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Harmful algal blooms significantly reduce the resource use efficiency in a coastal plankton community

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ABSTRACT

Harmful algal blooms (HABs) have been investigated for their catastrophic effects on public health and aquaculture intensively, but the research about HABs effects on the diversity patterns and intrinsic functions of the plankton community based on a species identification with high resolution and accuracy has been scarce. We therefore investigated the shifts of plankton diversity via pyrosequencing during and around a natural dinoflagellate (*Prorocentrum donghaiense*) bloom and analyzed the effect of *P. donghaiense* abundance on the operationally-defined resource use efficiency (RUE) of plankton community to test our hypothesis that outbreaks of HABs will reduce RUE of the plankton community via shifting the plankton community structure, species composition in particular. We found that the species diversity of eukaryotic plankton community was significantly decreased during the bloom, as reflected in OTU (operational taxonomic unit) richness, and Pielou’s evenness index. Principal coordinates analysis indicated significant difference in plankton community structure between blooming and non-blooming periods. As hypothesized, the species richness was positively correlated to RUE (defined as the ratio of phytoplankton biomass to total phosphorus), and more importantly, the cell density of *P. donghaiense* exhibited significant negative correlation with RUE. Our results explicitly demonstrated HABs reduce RUE via reducing species richness (corresponding to a less occupancy of the trophic niches), which supports the previously documented notion that niche partitioning enhances RUE (a key ecosystem function). Also, our work provides striking evidence for the relationship between
plankton species richness (or diversity) and community function (resource use efficiency) via studying on HABs, a natural but exceptional phenomenon, in addition to revealing a profound consequence of HABs.

Keywords: Ecosystem functions, Harmful algal blooms (HABs), Plankton diversity, Resource use efficiency (RUE), Trophic niche partitioning mechanism
1. Introduction

The most unique feature of Earth is the existence of life, and the most extraordinary feature of life is its diversity (Cardinale et al., 2012). The species diversity is vital to natural ecosystems, as it could enhance ecosystem productivity at higher levels of richness (Cardinale et al., 2012; Fraser et al., 2015; Grace et al., 2016; Isbell et al., 2015; Tilman et al., 2012; Willig, 2011), balance and increase stability at the levels of community and ecosystem (McCann, 2000), and underpin ecosystem services (Hooper et al., 2012; Oliver, 2016; Oliver et al., 2015). Plankton, as the vital components of marine ecosystem, underpin the global biological and geochemical processes (de Vargas et al., 2015). Due to the importance of plankton for marine ecosystem, plankton diversity has received considerable attention. Species diversity of plankton communities are, however, sensitive to changes in the environmental conditions of water bodies, reflecting the quality and health of water, and thus indicating the influence of environmental factors and processes (Bianchi et al., 2003; Cairns et al., 1993; Chai et al., 2018; Lee et al., 2012; Leppard and Munawar, 1992).

