



LETTER

Anthropogenic nutrient inputs affect productivity–biodiversity relationships in marine tintinnid assemblages

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Scientific Significance Statement

The productivity–biodiversity relationship (PBR) is highly controversial, yet insights into marine microzooplankton under varying anthropogenic nutrient inputs have been limited. We verified that PBRs varied across contrasting levels of anthropogenic nutrient input, and high productivity promoted greater diversity under low to medium levels than under high levels of anthropogenic nutrient input. Compared to conventional species richness, functional and phylogenetic diversity respond more quickly to nutrient changes and differently to productivity in both long-term and monthly trends, which deliver more information on PBRs and the underlying ecological processes. We also empirically addressed the previous concern regarding methodological inconsistencies in the meta-analysis of PBRs, providing one of the first consistent observational datasets on PBRs in marine unicellular secondary producers. Our study contributes to a comprehensive understanding of PBRs in microzooplankton under contrasting anthropogenic nutrient input conditions.

Abstract

Although the productivity–biodiversity relationship (PBR) has been a hot topic, few studies have considered how anthropogenic pressures affect PBRs in marine microzooplankton. Here, we provide the first insights into PBRs in tintinnid assemblages using 18-yr data from Jiaozhou Bay, a typical coastal bay in the Yellow Sea. We hypothesized and verified that PBRs vary across contrasting anthropogenic nutrient inputs and that functional and phylogenetic diversity would deliver more information than conventional species richness. High productivity promotes more diversity under low to medium rather than high anthropogenic nutrient inputs. Compared

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to species richness, functional and phylogenetic diversity reveal more PBR patterns and respond more quickly in response to varying anthropogenic inputs. A concave+ PBR is revealed for functional diversity in the ecozone with highly active water exchange. Our study contributes to the understanding of PBR in marine unicellular secondary producers and their responses to anthropogenic nutrient inputs in coastal ecosystems.

The productivity–biodiversity relationship (PBR) has been highly controversial over the past few decades (Whittaker 2010; Adler et al. 2011; Willig 2011; Grace et al. 2016; Brun et al. 2019). Five general patterns of PBRs have been distilled, including increasing (positive), humped (unimodal, concave–), decreasing (negative), U-shaped (concave+), and no relationship; however, these patterns, along with their causal processes, face critique (Gillman and Wright 2006; Whittaker 2010). Meta-analyses have been employed to discern the mechanisms underlying these patterns (Mittelbach et al. 2001; Gillman and Wright 2006), but they have raised new problems such as methodological inconsistencies, generating highly divergent outcomes, inconsistent classification of data sets, and mega-mistakes from one meta-analysis to the next (Whittaker 2010).

Biodiversity encompasses multiple attributes such as taxonomic, phylogenetic, and functional diversities, which may have contrasting effects on productivity (Naeem et al. 2016; Le Bagousse-Pinguet et al. 2019). Conventional biodiversity indices (e.g., species richness and Shannon index) without considering species similarity may lead to suspicion about their ecological significance, as species differ in their functions and ecological strategies (Stirling 2007; Leinster and Cobbold 2012). The niches and competitive abilities of species within a community are often reflected in functional and phylogenetic differences, suggesting that functional and phylogenetic diversity may provide a deeper understanding of PBRs (Mayfield and Levine 2010; Brun et al. 2019). These dimensions of biodiversity can reveal underlying ecological processes, such as environmental filtering, competitive exclusion, and evolutionary context (Mayfield and Levine 2010; Brun et al. 2019), as well as responses to nutrient availability and anthropogenic inputs (Prowe et al. 2012; Gamfeldt et al. 2015; Lehtinen et al. 2017), and further support the biodiversity–ecosystem multifunctionality relationship (Flynn et al. 2011; Naeem et al. 2016; Le Bagousse-Pinguet et al. 2019).

Most previous PBR studies have focused on terrestrial macroorganisms, especially multicellular plants, that is, primary producers (e.g., Adler et al. 2011; Willig 2011; Grace et al. 2016; Brun et al. 2019), whereas data on vast marine unicellular plankton remain limited (e.g., Irigoien et al. 2004; Witman et al. 2008). For marine phytoplankton, there is less data available, but a few large-scale studies also suggest that humped PBRs, with maximum diversity occurring at intermediate productivity levels (Li 2002; Irigoien et al. 2004; Smith 2007; Vallina et al. 2014), exhibit significant spatial and temporal variation and relate to nutrient availability

(Vadrucci et al. 2003; Lehtinen et al. 2017) or in some cases, show no relationship (Cermeño et al. 2013).

In contrast, current knowledge regarding the PBRs for microzooplankton (20–200 μm in size) remains limited. As the main consumers of marine primary production, microzooplankton can grow and reproduce rapidly, may respond more quickly to environmental changes, and are better coupled to ecological processes than slow-responding metazoans (Calbet and Landry 2004). However, PBRs of microzooplankton remain hitherto unexplored and highly fragmentary and are mainly based on meta-analyses and sensitive to heterogeneous datasets (Irigoien et al. 2004; Ibarbalz et al. 2019). As a model of plankton ecology, tintinnids are an ideal tool for microzooplankton to explore PBRs with functional and phylogenetic diversity, attributed to their loricae, which are of taxonomic significance and related to ecological characteristics and functional responses (Dolan et al. 2013). Tintinnids have been increasingly used as bioindicators of anthropogenic impacts and environmental changes in plankton ecosystems (e.g., Dolan et al. 2016; Al-Yamani et al. 2019; Wang et al. 2024).

