

# Phytoplankton as bioindicators of organic matter accumulation in rice-fish agro-ecosystems of Eastern Himalayas

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## Abstract

Rice-fish culture represents an ecologically sustainable farming system that integrates crop production with aquatic biodiversity. Within this system, phytoplankton act as the primary producers, which are highly responsive to environmental fluctuations and also serve as valuable bioindicators of water quality. The present study assessed phytoplankton abundance, diversity, and status of organic matter accumulation (OMA) in three experimental sites during the waterlogged phase of rice-fish fields in Ziro Valley, in the Eastern Himalayas. A total of 9,649 individuals per litre of phytoplankton were recorded, belonging to 73 genera, with Bacillariophyceae being the dominant class across all sites. Pearson correlation analysis revealed a significant positive relationship between turbidity and Shannon diversity, while total hardness was positively correlated with Pielou's evenness. However, Shannon diversity exhibited a strong negative correlation with Simpson dominance, confirming that higher diversity corresponds with reduced dominance by a few taxa. Palmer pollution index (PPI) used for OMA assessment resulted in high OMA scores (26-27) across all three sites, coinciding with Shannon-Weiner diversity index that reflected high algal diversity comprising OMA-tolerant genera (25.93%, McNaughton Dominance Index  $Y \geq 0.02$ ), indicating all three selected rice-fish fields of the Ziro valley experience high OMA.

## Introduction

Rice-fish culture is a traditional and ecologically sustainable farming practice that has been adopted for centuries across different parts of the world. In this integrated system, the interaction among rice, fish, and microorganisms plays an important role in nutrient recycling and maintaining the micro-habit of the field (Halwart, 1998). Fish such as common carp (*Cyprinus carpio* L.) are often raised in this system, which feeds on various algal life forms, constituting majorly of microalgae known as phytoplankton (Saikia and Das, 2009). In addition, the fish excreta, together with other dissolved nutrients, supports the growth of these phytoplankton. They form the base of the aquatic food chain and support the higher trophic levels (Saikia and Das, 2009; Ohadi *et al.*, 2021). Additionally, phytoplankton are widely recognised as sensitive and reliable bioindicators of aquatic ecosystem health

(Rishi *et al.*, 2016; Chandel *et al.*, 2023). Since they are the primary producers at the base of the aquatic food web, even subtle variations in physical or chemical parameters, such as nutrient availability, temperature, pH, or dissolved oxygen, can trigger measurable shifts in their community structure (Mukherjee *et al.*, 2010; Zhang *et al.*, 2018). These characteristics of phytoplankton help to detect early signs of ecosystem stress even before such changes become apparent at higher trophic levels.

In addition to their ecological sensitivity, phytoplankton-based monitoring is cost-effective and less labour-intensive compared to other methods of water quality assessment (Bellinger and Sigee, 2015). Even with minimal equipment and thorough microscopic identification, it can provide comprehensive insights into nutrient status, organic matter accumulation (OMA), or potential harmful algal blooms. As highlighted by Bellinger and Sigee (2015), Sharma and Tiwari



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(2018) and Huang *et al.* (2022), using phytoplankton as bioindicators reduces both the effort and financial resources needed for routine environmental monitoring, making them an efficient tool for long-term ecological surveillance and management.

Among the various environmental stressors, such as light intensity, hardness, dissolved oxygen (DO), temperature, water flow, or pH, previous studies suggest that higher OMA in the water bodies exerts a stronger influence on phytoplankton assemblages (Palmer, 1969) as higher OMA leads to microalgal bloom (Byod, 2015). This makes phytoplankton composition an important criterion for evaluating water quality. Palmer (1969) carried out an extensive review to assess the tolerance of algal species to organic pollution and identified 20 species of phytoplankton that are tolerant to higher organic enrichment and suggested the Palmer pollution index (PPI), which is one of the most widely applied biological indices for detecting organic pollution, by scoring tolerant algal genera. This method has been successfully applied in lakes, rivers, and canals as phytoplankton-based bioindicators for organic pollution (Thakur *et al.*, 2013; Zhang *et al.*, 2024). However, this could also be used as a potential method for OMA assessment in high-altitude rice–fish ecosystems to understand how algal communities respond to organic enrichment in these traditional farming systems.

Against this backdrop, investigations were conducted to understand if phytoplankton abundance and community composition can be used for OMA assessment in high-altitude rice–fish ecosystems, further applying PPI to determine the degree of OMA across different sampling sites. By analysing spatial variations in environmental

parameters, phytoplankton composition, and its tolerance, this research aims to provide insights into water quality conditions while demonstrating the effectiveness of phytoplankton as cost-effective bioindicators for sustainable management of rice–fish culture systems.