The relationship between species diversity and ecosystem functioning is a central issue in ecology (Bell et al., 2005; Brandenburg et al., 2018; Cardinale et al., 2006; McGrady-Steed et al., 1997; Ptacnik et al., 2008; Steiner et al., 2010). Studies based on artificial communities of phytoplankton and other microbes proved that community processes carried out by microbes were related to diversity, which were similar to those in macroscopic realm (Bell et al., 2005; Cardinale et al., 2006; McGrady-Steed et al., 1997; Steiner et al., 2010). However, such artificial
communities normally contain limited numbers of species that are significantly fewer than those in natural communities, raising a concern about to what extent such artificial communities represent natural responses. On the other hand, Ptacnik et al. (2008) investigated the relationship between phytoplankton diversity and ecosystem functions with over 3,000 natural phytoplankton samples collected from Scandinavian lakes and the Baltic Sea and, particularly, tested whether the variation in natural phytoplankton diversity affects resource use efficiency (RUE). RUE is an ecological concept that is defined as the amount of biomass produced per unit of supplied resource, which indicates that a higher proportion of resources turned into new biomass corresponds to a higher level of realized productivity and thus RUE (Hodapp et al., 2019). Although there are a number of inconsistencies regarding its application (e.g. highly divergent approaches are used to calculate RUE; using standing stock of biomass and total pools of nutrients instead of productivity rates and bioavailable nutrients for RUE calculation; application of RUE to single element rather than multiple co-limiting resources), the concept has been commonly applied to explain and understand the link between potential and realized productivity or biodiversity effects on ecosystem biomass production, which therefore constitutes a concept of major interest to study scientific questions in various ecological contexts (Hodapp et al., 2019 and references there). In freshwater and marine plankton ecosystems, phytoplankton biomass (e.g. particulate carbon, biovolume, or chlorophyll-α concentration) divided by the limiting resource (nitrogen or phosphorus) gives the yield as RUE for the community. Ptacnik et al. (2008) used the limiting resource
(total phosphorus, TP) as a proxy for potential system productivity and expressed the phytoplankton resource use efficiency ($RUE_{chl}$) as the ratio of phytoplankton biomass (chlorophyll-$a$, chl. $a$) to TP. By identifying and counting phytoplankton microscopically to the genus level, they found that $RUE_{chl}$ and the genus richness ($G$) of phytoplankton exhibited a highly positive correlation, i.e. phytoplankton resource use efficiency was directly linked to the diversity of phytoplankton communities (Ptacnik et al., 2008). This result provoked us to make a testable hypothesis about the effect of harmful algal blooms (HABs) on the function of plankton community (explained below).

During the past decades, coastal regions of the world have been affected by harmful algal blooms (HABs) seriously and caused massive losses in aquaculture, fisheries, human health, tourism, and ecosystems (Anderson et al., 2012). Most HABs are caused by rapid proliferation ("blooming") of one or a few toxic or deleterious species of microalgae (Anderson et al., 2012), although there is no universally accepted standard for the cell density that defines a bloom, as the cell density that a bloom can reach highly depends on what is the blooming species and particularly its harmful effects (i.e. some species such as *Dinophysis* spp. may cause serious toxic effects at cell densities of hundreds cells per liter) (Smayda, 1997). Studies on the negative effects of HABs have heretofore mainly focused on valuable animals and environmental factors such as dissolved oxygen and nutrients. However, investigations on the effect of HABs on plankton community structure and succession have been rare, and were generally based on single trophic level and microscopic
counting (Abbas et al., 2012), except for one recent publication reporting the effects
of a natural dinoflagellate bloom on the microbial community structure and
succession via metagenomic approach (Zhou et al., 2018). According to the
definitions of Pielou’s evenness index (Pielou, 1969), it can be logically inferred that
the evenness index should be decreased during HABs. From 1910s, the term "niche"
has been an *explanans* of species diversity: diverse species coexist because each
occupies its own niche (i.e. the niche is specific to each species, two species
coexisting in the same place must occupy different niches) (Pocheville, 2015).
Therefore, the change of species richness would give rise to the higher or lower niche
partitioning (or, niche occupancy). Previous studies have documented that niche
partitioning enhances resource use efficiency (Cardinale, 2011; Ye et al., 2019).
However, there has only one report about the species richness during HABs via
pyrosequencing (Nagai et al., 2019). While the bloom of a species will certainly lead
to the reduction of evenness, whether or not the bloom causes the reduction of
richness will depend on both the blooming intensity and, more importantly, whether
the blooming species has "harmful" effects on its competitors. Furthermore, to date,
no studies have investigated the effects of HABs on the resource use efficiency of
plankton communities. Therefore, based on the finding of Ptacnik et al. (2008)
described above, we hypothesized that outbreaks of HABs will reduce the RUE of the
plankton community via shifting the plankton community structure, species
composition in particular.