Our study provides one of the first empirical assessments of how PBRs incorporating multidimensional biodiversity respond to contrasting anthropogenic nutrient inputs (e.g., dissolved inorganic nitrogen and phosphate) in tintinnids over an 18-yr cycle. In addition to conventional species richness, we employed functional and phylogenetic diversity indices following Brun et al. (2019). Compared to the terrestrial multicellular primary producers studied by Brun et al. (2019), our work offers a new perspective on PBR patterns for marine unicellular secondary producers (microzooplankton) and examines their responses to anthropogenic nutrient inputs. To obtain convincing evidence, we used consistent, continuous observational sampling in a semi-enclosed coastal bay. Our study contributes to a comprehensive understanding of PBRs in marine microzooplankton, particularly in the context of varying anthropogenic nutrient inputs to temperate coastal ecosystems.

Methods

Study site

We examined tintinnid assemblages in 1280 continuous samples from Jiaozhou Bay Marine Ecosystem Research Station (<https://jzw.qdio.cas.cn/>) at 22 sites in Jiaozhou Bay (JZB, 120°04'E–120°23'E, 35°38'N–36°18'N, Fig. 1) from May 2003 to December 2020. Jiaozhou Bay is a semi-enclosed coastal bay with a narrow channel (about 3 km) connecting to the

Yellow Sea (Liu et al. 2004). With heavy anthropogenic impacts and frequent red tides, JZB has served as an ideal model system to explore the effects of ongoing climate changes and anthropogenic inputs on marine ecosystems (e.g., Feng et al. 2015; Guo et al. 2021; Wang et al. 2023). Anthropogenic nutrient inputs contribute more nutrients to JZB, primarily by rivers, than atmospheric deposition, accounting for most dissolved inorganic nitrogen and phosphate (Liu et al. 2005; Xu et al. 2020). The JZB witnessed the sharp decline of anthropogenic nutrient inputs in 2008 (Xu et al. 2020), with the decreasing phytoplankton, increasing zooplankton, and miniaturization of copepods during 2002–2016 (Wang et al. 2023). We split the sampling sites according to four levels of anthropogenic nutrient inputs based on the division of Zhao et al. (2020) from lowest AP1 to highest AP4 (Fig. 1, Table 1). Anthropogenic nutrient inputs were lowest in AP1 in the open waters outside the bay farthest from the rivers. The hydrodynamic condition was unique for AP2 in the bay mouth with highly active water exchange. Anthropogenic nutrient inputs were relatively high in AP3 surrounded by many seasonal rivers (medium level) and peaked in AP4 in the bay with the primary region for shellfish and algae farming and the largest river, Dagu River, flowing into.

Data collections and calculation

The sampling, identification, and enumeration of tintinnids were conducted and summarized in Part 1 in the

Supporting Information. The counting effort ranged from 1 to 330 individuals per sample. We used biomass as a proxy for plankton productivity, consistent with most empirical studies (e.g., Groner and Novoplansky 2003; Vadrucci et al. 2003; Irigoien et al. 2004; Smith 2007; Vallina et al. 2014). Tintinnid biomass was calculated using the equation: $C \text{ (pg)} = 444.5 + 0.053 \text{ LV}$ (Lorica Volume, Verity and Langdon 1984) μg carbon per L (Table S1). Functional traits and phylogenetic information of tintinnids were summarized (Tables S1, S2) based on microscope observations in this study with reference to the literature (Zhang et al. 2012; Dolan et al. 2013; Santoferrara and Mcmanus 2020; Agatha and Bartel 2022), encompassing vulnerability traits, life history traits, and habitat use.

For diversity, we focused on the common indices, including species richness and quadratic entropies of functional and phylogenetic diversity. To calculate functional and phylogenetic diversity, we employed the workflow of biodiversity space following Brun et al. (2019), by successively defining the scale of species similarity (δ), type of similarity (α), and species dominance effect (q) using functional traits and phylogenetic information (Tables S1, S2). We derived LC_{funct} ($\delta = 1$, $\alpha = 1$, $q = 2$) and LC_{phylo} ($\delta = 1$, $\alpha = 0$, $q = 2$) for functional and phylogenetic diversity, respectively, following Leinster and Cobbold (2012) and Chen and Grinfeld (2024), with detailed procedures in Part 1 in the Supporting Information. All steps were repeated for different groups of communities under four levels of anthropogenic groups inputs.