## Materials and methods

### Study area

The study was conducted in the rice–fish fields of Ziro Valley (Fig. 1), Lower Subansiri District, Arunachal Pradesh, India (93°49′10.8″E; 27°32′41.7″N; 1500 m a.s.l.). We selected three study locations from different villages within the valley, each located approximately 3 km apart from each other. Data were collected from June to August 2025, during the morning hours (06:00 hrs to 12:00 hrs) in the rice–fish fields during the waterlogged period, when fields were intentionally blocked to prevent the drainage of water and fish.

### Physicochemical analysis of water samples

Water samples were analysed for physicochemical parameters including water temperature, turbidity, and pH using a standard water quality analyser (Systronics 371). Total alkalinity and total hardness (TH) were determined following the standard methods for the examination of water and wastewater (APHA, 2023). Water samples from each field were collected in oxygen bottles for the estimation of dissolved oxygen (DO) and biological oxygen demand (BOD) and fixed immediately using Winkler’s reagents and analysed in the laboratory (Winkler, 1888). All the parameters were measured in triplicate.

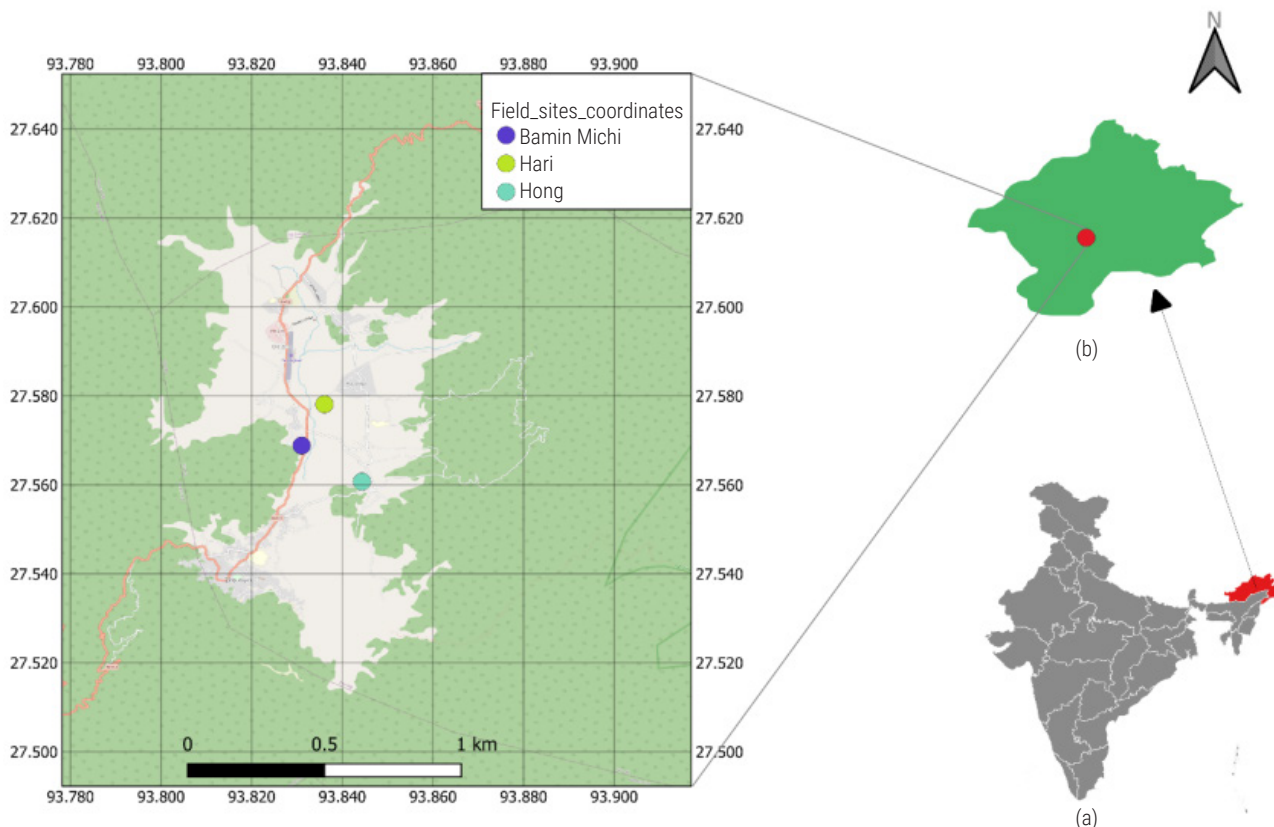


Fig. 1. Map of the study area showing (a) India, with the state of Arunachal Pradesh highlighted in red; (b) Lower-Subansiri District, indicating the location of Ziro valley; (c) Field sampling locations within Ziro valley