To test this hypothesis, we investigated the variations of planktonic diversity via
pyrosequencing amplicons of the 28S rRNA gene during and around a bloom caused
by the dinoflagellate Prorocentrum donghaiense, and analyzed the relationships
among the abundance of P. donghaiense, the species richness, and RUE$_{chl}$. We
believe testing this hypothesis is important in terms of understanding further the
relationship between species diversity and ecosystem functions, and the ecological
consequences of HABs.

2. Materials and methods

2.1. Sample sites

The study area Sansha Bay is located at the northeast to Ningde, Fujian Province
(26°44.5′-26°54.5′ N; 120°10.9′-120°11.3’ E), one of Fujian Province’s major
aquaculture waters in the East China Sea (Fig. 1), where blooms of the dinoflagellate
species, P. donghaiense and Karenia mikimotoi, are frequently observed (Lin et al.,
2014; Lu et al., 2005). From March to July, 2016, we conducted 5 cruises and
sampled four sites at every cruise in the study area (Table S1). The sample IDs
include sampling sites (A, B, C, D, or E), sampling dates (0331, 0422, 0503, 0513,
0719), and the lower-case letters referring to duplicate samples. For example, the
sample IDs A0331a and A0331b refers to the first and second samples from the site A
taken on March 31, respectively. We collected a total of 38 samples (excluding two
samples failed in DNA extraction).

2.2. Sample collection
Water samples were taken from 0.5 m below the surface and transferred into 5 L polyethylene buckets. Water samples (1 L) for counting cells of *P. donghaiense* were fixed with Lugol's iodine solution (final concentration, 2%), settled for 48 h, carefully concentrated to 50 mL via siphoning, and 1 mL of the 50 mL concentrated sample was counted using a Sedgewick-Rafter counting chamber under an inverted light microscope (IX73, Olympus, Japan). There were no duplicate samples for the cells counting, therefore, 20 water samples in total were collected for counting cells of *P. donghaiense*. Water samples for DNA extraction were collected by filtering 1.5 L water through a hydrophilic polycarbonate membrane (47 mm diameter, 0.4 μm pore size, Merck Millipore Ltd., Germany) with duplicates, put into an icebox (<10 °C) on the sampling boat and transferred to -20°C within 4 hours when coming back to the on-shore sampling base, and then transferred to -80 °C in the laboratory until DNA extraction. Samples for NO$_3^-$-N, NO$_2^-$-N, NH$_4^+$-N, PO$_4^{3-}$-P, and SiO$_2^{2-}$ measurements were filtered through Whatman GF/C filters (47 mm in diameter, 1.2 μm in pore size; Whatman, Kent, UK), and added 2 drops of chloroform per 100 mL sample (Grashoff et al., 1999; Murphy and Riley, 1956). Samples for total nitrogen (TN) and total phosphorus (TP) measurements were pretreated by adding 2 drops of 98% sulfuric acid per 100 mL sample (Grashoff et al., 1999; Valderrama, 1981). Water samples for Chl. *a* (500 mL each) were filtered onto Whatman GF/F glass fiber filters (47 mm in diameter, 0.7 μm in pore size; Whatman, Kent, UK) and stored frozen at -20 °C until analysis. All samples were immediately transported to the laboratory in cold conditions and subjected to measurements of the nutrients and Chl. *a*.
2.3. Environmental data measurement

Water temperature and salinity were measured on site using a hand-held thermometer (BoBang Ltd., China) and a digital refractometer (Atago Ltd., Japan), respectively. Measurements of NO$_3^-$-N, NO$_2^-$-N, NH$_4^+$-N and PO$_4^{3-}$-P, and SiO$_3^{2-}$ were conducted colorimetrically using a nutrient analyzer (Skalar Ltd., Netherland) according to the protocols of JGOFS report No. 19 (Knap et al., 1994). For TN and TP analyses, samples were digested using potassium persulfate under 115 °C for 30 min according to the protocol (Valderrama, 1981) and the digested samples were then analyzed colorimetrically using the abovementioned nutrient analyzer. Chl. $a$ filtered onto the glass fiber filters was extracted with 90% aqueous acetone overnight, analyzed using the acidified method fluorometrically with a Turner Designs fluorometer (Parsons et al., 1984). The ratio of TN to TP was calculated and compared to the Redfield Ratio (Redfield, 1958) to judge whether or not our study area was limited by nitrogen or phosphorus.