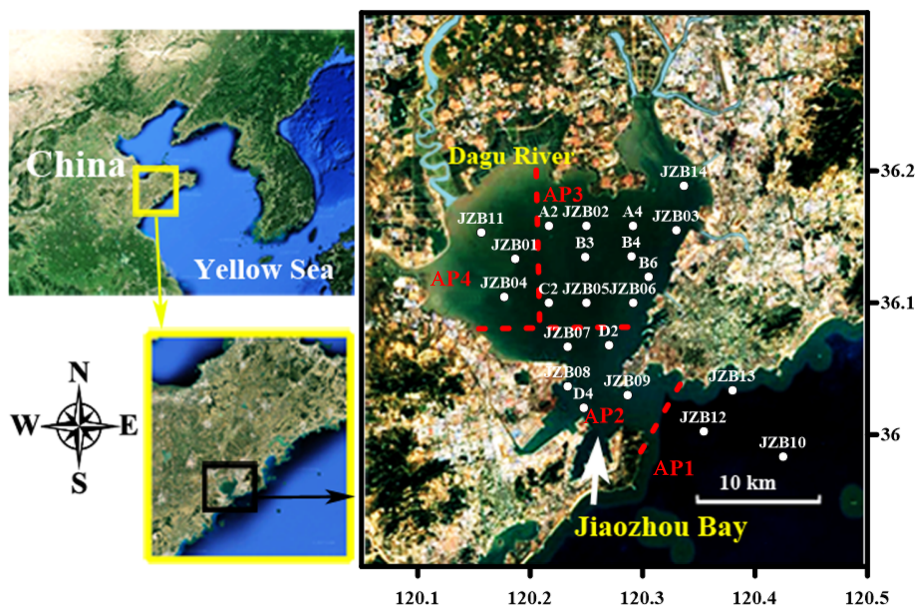


Fig. 1. Study area considering contrasting anthropogenic nutrient inputs in the coastal bay, Jiaozhou Bay (JZB) in the Yellow Sea in northern China from 2003 to 2020. Jiaozhou Bay is a semi-enclosed water body (390 km², averaged depth of 7 m) with a narrow channel (about 3 km) connecting to the Yellow Sea (Liu et al. 2004). Anthropogenic nutrient inputs were lowest in AP1 in the open waters outside the bay farthest from the rivers, increased from AP1 to AP4, and were heaviest in AP4 in the bay where the largest river, Dagu River, flows into (Zhao et al. 2020). The hydrodynamic condition was unique for AP2 in the bay mouth with highly active water exchange. Details are provided in Table 1.

Table 1. Basic information for the samplings under the four levels of anthropogenic nutrient inputs in the coastal bay, Jiaozhou Bay (JZB) in the Yellow Sea in northern China from 2003 to 2020. Samples were collected in different layers at 10-d intervals from 2003 to 2005, and at monthly intervals from 2005 to 2020. The mean values \pm standard deviations of productivity and biodiversity indices are provided, along with the *p* values from ANOVA multiple comparisons. LC_{func} and LC_{phylo} represent functional and phylogenetic diversity using LC biodiversity values at ($\delta = 1, \alpha = 1, q = 2$) and ($\delta = 1, \alpha = 0, q = 2$), respectively.

Region	Characteristics	Sites	Sampling periods	<i>n</i>	Productivity (log-transformed biomass)			Richness			LC_{func}			LC_{phylo}		
					Mean	<i>p</i> value		Mean	<i>p</i> value		Mean	<i>p</i> value		Mean	<i>p</i> value	
AP1	Offshore area of the Yellow Sea, representing open sea and adjacent waters.	JZB10, JZB12, JZB13	2003–2020	449	-0.73 \pm 0.71 (-2.83–1.53)	AP1-AP2 0.001, AP1-AP3 0.004, AP1-AP4 0.305	6.46 \pm 3.4 (1–21)	AP1-AP2 0.937, AP1-AP3 0.533, AP1-AP4 0.005	2 \pm 0.79 (1–6.26)	AP1-AP2 0.002, AP1-AP3 0.000, AP1-AP4 0.000	1.24 \pm 0.14 (1–1.69)	AP1-AP2 0.159, AP1-AP3 0.021, AP1-AP4 0.002				
AP2	Deep-water channel at bay mouth, with frequent water exchange and significant physical-biological changes.	A2, A4, B3, B4, C2, JZB07, JZB08, JZB09	2003–2005	181	-0.46 \pm 0.73 (-2.42–0.99)	AP2-AP3 0.500, AP2-AP4 0.000	6.28 \pm 3.04 (1–17)	AP2-AP3 0.979, AP2-AP4 0.049	1.77 \pm 0.64 (1–4.53)	AP2-AP3 0.996, AP2-AP4 0.188	1.21 \pm 0.13 (1–1.6)	AP2-AP3 1.000, AP2-AP4 0.269				
AP3	Inner bay, surrounded by many rivers, mostly seasonal rivers.	B6, D2, D4, JZB02, JZB03, JZB05, JZB06, JZB14	2003–2020	563	-0.56 \pm 0.82 (-3.03–1.47)	AP3-AP4 0.002	6.17 \pm 3.54 (1–21)	AP3-AP4 0.043	1.79 \pm 0.68 (1–4.63)	AP3-AP4 0.071	1.21 \pm 0.15 (1–1.69)	AP3-AP4 0.168				
AP4	Inner bay influenced by the largest river in Jiaozhou Bay (Dagu River), serving as a primary region for shellfish and algae farming.	JZB01, JZB04, JZB11	2003–2005	87	-0.89 \pm 1.01 (-3.05–1.19)		5.14 \pm 3.13 (1–12)		1.59 \pm 0.55 (1–2.92)		1.18 \pm 0.15 (1–1.63)					

n indicates the number of samplings. Productivity uses log-transformed biomass ($\mu\text{g/L}$). AP indicates anthropogenic nutrient input levels. Characteristics and division of anthropogenic nutrient input levels were sourced from Zhao et al. (2020), with consideration of Xu et al. (2020).