## Phytoplankton sampling and analysis

For the qualitative and quantitative plankton analysis, 120 l of water was filtered at each sampling site, using a 60 µm mesh plankton net. The concentrated sample was collected directly into a 15 ml Falcon tube, immediately preserved in 4% formalin, and later analysed in the laboratory. Plankton were identified and counted under a binocular light microscope (Nikon ECLIPSE E200) using 10× and 40× objectives. Identification of plankton taxa was carried out following standard taxonomic references (Needham and Needham, 1962; Tonapi, 1980; Botes, 2003; Nwankwo, 2004; Verlencar and Desai, 2004). Lackey's drop count method (Lackey, 1938) was followed to quantify the abundance of phytoplankton, and values were expressed as individuals per litre. The following formula was used to calculate plankton abundance:

$$N = n \times V_c / V_s \times V_o$$

Where, N = Abundance of phytoplankton (L); n = No. of organisms counted;  $V_c$  = Final concentrated volume (ml);  $V_s$  = Volume of original water sample (l);  $V_o$  = Actual volume of concentrate examined (ml).

## Diversity indices and calculation of organic matter accumulation (OMA)

Shannon–Wiener diversity index ( $H'$ ) (Shannon and Weiner, 1964), Pielou's evenness index (E) (Pielou, 1966), and Simpson's dominance index (D) (Simpson, 1949) were used to calculate phytoplankton diversity, evenness, and dominance, respectively. Diversity Indices were calculated using the following formulae:

$$\text{Shannon–Wiener diversity index } (H') = \sum (n_i / N) \ln (n_i / N)$$

where,  $n_i$  = No. of individuals of species  $i$ ; N = Total no. of individuals of all species

$$\text{Pielou's Evenness Index, } e = H' / \ln S,$$

where,  $H'$  = Shannon-Wiener diversity index; S = No. of species

$$\text{Simpson's dominance index } (D) = \sum (p_i)^2$$

where,  $p_i = n_i / N$  = Proportion of individuals belonging to species  $i$ ;  $n_i$  = No. of individuals of the  $i^{\text{th}}$  species (or genus, depending on the taxonomic resolution); N = Total no. of individuals of all species in the sample.

Furthermore, Palmer's Index was applied to evaluate the algal accumulation status in rice–fish ecosystem, based on the tolerance of different algal genera to high organic enrichment where, scores

below 10 indicate lack of OMA, scores between 10-15 reflect moderate accumulation, those from 15-20 suggest probable high accumulation, and values exceeding 20 confirm high OMA (Palmer, 1969). Further, the McNaughton dominance index ( $Y$ ) with dominance threshold ( $Y \geq 0.02$ ) was used to determine the dominance of OMA-tolerant genera (McNaughton, 1967) in the rice-fish field.

The following formula was used to calculate the McNaughton dominance index (McNaughton, 1967):

$$Y = (n_i / N \times f_i)$$

where:  $n_i$  = Total abundance of OMA tolerant genera  $i$ ; N = Total abundance of all genera combined;  $f_i$  = Frequency of occurrence (Number of samples where genus  $i$  appeared).

## Statistical analysis

Analysis of variance (ANOVA) was performed to determine the degree of variation in phytoplankton diversity indices among different sites at a 0.05 significance level. Furthermore, the Pearson correlation coefficient matrix was calculated to analyse the correlation between physico-chemical parameters, diversity indices, and the Palmer index at  $p < 0.05$  and  $p < 0.01$  levels. All the analyses (diversity indices, ANOVA, and Pearson correlation) were performed using *vegan*, *Biodiversity R*, and base R packages in R software (R Core Team, 2024).

## Results and discussion

### Physiochemical analysis of water

Physiochemical parameters from three sampling sites, Bamin Michi, Hari, and Hong, indicated that while some parameters (such as alkalinity and DO) were relatively stable, others (like turbidity, BOD, and pH) represented site-specific differences (Table 1).

### Spatial variation in phytoplankton diversity and abundance

#### Phytoplankton diversity

A total of 73 genera belonging to five algal classes, comprising 35 genera, were identified. Bacillariophyceae and Chlorophyceae were the dominant taxonomic groups, collectively accounting for about 93% of the total plankton counts. This may be attributed to their excellent adaptability to variable environmental conditions (Palupi, 2023). Among the 73 algal genera identified, 35 were common in all three sampling sites (Fig. 2). These results indicate

Table 1. Physiochemical parameters (Mean±SD) recorded during the study period.

