalytic potential of fruit peel-derived eco-enzymes for sustainable textile wastewater treatment. Results Eng 21: 101898. DOI: 10.1016/j.rineng.2024.101898.

Dilys R. 2019. Biodiversity loss-more than an environmental emergency. Lancet Planet Health 3: e287-e289. DOI: 10.1016/S2542-5196(19)30113-5.

Dondajewska R, Kozak A, Rosińska J, Gołdyn R. 2019. Water quality and phytoplankton structure changes under the influence of Effective Microorganisms (EM) and barley straw-Lake restoration case study. Sci Total Environ 660: 1355-1366. DOI: 10.1016/j.scitotenv.2019.01.071.

Enawgaw Y, Wagaw S. 2023. Phytoplankton communities and environmental variables as indicators of ecosystem productivity in a shallow tropical lake. J Freshw Ecol 38: 2216244. DOI: 10.1080/02705060.2023.2216244.

Galintin O, Rasit N, Hamzah S. 2021. Production and characterization of eco enzyme produced from fruit and vegetable wastes and its influence on the aquaculture sludge. Biointerface Res Appl Chem 11: 10205-10214. DOI: 10.33263/BRIAC113.1020510214.

Gao W, Xiong F, Lu Y, Xin W, Wang H, Feng G, Kong C, Fang L, Gao X, Chen Y. 2024. Water quality and habitat drive phytoplankton taxonomic and functional group patterns in the Yangtze River. Ecol Proc 13: 11. DOI: 10.1186/s13717-024-00489-6.

Garg A, Yadav BK, Das DB, Wood PJ. 2022. Improving the assessment of polluted sites using an integrated bio-physico-chemical monitoring framework. Chemosphere 290: 133344. DOI: 10.1016/j.chemosphere.2021.133344.

Glibert PM, Mitra A. 2022. From webs, loops, shunts, and pumps to microbial multitasking: Evolving concepts of marine microbial ecology, the mixoplankton paradigm, and implications for a future ocean. Limnol Oceanogr 67: 585-597. DOI: 10.1002/lno.12018.

Groß E, Di Pane J, Boersma M, Meunier CL. 2022. River discharge-related nutrient effects on North Sea coastal and offshore phytoplankton communities. J Plankton Res 44: 947-960. DOI: 10.1093/plankt/fbac049.

Gu S, Xu D, Zhou F, Chen C, Liu C, Tian M, Jiang A. 2021. The garbage enzyme with Chinese hoenylocust fruits showed better properties and application than when using the garbage enzyme alone. Foods 10: 2656. DOI: 10.3390/foods10112656.

Harris TD, Reinl KL, Azarderakhsh M, Berger SA, Berman MC, Bizic M, Bhattacharya R, Burnet SH, Cianci-Gaskill JA, de Senerpont DLN, ElfferichI, Ger KA, Grossart H-PF, Ibelings BW, Ionescu D, Kouhanestani ZM, Mauch J, McElarney YR, Nava V, North RL, Ogashawara I, Paule-Mercado MCA, Soria-Píriz S, Sun X, Trout-Haney JV, Weyhenmeyer GA, Yokota K, Zhan Q. 2024. What makes a cyanobacterial bloom disappear? A review of the abiotic and biotic cyanobacterial bloom loss factors. Harmful Algae 133: 102599. DOI: 10.1016/j.hal.2024.102599.

Hou G, Shi P, Zhou T, Sun J, Zong N, Song M, Zhang X. 2023. Dominant species play a leading role in shaping community stability in the northern Tibetan grasslands. J Plant Ecol 16: rtac110. DOI: 10.1093/jpe/rtac110.

Hudson JJ, Taylor WD, Schindler DW. 1999. Planktonic nutrient regeneration and cycling efficiency in temperate lakes. Nature 400: 659-661. DOI: 10.1038/23240.

Ihtiar A, Vira TD, Faizsyahrani LP, Anggraini N, Azuhro V, Dewi ERS, Nurwahyunani A. 2023. The utilization of household waste through ecoenzymes. Intl J Hum Soc Sci Bus 2: 239-249. DOI: 10.54443/injoss.v2i2.75.

Jiang J, Cui H, Bhople P, Chater CCC, Yu F, Liu D. 2024. Biochar combined with garbage enzyme enhances nitrogen conservation during sewage sludge composting: evidence from microbial community and enzyme activities related to ammonization. Agronomy 14: 1162. DOI: 10.3390/agronomy14061162.

Koffel T, Umemura K, Litchman E, Klausmeier CA. 2022. A general framework for species-abundance distributions: Linking traits and dispersal to explain commonness and rarity. Ecol Lett 25: 2359-2371. DOI: 10.1111/ele.14094.