Functional traits and phylogenetic information of tintinnids were summarized (Tables S1, S2) based on microscope observations conducted in this study, with reference to the literature (Zhang et al. 2012; Dolan et al. 2013; Santoferrara and Mcmanus 2020; Agatha and Bartel 2022). These summaries encompassed vulnerability traits, life history traits, and habitat use.

Statistical analyses

We first assessed the significant long-term trends for productivity (biomass) and biodiversity with the Mann–Kendall test using the R package “Kendall” (Frenken et al. 2023). Then we explored the PBRs using generalized additive models (GAMs, *see* details in Part 1 in the Supporting Information) in R after checking for the outliers (Adler et al. 2011). MANOVA was applied to test the effects of anthropogenic nutrient inputs on productivity and biodiversity. Akaike’s Information Criterion (AIC) and explained variances were deployed to evaluate the models, excluding variables with high concurrency (the GAM equivalent of collinearity). No significant spatial autocorrelation was found after including latitude and longitude as extra predictors in the models, following Le Bagousse-Pinguet et al. (2019).

We used REML to estimate model coefficients. The PBRs were then classified into different classes based on the goodness of fit (R^2) and curve shape criteria (Table S5) according to Brun et al. (2019). Those curves with $R^2 < 0.15$ were labeled as non-significant as per Brun et al. (2019).

Results

General variations of productivity and biodiversity

The productivity and three focal diversity measures of the tintinnid assemblages varied significantly across the four levels of anthropogenic nutrient inputs (MANOVA, $p < 0.01$). Tintinnids under the highest anthropogenic nutrient input (AP4) showed relatively low productivity and diversity compared to those under medium levels (AP2 and AP3), with significant differences in productivity ($p < 0.01$) and species richness ($p < 0.05$). However, no significant differences were observed in the functional or phylogenetic diversity (Table 1; $p > 0.05$). In contrast, at the lowest anthropogenic input level (AP1), tintinnids exhibited significantly lower productivity than at medium levels (AP2 and AP3, $p < 0.01$), but higher values at all three biodiversity levels than at other levels. Significant differences were observed in terms of functional diversity between AP1 and each of the other three levels ($p < 0.001$) but only between AP1 and AP4 in terms of species richness ($p < 0.01$).

In addition, the GAM results revealed that temporal variability (both long-term and monthly) affected the productivity and diversity of tintinnid assemblages (Table S3). Similar to productivity, species richness and phylogenetic diversity showed a small peak in 2008, followed by a general decrease,

whereas functional diversity showed a general increase (Fig. S1).

Effect of anthropogenic nutrient inputs on the PBRs

As for the PBRs, the GAM results (Table S4) showed that levels of anthropogenic nutrient inputs and the interaction effects of productivity, month, and year were all significant predictors of species richness ($R^2 = 0.64$; deviance explained 65%), functional diversity ($R^2 = 0.22$; deviance explained 23%), and phylogenetic diversity ($R^2 = 0.34$; deviance explained 36%). Similar trends in the interaction between productivity and month were revealed for the three biodiversity indices, showing greater effects on diversity in August, with the highest productivity. In contrast, functional and phylogenetic diversity responded quickly to the sharp decline in anthropogenic nutrient inputs in 2008, with the former increasing (Fig. 2d) and the latter decreasing (Fig. 2f). Species richness responded more slowly and increased only after 2010 (Fig. 2b).

After keeping the other parameters constant, the relationships between productivity and the three biodiversity indices displayed contrasting patterns across different levels of anthropogenic nutrient input (Fig. 3). For species richness, increasing PBRs prevailed at low to medium levels of anthropogenic nutrient inputs (Fig. 3a–c), whereas a concave– PBR was revealed in AP4, which had the highest anthropogenic nutrient inputs (Fig. 3d). Similarly, under the highest level of anthropogenic nutrient input, concave– PBRs were found for both functional and phylogenetic diversity (Fig. 3h,i). All three diversity indices showed a concave– PBR at the highest level, revealing that high anthropogenic nutrient inputs promoted decreased diversity at high productivity.

Compared to species richness, functional and phylogenetic diversity revealed a new perspective on PBRs under different levels of anthropogenic nutrient inputs. Species richness failed to show evident changes in PBRs (all increasing) between AP1 and AP3; however, both functional and phylogenetic diversity revealed different PBRs. For AP1, under the lowest anthropogenic inputs, both functional and phylogenetic diversity revealed a concave– PBR (Fig. 3e,i), showing decreasing functional and phylogenetic diversity along with increasing species richness at high productivity. Notably, functional diversity revealed a distinct PBR pattern from others, concave + PBRs, in AP2 at the bay mouth with highly active water exchange and medium anthropogenic inputs, showing low functional diversity at intermediate levels of productivity (Fig. 3f).