Parameter	Bamin Michi	Hari	Hong	Mean±SD
Alkalinity (mg l <sup>-1</sup> )	33.33±14.43	33.33±14.43	33.33±14.43	33.33±14.43
BOD (mg l <sup>-1</sup> )	3.53±0.42	3.00±0.20	2.37±0.32	2.97±0.58
DO (mg l <sup>-1</sup> )	9.20±0.87	9.00±0.53	9.43±0.72	9.21±0.69
Total hardness (mg l <sup>-1</sup> )	65.00±8.66	75.00±15.00	70.00±8.66	70.00±11.34
Turbidity (NTU)	85.00±62.45	35.00±40.41	3.33±5.77	41.11±47.55
pH	7.17±1.15	6.33±0.58	6.17±0.29	6.56±0.92
Water temperature (°C)	27.67±1.15	24.67±0.58	25.67±1.15	26.00±1.53

that although certain genera were consistent across multiple sites, the composition of rare taxa varied between locations, reflecting localised ecological differences (Table 2). The diversity analysis showed slight variation among sites (Table 3), but ANOVA revealed no statistically significant differences among sampling locations ( $p > 0.05$ ). Overall, higher Shannon diversity ( $H' = 3.18-3.38$ ) among the field sites may suggest a balanced community structure consistent

with moderately enriched but not severely eutrophic conditions (Magurran, 2013). Evenness values ( $E = 0.85-0.89$ ) showed species were fairly evenly distributed, likely due to stable water environmental conditions, consistent organic matter inputs from rice residues, and fish waste (Tilman *et al.*, 2001). Furthermore, comparable differences in the dominance index (D) may imply that the phytoplankton community were strongly influenced by a few dominant species at some sites where diversity is low (Reinl *et al.*, 2022).