2.4. RUE$_{chl}$ definition and calculation

The definition and calculation of RUE$_{chl}$ was adopted from Ptacnik et al. (2008), using the limiting resource, TP, as a proxy for potential system productivity (i.e. resource availability) and expressing the phytoplankton RUE as the ratio of phytoplankton biomass (chl. $a$) to TP (RUE$_{chl}$ = chl. $a$ : TP).
2.5. Primer design, DNA extraction, PCR amplification, pyrosequencing and pyrosequencing data preprocessing

The primer design, DNA extraction, PCR amplification, pyrosequencing and pyrosequencing data preprocessing were the same as described in our previous study (Chai et al., 2018). The detailed preprocessing of pyrosequencing data were as follows: all the raw data were processed using the UPARSE pipeline for 28S rRNA data sets ([http://drive5.com/uparse/](http://drive5.com/uparse/)) (Edgar, 2013); The adapters and barcodes of sequences were trimmed off using the default parameters; Sequence length and quality were evaluated for each read; and those sequences were removed if they contained ambiguous base calls, were shorter than 200 bps, had the average SFF quality score <20. Also, PCR chimeras were filtered out using Chimera Slayer (Haas et al., 2011). Sequences were denoised using the “pre.cluster” command which applies a pseudo-single linkage algorithm with the goal of removing sequences that are likely due to pyrosequencing errors (Huse et al., 2010; Roeselers et al., 2011; Zhang et al., 2012).

2.6. Statistical and bioinformatic analyses

After the above-described pyrosequencing data preprocessing, to fairly compare the 38 samples at the same sequencing depth, a normalization of the sequence reads was conducted by extracting the minimum number of sequences in each sample for all the following analyses (i.e. the reads number of each taxon is a relative number out of the normalized total reads in a sample (10,000 reads)). Aligned sequences were
clustered into operational taxonomic units (OTUs) based on the widely used 97% similarity (identity) criterion (e.g. Buée et al., 2009; Campbell and Kirchman, 2013; Chai et al., 2018; Fortunato et al., 2012; Herlemann et al., 2011; Öpik et al., 2009) using the average neighbor algorithm. It is noteworthy that while a higher criterion of similarity (e.g. 99%) would reveal more realistic genetic diversity in populations and the communities, as applied in the recently published investigations (Sildever et al., 2019; Nagai et al., 2019), the widely used criterion of 97% similarity is not likely to lose the species diversity because 97% similarity has been commonly used as a threshold to define a species (e.g. Albanese et al., 2015), although exceptions exist in the literature. The taxonomic annotation of OTUs was done by Global Alignment for Sequence Taxonomy (GAST) process (Huse et al., 2008) against the NCBI GenBank database. Based on our previously published study (Chai et al., 2018), all the dinoflagellates in this study were categorized as phytoplankton in terms of their taxonomic status (i.e. generally called "microalgae") but not in their ecological functions. The taxonomic levels we used to group OTUs for community structure analyses ranged from “species” to “class” level. The group annotation (i.e. whether an OTU belongs to phytoplankton, protist, or zooplankton) of those OTUs with identities to their respective reference sequences in the GenBank ranging from 90% to 97% was based on the species assignment of the reference sequence, while that of those OTUs with identities to their reference sequences ranging from 80% to 90% was based on the genus assignment. Those OTUs that could not be annotated to species or genus levels with certainty (with identities to reference sequences below 80%) had to be
categorized as “unclassified” group because of the highly limited availability of reference sequences in the GenBank at present. The Pielou’s evenness index was used as the community diversity parameter and calculated as described in the Mothur software manual (http://www.mothur.org/) (Schloss et al., 2009). Principal coordinates analysis (PCoA) based on the Bray–Curtis distance matrix was conducted at the OTU level with the community ecology package (http://www.mothur.org/) (Gower, 2005; Schloss et al., 2009). The linear regression coefficients between two variables and their significance tests were calculated using the software SPSS 22.0. The significance level was set at 0.05 for all tests unless otherwise stated.