The interaction between productivity and month significantly affected diversity (Table S4; $p < 0.01$), with its effects varying across the four levels of anthropogenic nutrient input (Fig. 4). For species richness, a pronounced effect was observed around August, coinciding with high productivity. In contrast, the effects were broader for functional and phylogenetic diversity, particularly for functional diversity at high levels of anthropogenic nutrient input (Fig. 4h) and phylogenetic

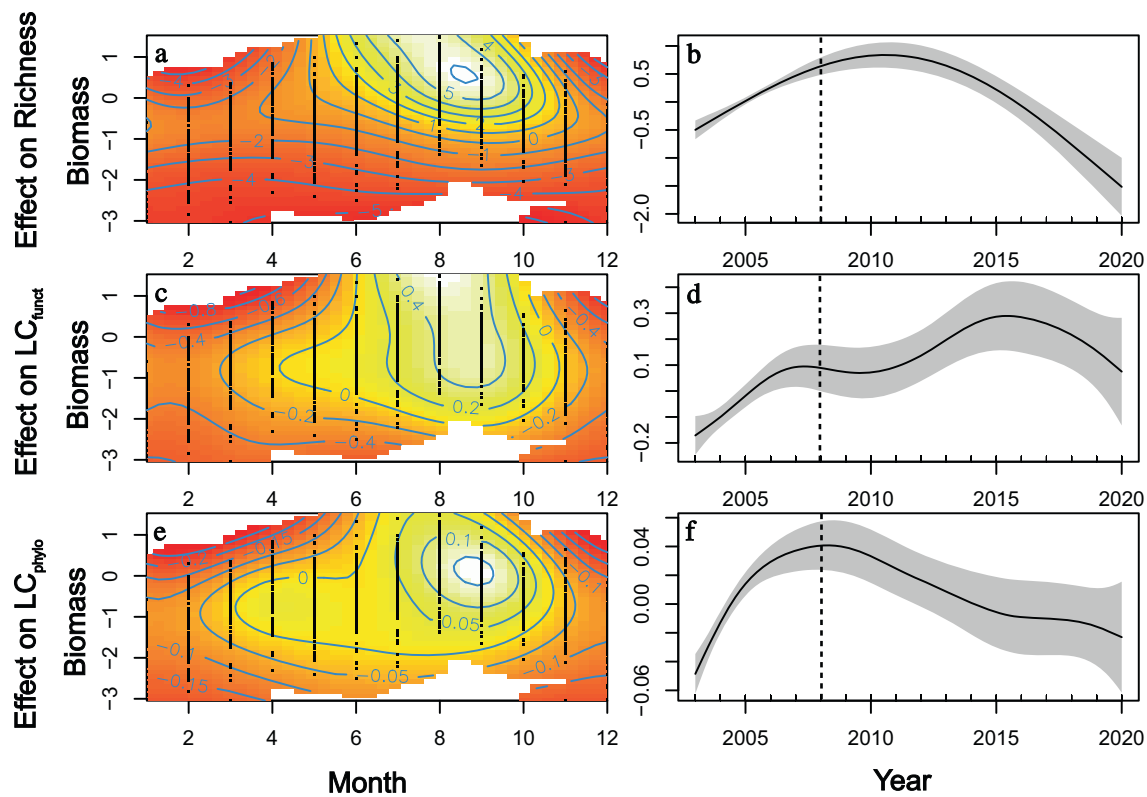


Fig. 2. Generalized additive model response of diversity to the interactions between productivity and month, year, and levels of anthropogenic nutrient inputs (AP) in Jiaozhou Bay during the studied period. Biomass (log-transformed, $\mu\text{g/L}$) was used as a proxy of productivity following Irigoien et al. (2004). Diversity including species richness (a–b), functional diversity (LC_{func}, c–d), and phylogenetic diversity (LC_{phylo}, e–f) are compared among months and years. Overlaid curves show the partial effect of productivity (log-transformed biomass) on the diversity in GAM fits with 95% confidence intervals. LC_{func} and LC_{phylo} represent functional and phylogenetic diversity using LC biodiversity values at ($\delta = 1$, $\alpha = 1$, $q = 2$) and ($\delta = 1$, $\alpha = 0$, $q = 2$), respectively.

diversity at medium to high levels of anthropogenic nutrient input (Fig. 4k,l).

Discussion

Our study firstly verified that PBRs varied across contrasting levels of anthropogenic nutrient input in unicellular microzooplankton assemblages in coastal marine ecosystems. We found that increasing PBRs prevail at low to medium levels of anthropogenic nutrient input for species richness, indicating that high productivity promotes diverse communities with low to medium levels of anthropogenic nutrient input. This corresponds to the niche differentiation and limiting similarity hypotheses, which assume that competitive exclusion favors complementary strategies and promotes assemblages with diverse traits (Pacala and Tilman 1994; Brun et al. 2019).