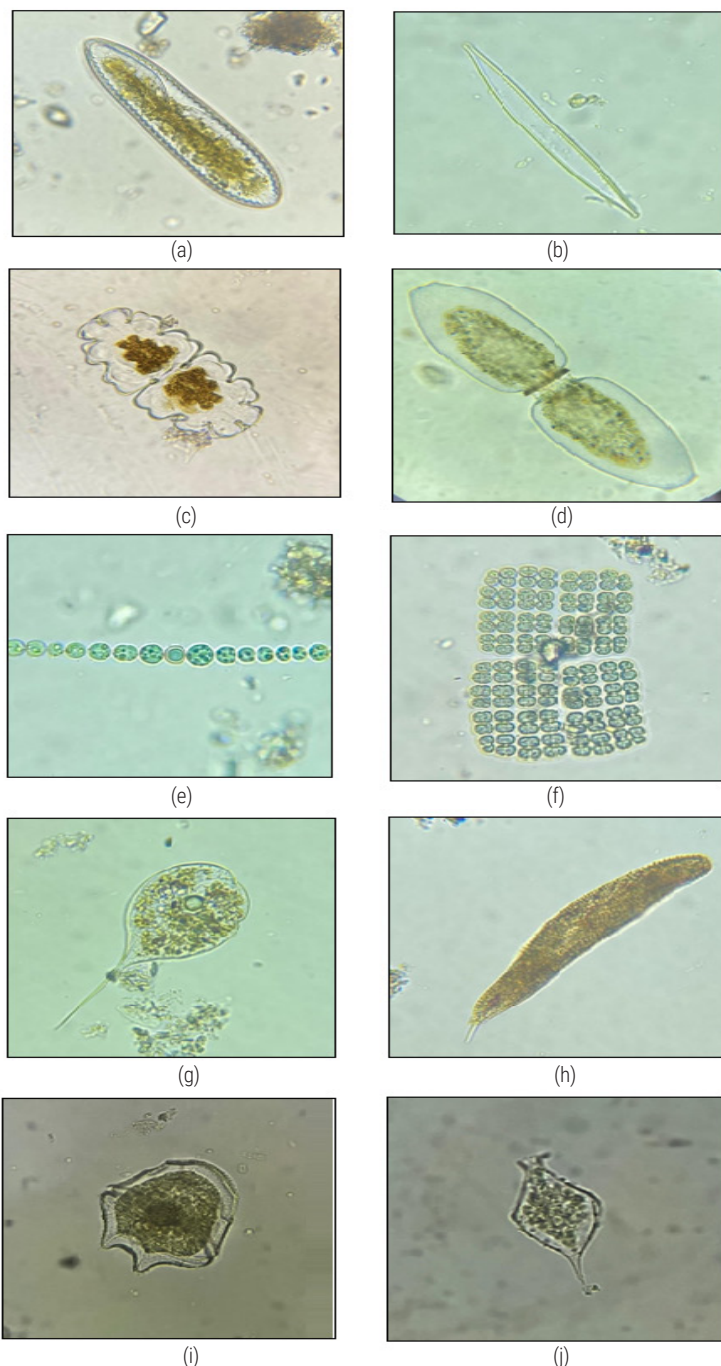


Fig. 2. Representative phytoplankton species belonging to different taxonomic classes recorded from the rice-fish fields of Ziro Valley. (a-b) Bacillariophyceae; (c-d) Chlorophyceae; (e-f) Cyanophyceae; (g-h) Euglenophyceae; (i-j) Dinophyceae. (a) *Surirella tenera*; (b) *Nitzschia* sp.; (c) *Euastrum oblongum*; (d) *Pleurotaenium ovatum*; (e) *Anabaena* sp.; (f) *Merismopedia* sp.; (g) *Phacuss* sp.; (h) *Lepocinclis spirogyroides*; (i) *Peridinium* sp.; (j) *Strombomonas* sp.

Table 2. List of algal taxa recorded in the rice-fish culture systems at the three sampling stations

Species	Bamin Michi	Hari	Hong
<b>Bacillariophyceae</b>			
<i>Amphora</i> spp.	+	+	+
<i>Aulacosira</i> spp.	+	+	+
<i>Caloneis</i> spp.	+	+	-
<i>Cocconeis</i> spp.	+	-	-
<i>Craticula</i> spp.	+	-	-
<i>Cymbella</i> spp.	+	+	+
<i>Cymbopleura</i> spp.	+	+	+
<i>Encyonema</i> spp.	+	+	+
<i>Eunotia</i> spp.	+	+	+
<i>Eunotogramma</i> spp.	+	+	+
<i>Frustulia</i> spp.	+	+	+
<i>Gomphonema</i> spp.	+	+	+
<i>Gyrosigma</i> spp.	+	+	-
<i>Hantzschia</i> spp.	+	+	-
<i>Hyalotheca</i> spp.	-	+	-
<i>Lemnicola</i> spp.	-	+	-
<i>Melosira</i> spp.	-	-	+
<i>Navicula</i> spp.	+	+	+
<i>Neidium</i> spp.	+	+	+
<i>Nitzschia</i> spp.	+	+	+
<i>Pinnularia</i> spp.	+	+	+
<i>Placoneis</i> spp.	+	-	-
<i>Planothidium</i> spp.	-	+	+
<i>Rhopalodia</i> spp.	+	-	-
<i>Sellaphora</i> spp.	+	+	+
<i>Seminavis</i> spp.	+	+	+
<i>Stauroneis</i> spp.	+	+	+
<i>Staurosira</i> spp.	+	+	+
<i>Surirella</i> spp.	+	+	+
<i>Synedra</i> spp.	+	+	+
<i>Tabellaria</i> spp.	+	+	+
<i>Tetrastrum</i> spp.	-	+	-
<i>Triploceras</i> spp.	-	+	-
<i>Tryblionella</i> spp.	+	-	-
<i>Ulnaria</i> spp.	+	-	+
<b>Chlorophyceae</b>			
<i>Actinotaenium</i> spp.	+	+	+
<i>Ankistodesmus</i> spp.	-	+	+
<i>Ankyra</i> spp.	-	+	+
<i>Characium</i> spp.	+	-	-
<i>Closteriopsis</i> spp.	-	-	+
<i>Closterium</i> spp.	+	+	+
<i>Cosmarium</i> spp.	+	+	+
<i>Desmidiium</i> spp.	+	+	+
<i>Desmodesmus</i> spp.	+	+	+
<i>Euastrum</i> spp.	+	+	+
<i>Gonatozygon</i> spp.	+	-	+
<i>Hydrodictyon</i> spp.	+	-	-
<i>Micrasteriasis</i> spp.	+	-	-
<i>Oedogonium</i> spp.	+	+	+
<i>Oocystis</i> spp.	+	+	+
<i>Pediastrum</i> spp.	+	+	+
<i>Penium</i> spp.	+	-	+
<i>Pleurotaenium</i> spp.	+	+	+
<i>Scenedesmus</i> spp.	+	-	-
<i>Spirogyra</i> spp.	+	+	+
<i>Staurastum</i> spp.	+	+	+
<i>Stauromesmus</i> spp.	-	+	+
<i>Stigeoclonium</i> spp.	-	-	+
<i>Tetraselmis</i> spp.	-	+	-
<i>Xanthidium</i> spp.	-	+	-
<i>Zygnema</i> spp.	-	+	+
<b>Cyanophyceae</b>			
<i>Anabaena</i> spp.	+	+	+
<i>Gleotrichea</i> spp.	+	-	-
<i>Merismopedia</i> spp.	+	+	+
<i>Occilatoria</i> spp.	+	+	+
<i>Phormidium</i> spp.	-	+	-
<b>Dinophyceae</b>			
<i>Peridinium</i> spp.	-	+	+
<i>Strombomonas</i> spp.	-	+	-
<b>Euglenophyceae</b>			
<i>Euglena</i> spp.	+	+	+
<i>Lepocinlis</i> spp.	+	+	+
<i>Monomorpha</i> spp.	+	+	-
<i>Phacus</i> spp.	+	+	-
<i>Trachelomonas</i> spp.	+	+	+
Total Genera	56	57	50