3. Results

3.1. Effects of HAB on the community structure of eukaryotic plankton

The rarefaction curve of high-throughput sequencing reached saturation (Fig. S1), indicating sufficient sequencing depth for the samples. After filtering the low-quality reads using the UPARSE pipeline, trimming the adapters and barcodes, and removing chimeras, 2.4 million sequences (2,984 OTUs) were obtained from 38 samples (including replicates). After blasting against the GenBank of NCBI using BLASTn, all OTUs were categorized into five groups: phytoplankton (including dinoflagellates), protists (excluding dinoflagellates), other zooplankton (excluding protists), fungi, and unclassified, with the number of OTUs being 1,361, 76, 379, 20, and 1,162, respectively. The average cell densities of *P. donghaiense* in April 22 (3.24×10^4 cells mL^-1), May 3 (16.96×10^4 cells mL^-1), and May 13 (2.22×10^4 cells
mL\(^{-1}\)) were significantly higher than those in March 31 (0.71\(\times\)10\(^3\) cells mL\(^{-1}\)) and July 19 (0.11\(\times\)10\(^3\) cells mL\(^{-1}\)) \((P<0.001\), one-way ANOVA\), and this trend was similar to the reads number of \(P.\) donghaiense in the pyrosequencing data. The maximum cell density of \(P.\) donghaiense in March 31 and July 19 was 1.15\(\times\)10\(^3\) cells mL\(^{-1}\), while the cell densities of \(P.\) donghaiense of the most samples in April 22, May 3 and May 13 were over 1.5\(\times\)10\(^4\) cells mL\(^{-1}\). Therefore, in this study, the cell density of 10\(^3\) cells mL\(^{-1}\) was defined as a cut-off between non-bloom and bloom of \(P.\) donghaiense. A previous study on \(P.\) donghaiense blooms in China also defined a cell density of 10\(^3\) cells mL\(^{-1}\) as a cut-off between non-bloom and bloom of \(P.\) donghaiense (Lu et al., 2014). The cell density of \(P.\) donghaiense had a significant positive correlation with the reads number of \(P.\) donghaiense \((n=38, R^2=0.5649, P<0.0001)\) (Fig. S2). Therefore, the dates March 31 and July 19 were categorized respectively as pre-blooming and after-blooming periods of \(P.\) donghaiense while the dates April 22, May 3, and May 13 were categorized as the blooming period. The plankton community structures during the blooming period were significantly different from that in pre-blooming and after-blooming periods (Fig. S3; Fig. 2a). Redundancy analysis showed that the reads number of \(P.\) donghaiense (a relative reads number out of the normalized total reads of sequences (i.e. 10,000 reads)) was the most critical factor in controlling the dynamics of eukaryotic plankton communities in the studied coastal ecosystem (Fig. S4). The Pielou’s evenness index during blooming period was significantly lower than that in pre-blooming and after-blooming periods, and this diversity index was strongly and negatively correlated to the reads number of \(P.\)
The species richness (expressed as the number of OTUs) showed a significant negative correlation with the reads number of *P. donghaiense* (\(P<0.001\); Fig. 2b), especially during the blooming period (\(P=0.0005\); Figs. 2c and 2d). To clarify whether or not the negative correlation between the dominance of *P. donghaiense* vs richness (OTUs) was caused by autocorrelation, we analyzed the correlations between the richness and the reads number of two diatoms ("Uncultured Chaetoceros" and *Chaetoceros tenuissimus*) that were the second and third most common phytoplankton species in our samples and were close to blooming levels in the samples of July 19 (posterior to *P. donghaiense* bloom). We also did the similar correlation analyses for an "Uncultured Prasinophyceae" and an "Unclassified alveolate" that were common in the samples prior to *P. donghaiense* bloom and abundant in the samples of March 31. As shown in Fig. S6, the abundances of "Uncultured Chaetoceros" and *Chaetoceros tenuissimus* exhibited either no significant or even positive correlation with the richness, while the cell abundances of both the "Uncultured Prasinophyceae" and "Unclassified alveolate" had a negative correlation with the richness. However, it is noteworthy that the significance levels of negative correlation for *P. donghaiense* were higher than that for "Uncultured Prasinophyceae" and "Unclassified alveolate" during their respective blooming periods (*P. donghaiense*: \(n=19, R^2=0.517, P=0.0005\); Uncultured Prasinophyceae: \(n=8, R^2=0.6884, P=0.01085\); Unclassified alveolate: \(n=8, R^2=0.4505, P=0.0684\)).