In contrast, concave– (humped) PBRs prevailed in AP4 for all three diversity indices, indicating that with high anthropogenic nutrient inputs, diversity increased at low productivity and decreased at high productivity. This is consistent with the

speculation that humped PBRs in marine zooplankton result from a balance between food limitations at low population levels and selective predation at high population levels (Irigoien et al. 2004; Vallina et al. 2014). According to the most prominent historical hypothesis, biodiversity is low in unproductive environments because of environmental filtering, increases with increasing resource supply until it reaches the coexistence limits at high productivity, and declines because of increased competition and competitive exclusion (Grime 1973; Al-Mufti et al. 1977; Grace et al. 2016; Brun et al. 2019). Productive communities are less diverse with high anthropogenic nutrient inputs, probably because competition can eliminate more distinct and less related taxa (Mayfield and Levine 2010). In addition, this result may provide another possible explanation for the negative PBRs of global marine plankton (Ibarbalz et al. 2019), since they used phylogenetic diversity, rather than taxonomic diversity, which is more related to previously reported functional traits.

We further verified that, in response to varying anthropogenic nutrient inputs, functional and phylogenetic diversity offers more insights into PBRs and underlying ecological

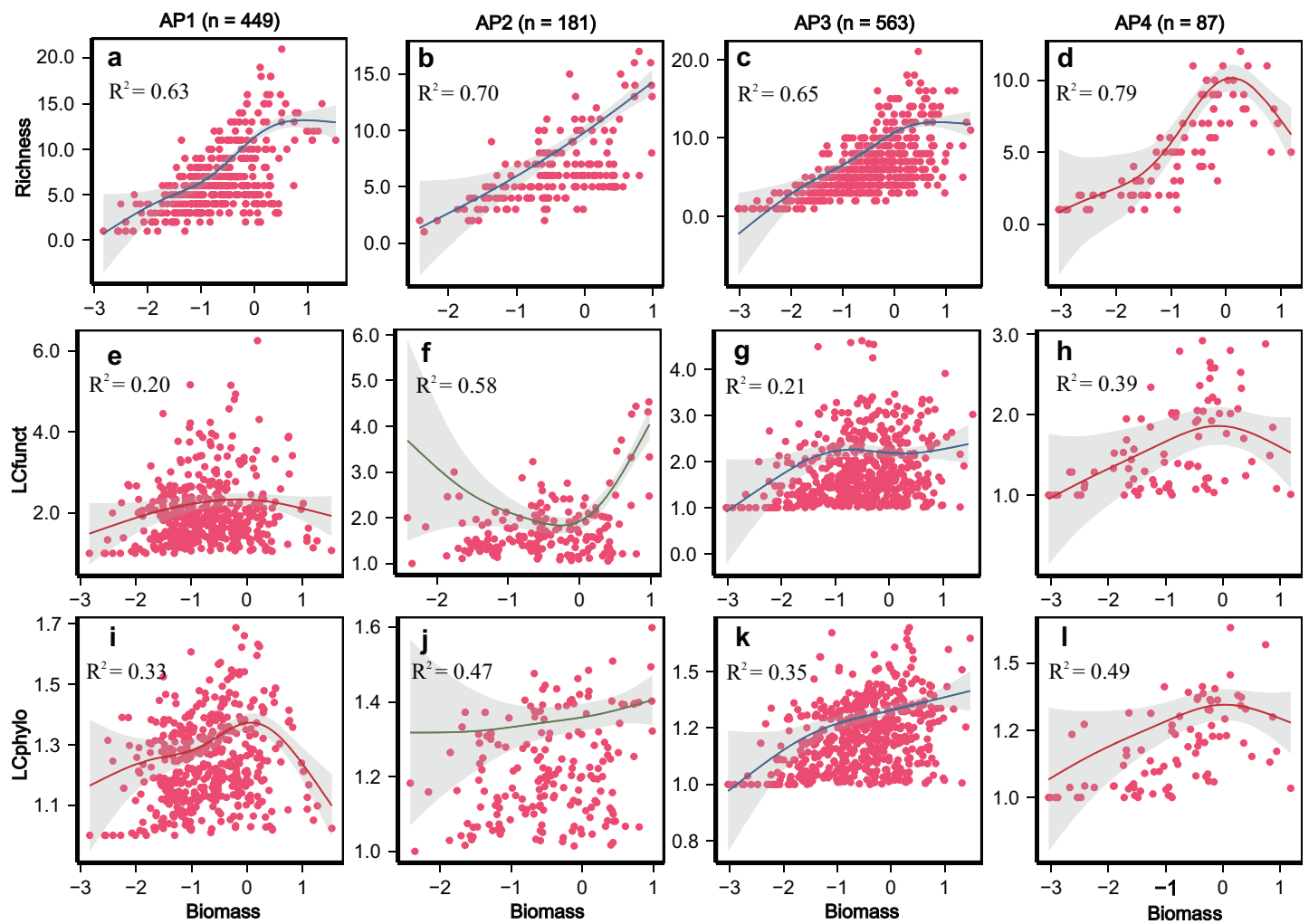


Fig. 3. Generalized additive model response curves of diversity to productivity of tintinnid assemblages at four levels of anthropogenic nutrient inputs in Jiaozhou Bay during the studied period. Biomass (log-transformed, $\mu\text{g/L}$) was used as a proxy of productivity following Irigoien et al. (2004). Diversity including species richness (a–d), LC_{funct} (e–h), and LC_{phylo} (i–l) are compared between communities of different levels of anthropogenic nutrient inputs: low (AP1, a, e, i), medium with active water exchange (AP2), medium (AP3), and high (AP4). Overlaid curves show the partial effect of productivity (log-transformed biomass) on the diversity in GAM fits with 95% confidence intervals. Overlaid curves are colored according to curve type classification: red is concave–; blue is increasing; dark green is Concave+. LC_{funct} and LC_{phylo} represent functional and phylogenetic diversity using LC biodiversity values at ($\delta = 1, \alpha = 1, q = 2$) and ($\delta = 1, \alpha = 0, q = 2$), respectively.