Table 3. Diversity indices across the three field locations

Site	Shannon index (H')	Evenness index (E)	Dominance index (D)
Bamin Michi	3.38	0.89	0.06
Hari	3.28	0.87	0.07
Hong	3.18	0.85	0.08

### Phytoplankton abundance

The analysis revealed a total of 9649 individual l<sup>-1</sup> across all the locations, of which Bamin Michi exhibited the highest abundance (4203 individuals), followed by Hari and Hong (Table 4). Furthermore, a reduction in the abundance of almost all taxonomic classes was observed across all field sites during July (Fig. 3), likely due to the overflow of the rice-fish fields following heavy rainfall. In Bamin Michi and Hari, *Pinnularia* spp. (Bacillariophyceae) was the dominant taxon, accounting for 13% and 11% of the total phytoplankton abundance, respectively (Fig. 3). In contrast, *Synedra* spp. was the most abundant taxon in Hong, constituting 15% of the total individual count. The comparatively higher phytoplankton abundance in Bamin Michi may be attributed to the stagnant water conditions in the location, as compared to the other two sampling locations. Recent studies (Ding *et al.*, 2024; Li *et al.*, 2024; Zhang *et al.*, 2024) suggest that stagnant water bodies tend to hold phytoplankton within the photic zone for longer periods, supporting continuous photosynthesis and growth, while allowing the buildup of nitrogen and potassium, which can enhance algalbiomassa. Further, reduced grazing pressure by fish may contribute to algae to increased phytoplankton proliferation (Beman *et al.*, 2005; Wijewardene *et al.*, 2021).

Table 4. Phytoplankton abundance across different field sites (Individual l<sup>-1</sup>)

Class	Bamin Michi	Hari	Hong	Mean	%
Bacillariophyceae	2668	1666	1406	1913	53.5
Chlorophyceae	1266	1219	660	1408	39.4
Cyanophyceae	75	152	54	94	2.6
Dinophyceae	0	21	25	15	0.4
Euglenophyceae	194	175	68	146	4.1
Total individual L <sup>-1</sup>	4203	3233	2213	3576	100

### Phytoplankton tolerance and OMA in rice fields Palmer index and McNaughton dominance index

Out of the 20 genera identified by Palmer (1969) as bioindicators of OMA, 14 were recorded in this study, and eight of these genera (*Closterium* spp., *Euglena* spp., *Gomphonema* spp., *Lepocinlis* spp., *Navicula* spp., *Nitzschia* spp., *Oscillatoria* spp. and *Synedra* spp.) were consistently observed across all three study sites (Table 5). The high Palmer scores (>20) at all sites indicate consistently elevated OMA (Table 5). According to the McNaughton dominance index, OMA-tolerant taxa accounted for 25.93% (Y≥0.02) of the total phytoplankton community, implying that roughly one-quarter of all observed species were strongly dominant, tolerant forms. The presence of these taxa indicates substantial OMA inputs, potentially derived from decomposed rice straw, fish excreta and agricultural runoff. Such conditions enhance phytoplankton proliferation and shift community composition towards tolerant taxa (Wijewardene *et al.*, 2021; Wang *et al.*, 2022).