Therefore, these results indicated that it was the bloom of *P. donghaiense*, rather than the autocorrelation, that caused the significant decrease of species richness due to the
harmful effects of the species.

3.2. Species richness as a predictor of RUE

The TN and TP concentrations of all samples ranged from 18.54 \( \mu \text{M} \) to 199.23 \( \mu \text{M} \) and from 0.58 \( \mu \text{M} \) to 11.64 \( \mu \text{M} \), respectively, and the molar ratios of TN to TP ranged from 17.11:1 to 87.68:1, which were greater than the Redfield Ratio (16:1) and thus indicated the limiting resource in our research area was phosphorus. RUE\(_{\text{chl}}\) (= chl. a: limiting resource (TP)) exhibited a significant positive correlation with the species richness (number of OTUs) of all eukaryotic plankton (phytoplankton, zooplankton, fungi, and unclassified taxa; ranged from 82 to 821) for the entire dataset (n=38, \( R^2=0.1384, P<0.05 \); Fig. 3). Considering the zooplankton community only, the number of OTUs varied from 9 to 235, which was also significantly correlated with RUE\(_{\text{chl}}\) (n=38, \( R^2=0.2220, P<0.01 \); Fig. 3). The number of OTUs for the phytoplankton community, ranging from 58 to 507, was also positively correlated to RUE\(_{\text{chl}}\) (n=38, \( R^2=0.1092, P=0.052 \); Fig. 3). These results demonstrated that species richness acted as an important predictor of the RUE\(_{\text{chl}}\) of phytoplankton community.

3.3. Blooms of P. donghaiense significantly reduced RUE\(_{\text{chl}}\)

Based on the cell densities of P. donghaiense, all samples were categorized into bloom group (cell density > 1000 cells mL\(^{-1}\)) and non-bloom group (cell density < 1000 cells mL\(^{-1}\)) (Fig. 4a), a grouping consistent with the non-significant correlation
between the cell density of *P. donghaiense* and RUE$_{chl}$ (Fig. 4b; the dots are separated as blooming and non-blooming groups). The non-bloom samples were collected on March 31 (pre-blooming period) and July 19 (after-blooming period), while all samples collected on April 22, May 13, and May 31 belonged to the bloom group except for one control sample from May 13 (see explanation below). The cell density of *P. donghaiense* in blooming samples exhibited a strong negative correlation with RUE$_{chl}$ (n=10, R$^2$=0.8360, P=0.0002; Fig. 4c), while the cell density of *P. donghaiense* in non-blooming (including pre- and post-blooming) samples was also negatively correlated with RUE$_{chl}$ (n=9, R$^2$=0.6562, P<0.0001; Fig. 4d). The R$^2$ value for the blooming samples (0.8360) was much higher than that for non-blooming samples (0.6562), indicating a more pronounced effect of *P. donghaiense* on RUE$_{chl}$ during blooming period. These results demonstrated that the cell density of *P. donghaiense* almost entirely explained the RUE$_{chl}$ variation in blooming samples (i.e. the higher cell density of *P. donghaiense*, the lower the value of RUE$_{chl}$ of the plankton community), suggesting that it was the bloom of *P. donghaiense* that significantly reduced the RUE. Note that the correlation between the cell density of *P. donghaiense* and RUE was not significant if both the blooming and non-blooming samples were plotted together, which will be discussed below.