Kumar N, Rajshree YA, Yadav A, Malhotra NM, Gupta N, Pudhp P. 2019. Validation of eco-enzyme for improved water quality effect during large public gathering at river bank. Intl J Hum Cap Urban Manag 3: 181-188. DOI: 10.22034/IJHCUM.2019.03.03.

Leong RZL, Tee JJ, Teo SS. 2023. Decolorization of crystal violate and methylene blue wastewater using anaerobic fermented bio-waste. Water Conserv Sci Eng 8: 15. DOI: 10.1007/s41101-023-00189-w.

Li Y, Geng M, Yu J, Du Y, Xu M, Zhang W, Wang J, Su H, Wang R, Chen F. 2022. Eutrophication decreases compositional dissimilarity in freshwater plankton communities. Sci Total Environ 821: 153434. DOI: 10.1016/j.scitotenv.2022.153434.

Low CW, Ling RLZ, Teo SS. 2021. Effective microorganisms in producing eco-enzyme from food waste for wastewater treatment. Appl Microbiol Theory Technol 2: 28-36. DOI: 10.37256/amtt.212021726.

Luo L, Meng H, Gu JD. 2017. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. J Environ Manag 197: 539-549. DOI: 10.1016/j.jenvman.2017.04.023.

Luong HA, Rohlfs A-M, Facey JA, Colville A, Mitrovic SM. 2024. Long-term study of phytoplankton dynamics in a supply reservoir reveals signs of trophic state shift linked to changes in hydrodynamics associated with flow management and extreme events. Water Res 256: 121547. DOI: 10.1016/j.watres.2024.121547.

Meng F, Li Z, Li L, Lu F, Liu Y, Lu X, Fan Y. 2020. Phytoplankton alpha diversity indices response the trophic state variation in hydrologically connected aquatic habitats in the Harbin Section of the Songhua River. Sci Rep 10: 21337. DOI: 10.1038/s41598-020-78300-7.

Morris A, Meyer K, Bohannan B. 2020. Linking microbial communities to ecosystem functions: what we can learn from genotype-phenotype mapping in organisms. Philos Trans Royal Soc B 375: 20190244. DOI: 10.1098/rstb.2019.0244.

Naselli-Flores L, Padisák J. 2022. Ecosystem services provided by marine and freshwater phytoplankton. Hydrobiologia 850: 2691-2706. DOI: 10.1007/s10750-022-04795-y.

Neijnens FK, Moreira H, de Jonge MM, Linssen BBHP, Huijbregts MAJ, Geerling GW, Schipper AM. 2023. Effects of nutrient enrichment on freshwater macrophyte and invertebrate abundance: A meta-analysis. Glob Change Biol 30: e17094. DOI: 10.1111/gcb.17094.

Ni G, Leung PM, Daebeler A, Guo J, Hu S, Cook P, Nicol GW, Daims H, Greening C. 2023. Nitrification in acidic and alkaline environments. Essays Biochem 67: 753-768. DOI: 10.1042/EBC20220194.

Otero J, Alvarez-Salgado XA, Bode A. 2020. Phytoplankton diversity effect on ecosystem functioning in a coastal upwelling system. Front Mar Sci 7: 592255. DOI:10.3389/fmars.2020.592255.

Paerl HW, Otten TG. 2013. Harmful cyanobacterial blooms: Causes, consequences, and controls. Microb Ecol 65: 995-1010. DOI: 10.1007/s00248-012-0159-y.

Pasalari H, Moosavi A, Kermani M, Sharifi R, Farzadkia M. 2024. A systematic review on garbage enzymes and their applications in environmental processes. Ecotoxicol Environ Saf 277: 116369. DOI: 10.1016/j.ecoenv.2024.116369.

Philippot L, Griffiths BS, Langenheder S. 2021. Microbial community resilience across ecosystems and multiple disturbances. Microbiol Mol Biol Rev 31: e00026-20. DOI: 10.1128/MMBR.00026-20.

Pinheiro JPS, Windsor FM, Wilson RW, Tyler CR. 2021. Global variation in freshwater physico-chemistry and its influence on chemical toxicity in aquatic wildlife. Biol Rev 96: 1528-1546. DOI: 10.1111/brv.12711.

Pratiwi D, Oktavia D, Sumiarsa D, Sunardi. 2024. Water quality assessment of river based on phytoplankton biological integrity index in rural areas of the upstream Citarum River, West Java, Indonesia. Biodiversitas 25 (2): 881-889. DOI: 10.13057/biodiv/d250248.

Ramlee A, Suhaimi H, Rasdi NW. 2022. Diversity and abundance of plankton in different habitat zonation of Papan River, Lake Kenyir, Malaysia. Biodiversitas 23 (1): 212-221. DOI: 10.13057/biodiv/d230127.