processes than conventional species richness, especially at low to medium levels of anthropogenic nutrient inputs. We found more PBR patterns in functional and phylogenetic diversity at low to medium levels of anthropogenic nutrient input, which were not revealed for species richness. Furthermore, functional and phylogenetic diversity responded faster than species richness to the reduction in anthropogenic nutrient loads around 2008. This is consistent with the expected importance of functional and phylogenetic diversity in supporting the biodiversity–ecosystem multifunctionality relationship (Flynn et al. 2011; Le Bagousse-Pinguet et al. 2019).

Notably, the concave+ (U-shaped) PBR in AP2 had not been extensively discussed before the meta-analysis work by Mittelbach et al. (2001), and the interaction of productivity-

driven processes with other processes may be a probable explanation (Scheiner and Jones 2002). This also explains why we only found the concave+ PBR in AP2, the ecozone of all the other three levels of anthropogenic nutrient inputs and highly active water exchange. Nevertheless, different scales and statistical methods have been reported to lead to different classifications of concave+ PBRs (Mittelbach et al. 2001), and some have been classified as humped or having no relationship (Mittelbach et al. 2001; Gillman and Wright 2006; Whittaker 2010). The classification and causal processes of PBRs remain highly controversial (Gillman and Wright 2006). Most of the associated hypotheses on PBRs are derived from terrestrial systems and are probably limited when applied to marine systems.

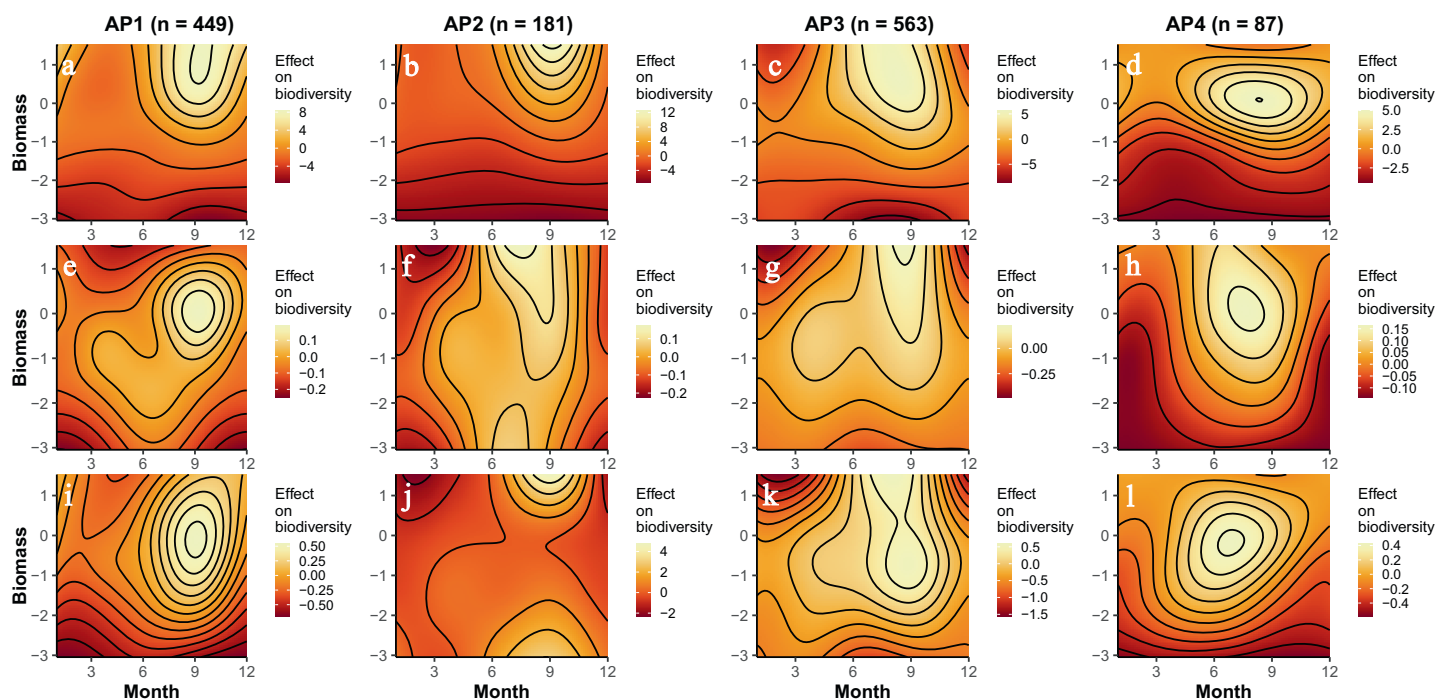


Fig. 4. Generalized additive model response of diversity to the interaction between productivity and month at four levels of anthropogenic inputs in Jiaozhou Bay during the studied period. Biomass (log-transformed, $\mu\text{g/L}$) was used as a proxy of productivity following Irigoien et al. (2004). Diversity including species richness (a–d), LC_{funct} (e–h), and LC_{phylo} (i–l) are compared between communities of different levels of anthropogenic nutrient inputs: low (AP1, a, e, i), medium with active water exchange (AP2), medium (AP3), and high (AP4). LC_{funct} and LC_{phylo} represent functional and phylogenetic diversity using LC biodiversity values at ($\delta = 1, \alpha = 1, q = 2$) and ($\delta = 1, \alpha = 0, q = 2$), respectively.