During the blooming period, one sample (A0513a) was collected on May 13 from a non-blooming area as a control (cell density of *P. donghaiense* 30 cells mL$^{-1}$). When we analyzed the correlation between RUE$_{chl}$ and the cell density of *P. donghaiense* with this sample included, the R$^2$ value was dramatically reduced from 0.8360 (Fig.
4c) to 0.0134 (Fig. 4e, $P > 0.05$). If this sample was included in the regression analysis between RUE$_{chl}$ and the cell density of $P. $donghaiense for non-blooming samples, the $R^2$ value was reduced from 0.6532 (Fig. 4d) to 0.3829 (Fig. 4f, $P =$ 0.0047). The significant effect of this single "outlier" on the significance of regression analysis clearly demonstrated that the bloom (i.e. high cell density) of $P. $donghaiense played a critical role in regulating RUE$_{chl}$ of the phytoplankton community, while other factors also played important roles for samples from non-blooming period and area, which is consistent with the above-described fact that the correlation between the cell density of $P. $donghaiense and RUE was not significant when both the blooming and non-blooming samples were plotted together (Fig. 4b).

4. Discussion

4.1. HABs affect the plankton community structure

OTU clustering is a key step for the definition of the microbial diversity and taxonomic composition of the analyzed samples. In our study, aligned sequences were clustered into operational taxonomic units (OTUs) based on the widely used 97% similarity (identity) criterion. We noticed Sildever et al. (2019) and Nagai et al. (2019) used $\geq 99.1\%$ similarity as the threshold to define their OTUs, which would reveal more realistic intra-populational and intra-specific genetic diversities. However, 97% has been a widely used threshold to cluster sequences into OTUs in numerous environmental genomic studies (Buée et al., 2009; Campbell and Kirchman, 2013; Chai et al., 2018; Fortunato et al., 2012; Herlemann et al., 2011;
Öpik et al., 2009; Sun et al., 2014; Wu et al., 2012; Zhou et al., 2018), because 97% similarity represents the common working criterion for conspecific entities (Albanese et al., 2015). Powell et al. (2011) characterized AM fungal diversity in natural systems using both 97% and 99% similarity criteria to define OTUs and found ≥ 99% similarity threshold is likely overestimating species richness.