### Relationship between physicochemical parameters, phytoplankton diversity and OMA

Pearson correlation analysis was used to examine the relationships among physicochemical parameters, diversity indices and the

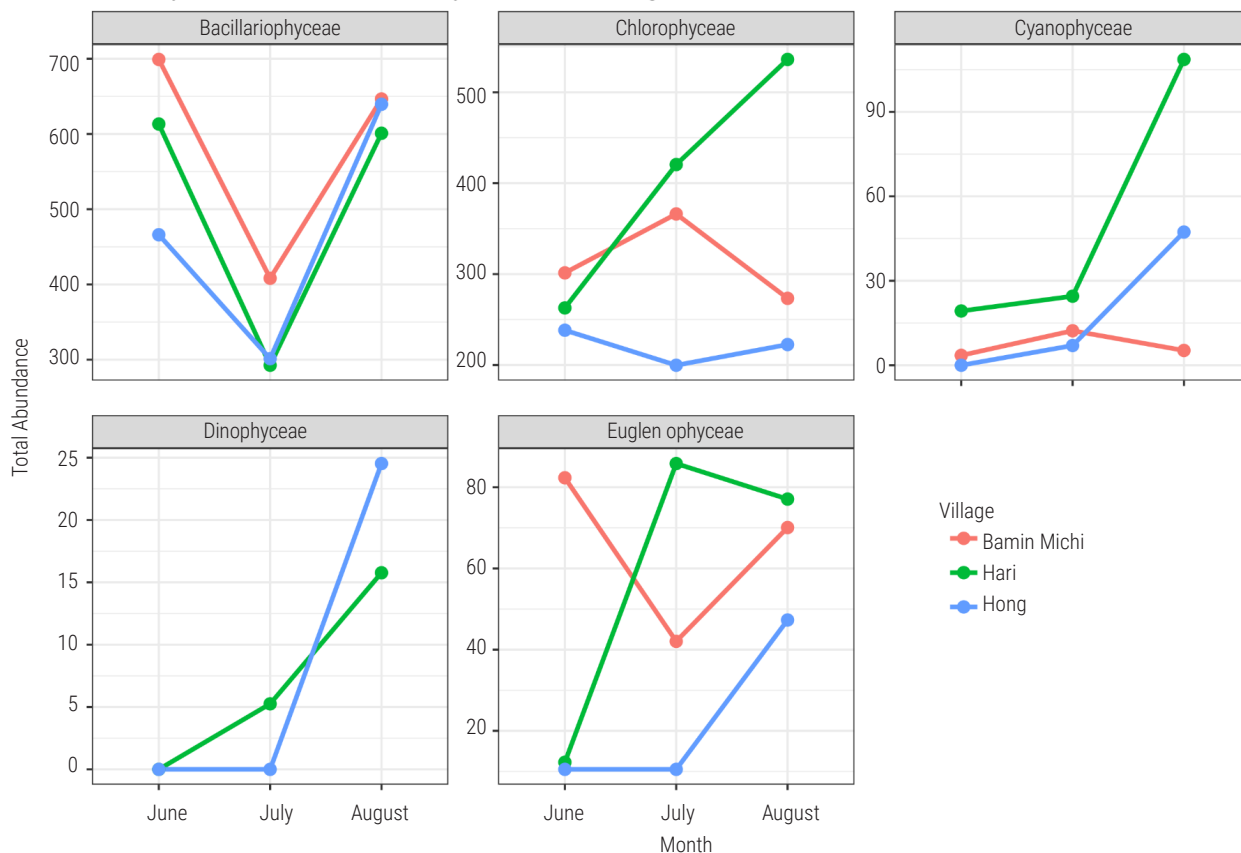


Fig. 3. Decline in the abundance of phytoplankton classes across all sampling sites during July, likely associated with overflow of the rice-fish fields

Table 5. OMA-tolerant phytoplankton recorded across the study sites, along with their Palmer score and dominance values based on McNaughton dominance index (Y)

Genera	Palmer score			Dominance index (Y)		
	Bamin Michi	Hari	Hong	Bamin Michi	Hari	Hong
<i>Ankistrodesmus</i>	-	2	2	-	0.02	0.01
<i>Closterium</i>	1	1	1	0.03	0.01	0.001
<i>Euglena</i>	5	5	5	0.005	0.02	0.02
<i>Gomphonema</i>	1	1	1	0.13	0.11	0.123
<i>Lepocinclis</i>	1	1	1	0.05	0.07	0.03
<i>Melosira</i>	-	-	1	-	-	0.002
<i>Navicula</i>	3	3	3	0.15	0.17	0.25
<i>Nitzschia</i>	3	3	3	0.13	0.13	0.08
<i>Oscillatoria</i>	5	5	5	0.001	0.01	0.01
<i>Phacus</i>	2	2	-	0.06	0.01	-
<i>Phormodium</i>	-	1	-	-	0.003	-
<i>Scenedesmus</i>	4	-	-	0.06	-	-
<i>Stigeoclonium</i>	-	-	2	-	-	0.09
<i>Synedra</i>	2	2	2	0.10	0.06	0.44
Total	27	26	26	0.05	0.04	0.07