Anthropogenic nutrient inputs would affect the PBRs of plankton communities through trophic links, including top-down and bottom-up controls (Vallina et al. 2014). Serving as secondary producers and key links between phytoplankton and copepods, tintinnid communities decreased in productivity, species richness, and phylogenetic diversity, in line with generally decreasing nutrients and phytoplankton, and increasing zooplankton during the investigated period (Wang et al. 2023). However, our study also found increasing functional diversity for tintinnids, especially after 2008; that is, tintinnids were more diverse in ecological functions and less diverse in richness and phylogeny, which might be due to the generally decreasing abundance ratio of large-sized copepods. The competition vulnerability trade-off (top-down control) allows stable predator-mediated coexistence of species within and across functional groups, whereas the growth-affinity trade-off (bottom-up control) allows the non-equilibrium coexistence of functional groups ranging from nutrient specialists to nutrient opportunists, resulting in humped PBRs in phytoplankton (Vallina et al. 2014). The positive effect of nutrient supply on species diversity only occurred in the presence of predators because the two processes do not act in isolation (Worm et al. 2002). Other processes should be considered to explain the potential contrasting responses of PBRs to anthropogenic nutrient inputs, including the

sampling regime, scale of analysis, and processes such as community assembly.

We acknowledge the limitation of the use of tintinnid biomass as a productivity proxy. In PBR studies, productivity is estimated either directly from the organisms' biomass or energy, or indirectly through lower trophic level productivity and surrogates, with the PBR pattern being sensitive to the directness of productivity estimates in animal studies but not in plant studies (Groner and Novoplansky 2003). Herein we used biomass as a productivity proxy, consistent with most empirical studies (e.g., Irigoien et al. 2004; Vallina et al. 2014), but there are limitations to this approach. We stressed biomass (for more contribution in the productivity) rather than rate, among the three aspects of productivity (Clarke et al. 1946), because rate relies on empirical data and temperature and might introduce more bias in the productivity estimation in our long-term samples spanning large temperature ranges. Rate (production rate) is also important for tintinnid productivity, calculated as the product of biomass and empirically specific growth rates (g) for tintinnids (Godhantaraman 2002). However, g was derived from freshwater ciliates, which differ from marine tintinnids in our study in thermal performance and growth rates (Lukić et al. 2022). Furthermore, growth rates differ between tintinnids and ciliates: they increase allometrically with cellular biomass for tintinnids

(Heinbokel 1978; Stoecker et al. 1983; Verity 1985, 1986; Godhantaraman 2002), but not for all planktonic ciliates (Lukić et al. 2022; Weisse 2024). Allometric considerations remain important in planktonic food webs, as direct methods for measuring tintinnid growth in natural assemblages are still limited (Dolan et al. 2013). In summary, this proxy should be used with caution, and additional field data are needed for more robust conclusions on tintinnids and other microzooplankton.

Additionally, meta-analyses have been increasingly employed as powerful tools for PBR studies, but they have also been criticized for relying on data collected from previous studies using inconsistent methods (Whittaker 2010; Adler et al. 2011). Our study provides one of the first insights into PBRs in marine microzooplankton using continuous data obtained by conducting consistent and continuous observational sampling, which further empirically contributes to addressing methodological inconsistencies and convincing conclusions. Nevertheless, it is important to acknowledge that standardized observational studies have limitations, such as uncontrolled environmental factors, which restrict the analysis to correlations rather than causations and fail to address the paradox: Is diversity the cause or consequence of productivity? (Cardinale et al. 2009). Additionally, in relation to resource use strategies and ecological processes, the functional traits in our study were limited, lacking aspects such as trophic structure and disturbance (Hillebrand and Matthiessen 2009; Grace et al. 2016), which also led to limitations in the statistical analysis. Further exploration and integrative modeling are required to confirm our findings and explore the variation in PBRs under anthropogenic impacts in coastal marine ecosystems.

Author Contributions

Meiping Feng and Bingzhang Chen conceived of the presented idea. Meiping Feng, Wenhua Bian, Wuchang Zhang, Chaofeng Wang, Xiaoxia Sun, and Shujin Guo conducted the field samplings and lab work. Meiping Feng, Wenhua Bian, Jichen Qiu, Yunfan Zhang, Siting Hu, Yijia Yang, Na Li, and Jun Lin compiled the original data. Jichen Qiu, Meiping Feng, Bingzhang Chen, and Kailin Liu performed statistical analysis. Meiping Feng wrote the manuscript with support from Bingzhang Chen. All authors critically reviewed and approved the manuscript.

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Conflicts of Interest

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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