Reinl KL, Harris TD, Elfferich I, Coker A, Zhan Q, Domis LNDS, Morales-Williams AM, Bhattacharya R, Grossart HP, North RL, Sweetman JN. 2022. The role of organic nutrients in structuring freshwater phytoplankton communities in a rapidly changing world. Water Res 219: 118573. DOI: 10.1016/j.watres.2022.118573.

Roswell M, Dushoff J, Winfree R. 2021. A conceptual guide to measuring species diversity. Oikos 130: 321-338. DOI: 10.1111/oik.07202.

Rungta S, Ojha A, Mishra SK. 2022. Study of physico-chemical property of bioenzymes produced from organic household waste and their application in daily life. Discovery 58 (315): 228-234.

Streble H, Krauter D. 2010. Das Leben im Wassertropfen: Mikroflora und Mikrofauna des Süßwassers; Ein Bestimmungsbuch. Kosmos, Stuttgart.

Tambaru R, Burhanuddin AI, Haris A, Amran MA, Massinai A, Muhiddin AH, Yaqin K, Firman, Yuliana. 2024. Diversity and abundance of phytoplankton in Bone Bay, South Sulawesi, Indonesia and its relationship with environmental variables. Biodiversitas 25 (2): 624-631. DOI: 10.13057/biodiv/d250221.

Trommer G, Poxleitner M, Stibor H. 2020. Responses of lake phytoplankton communities to changing inorganic nitrogen supply forms. Aquat Sci 82: 22. DOI: 10.1007/s00027-020-0696-2.

Tuhumury NC, Tuahatu J, Manuputty GD. 2024. Effect of eco-enzyme on water quality parameters in some rivers disembogued at Ambon Bay. J Nat Resour Environ Manag 14 (4): 824-835. DOI: 10.29244/jpsl.14.4.824.

Varshini B, Gayathri V. 2023. Role of eco-enzymes in sustainable development. Nat Environ Pollut Technol 22 (3): 1299-1310. DOI: 10.46488/NEPT.2023.v22i03.017.

Vidalia C, Angeina E, Hans J, Field LH, Santo NC, Rukmini E. 2023. Eco-enzyme as disinfectant: a systematic literature review. Intl J Publ Health Sci 12 (3): 1171-1180. DOI: 10.11591/ijphs.v12i3.22131.

Wang J, Zhang Z. 2020. Phytoplankton, dissolved oxygen and nutrient patterns along a eutrophic river-estuary continuum: Observation and modeling. J Environ Manag 261: 110233. DOI: 10.1016/j.jenvman.2020.110233.

Wani ZA, Khan S, Bhat JA, Malik AH, Alyas T, Pant S, Siddiqui S, Moustafa M, Ahmad AE. 2022. Pattern of β-diversity and plant species richness along vertical gradient in Northwest Himalaya, India. Biology 11 (7): 1064. DOI: 10.3390/biology11071064.

Wear SL, Acuña V, McDonald R, Font C. 2021. Sewage pollution, declining ecosystem health, and cross-sector collaboration. Biol Conserv 255: 109010. DOI: 10.1016/j.biocon.2021.109010.

Yang J, Yu X, Liu L, Zhang W, Guo P. 2012. Algae community and trophic state of subtropical reservoirs in southeast Fujian, China. Environ Sci Pollut Res 19: 1432-1442. DOI: 10.1007/s11356-011-0683-1.

Yustiani YM, Nugroho FL, Murtadho FZ, Dijayadisastra AT. 2023. Use of eco enzyme to reduce the chemical oxygen demand of synthetic river water. J Eng Technol Sci 55: 91-97. DOI: 10.5614/j.eng.technol.sci.2023.55.1.9.

Zhang Y, Yu H, Liu J, Guo Y. 2024. Analysis of water quality and the response of phytoplankton in the low-temperature environment of Majiagou Urban River, China. Heliyon 10: e25955. DOI: 10.1016/j.heliyon.2024.e25955.

Zhikharev V, Vodeneeva E, Kudrin I, Gavrilko D, Startseva N, Kulizin P, Erina O, Tereshina M, Okhapkin A, Shurganova G. 2023. The species structure of plankton communities as a response to changes in the trophic gradient of the mouth areas of large tributaries to a lowland reservoir. Water 15 (1): 74. DOI: 10.3390/w15010074.

Zhu J, Yu Z, Cao X, Jiang W, He L, Zang X, Song X. 2024. Double effects of mitigating cyanobacterial blooms using modified clay technology: Regulation and optimization of the microbial community structure. Front Microbiol 15: 1480069. DOI: 10.3389/fmicb.2024.1480069.

Zhu K, Wu Y, Li C, Xu J, Zhang M. 2020. Ecosystem-based restoration to mitigate eutrophication: A case study in a Shallow Lake. Water 12: 2141. DOI: 10.3390/w12082141.