‘-’ Indicates absence of genera

Palmer pollution index (Fig. 4). Significant correlations were considered at  $p < 0.05$  and  $p < 0.01$ . A strong and highly significant negative correlation was observed between Shannon diversity and Simpson dominance ( $r = -0.919$ ,  $p = 0.00046$ ), indicating that phytoplankton communities with higher diversity tend to exhibit lower dominance by a few taxa. This pattern suggests a more balanced and stable ecosystem, which

can support improved water quality and enhanced food availability for fish, while high dominance by a limited number of species may increase the risk of ecological imbalance and water quality deterioration. Additionally, a significant positive correlation between Shannon diversity and turbidity ( $r = 0.672$ ,  $p = 0.047$ ) may suggest that organic matter can stimulate diverse phytoplankton assemblages.

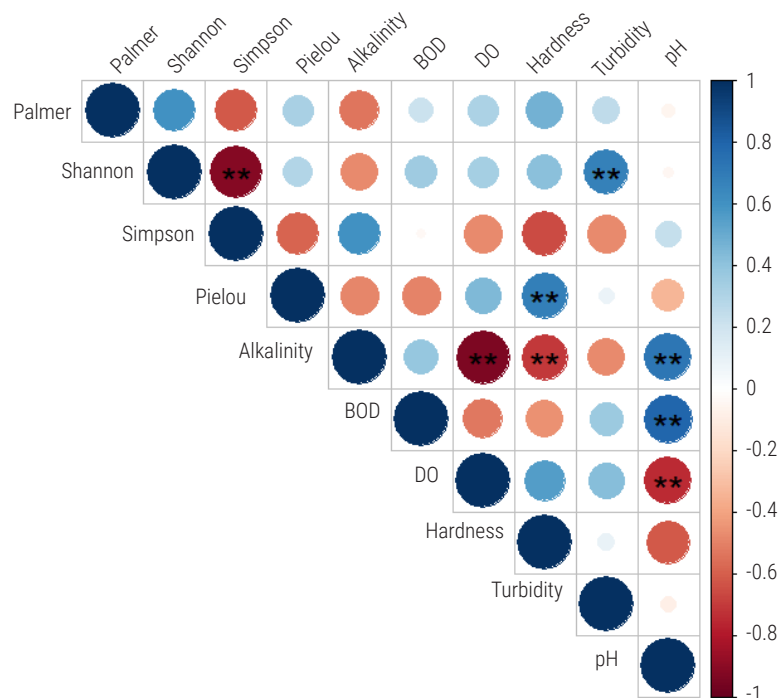


Fig. 4. Pearson correlation matrix showing the relationships among diversity indices, Palmer's score and water quality parameters. Double asterisk (\*\*) indicates a highly significant correlation between variables ( $p < 0.01$ )

Furthermore, a significant positive correlation between BOD and pH ( $r=0.793$ ,  $p=0.011$ ) suggests that higher pH values in the rice-fish fields were associated with increased BOD. Since BOD reflects the amount of biodegradable organic matter being decomposed by microorganisms and pH indicates the acidity/alkalinity of water, this relationship indicates that organic decomposition and the associated oxygen demand may be greater under relatively alkaline conditions. Such a pattern could be linked to enhanced microbial activity or increased nutrient inputs that elevate both organic matter decomposition and pH levels. Together with the consistently high Palmer's index values (Table 5), these findings suggest that the phytoplankton community is responding to substantial organic matter enrichment (OMA) across the study sites.

This study demonstrates that the rice-fish fields of Ziro valley support diverse phytoplankton communities dominated by Bacillariophyceae and Chlorophyceae, indicating their adaptability to variable environmental conditions. High Shannon diversity, evenness, and Palmer scores ( $>20$ ) revealed moderately organic matter enriched yet ecologically balanced systems, where OMA sustains phytoplankton diversity while favouring tolerant genera such as *Gomphonema*, *Lepocinclis*, *Navicula*, *Nitzschia*, *Scenedesmus*, and *Synedra*. Spatial differences, especially the higher abundance and diversity observed at Bamin Michi, are likely associated with stagnant water condition, greater nutrient retention, and reduced grazing pressure. The study also highlights the sensitivity of phytoplankton to OMA, turbidity, and dissolved oxygen dynamics. Overall, phytoplankton composition and diversity

emerged as reliable and cost-effective indicators of OMA in rice-fish agroecosystems. Sustainable management of organic inputs from rice residues, fish waste, and surface runoff coupled with long-term monitoring, will be critical for maintaining ecological balance and enhancing system productivity.

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