In our study, the primers amplified the most dominant groups: dinoflagellates, diatoms, and copepods. It has been well known that dinoflagellates, diatoms, and copepods are the most dominant groups in coastal waters (Beaugrand et al., 2014; Blaxter et al., 1998; Heiskanen, 1998), indicating that our primers are universal enough to amplify all eukaryotic plankton presented in our samples. This study demonstrated that the bloom of *P. donghaiense* affected the structure of eukaryotic plankton community (including phytoplankton, zooplankton, fungi, and unclassified eukaryotes) in terms of reducing the species richness (as reflected in the number of OTUs) and Pielou’s evenness index. All these parameters showed a significant negative correlation with the reads number of *P. donghaiense* (equivalent to the cell abundance). Our correlation analyses for other common and abundant species that were close to blooming levels prior or posterior to the bloom of *P. donghaiense* indicated that the significant negative correlation between the reads number of *P. donghaiense* and richness was mainly caused by the "harmful" effects of *P. donghaiense* bloom rather than autocorrelation. Furthermore, the composition of plankton community changed with the transition or development of *P. donghaiense* bloom. For example, the plankton community was respectively dominated by a genus
which has not been well described (annotated as "Eukaryota-noname"), *Prorocentrum*, and *Chaetoceros* for the periods of pre-, during, and after-blooming. As shown in PCoA analysis, the plankton community during the blooming period was significantly different from that before and after blooming. When the abundance of *P. donghaiense* was low (before and after the bloom), the species richness, and Pielou’s evenness index varied significantly, understandably, due to other factors such as water temperature and nutrients availability. While these results are not greatly surprising, in the literature, however, there has been a very few investigations on the effects of HABs on plankton community structure and succession (Nagai et al., 2019; Zhou et al., 2018), particularly so for the study having a comprehensive inventory of all planktonic species obtained via the high throughput next generation sequencing approach. Both of the two recent work reported the effects of *Alexandrium catenella* bloom at the microbial community level via pyrosequencing and correlation approaches, which demonstrated that the microbial community structure (species composition, etc.) was strongly linked to the bloom progression of *Alexandrium catenella* (Nagai et al., 2019; Zhou et al., 2018), which is generally consistent to the results presented in this study. While both abiotic and biotic factors affect simultaneously the structure and dynamics of plankton community (Lima-Mendez et al., 2015), however, abiotic factors (e.g. temperature, salinity, and nutrient availability) have been historically investigated much more intensively and appreciation of biotic factors (e.g. species competition) has been growing only recently (Chai et al., 2018; Lima-Mendez et al., 2015; Worden et al., 2015; Zhou et
al., 2018). Our study demonstrated that a harmful algal bloom can be a vital driving force for the transition of entire plankton community (i.e. from high to lower evenness and species richness and thus high to lower resource use efficiency) due to the biological features of the blooming species (e.g. potent allelopathy, higher nutrient affinity and growth rate, etc.). For example, most of HABs-causing species have been demonstrated to be allelopathic (i.e. interactions among phytoplankton via releasing chemicals) to other co-occurring phytoplankton via releasing allelochemicals (Felpeto et al., 2018; Leao et al., 2012; Leflaive and Ten-Hage, 2007; Legrand et al., 2003).

4.2. HAB reduces \( RUE_{chl} \) of phytoplankton community via reducing species richness

\( RUE_{chl} \) showed strong and positive relationships with species richness (i.e. number of OTUs in this study) in phytoplankton, zooplankton, and overall plankton communities, indicating that species richness acted as a predictor of \( RUE \) in plankton community. In our study, OTUs-level diversity estimates could reflect not only species-level diversity, but also sub-species level diversity (e.g. ribotype diversity) of plankton community, which might be more precise than previous work based on genus-level diversity estimates (Ptacnik et al., 2008). Our results from the natural plankton communities soundly proved that the increasing species richness in community can result in higher resource use efficiency, as reviewed for artificial ecosystems and theoretical studies (Bolnick et al., 2011; Cardinale, 2011; Hooper et al., 2005). Although \( RUE_{chl} \) by definition, represents the resource use efficiency of phytoplankton only, because of that zooplankton graze on phytoplankton (i.e.
bottom-up, or cascading, mechanism; phytoplankton act as the resource of zooplankton; Filstrup et al., 2014; Gasol et al., 1997; Sinistro, 2009; Yuan and Pollard, 2018), and analogous to H. T. Odum’s concept of transfer efficiency (Hodapp et al., 2019), the positive correlation between RUE$_{chl}$ and zooplankton richness was also observed. The positive correlation between species richness and RUE$_{chl}$ was the most pronounced for zooplankton community, amongst phytoplankton, zooplankton, and overall plankton, while that between RUE$_{chl}$ and species richness of phytoplankton is more pronounced than that for the overall plankton community. In addition, the positive relationship became more pronounced in community with lower species richness, indicating the effect of diversity on resource use efficiency is stronger for less diverse communities. These results are well in agreement with the previous study (Ptacnik et al., 2008) in terms of the relationship between phytoplankton diversity and RUE$_{chl}$.

Most importantly, the *P. donghaiense* cell density in blooming samples showed a strong negative correlation with RUE$_{chl}$ which indicates that HABs could significantly reduce the RUE of plankton community. The only study on the effects of HABs on the level of microbial community mentioned above was focusing on the community succession along the progression of a bloom of *Alexandrium catenella* (Zhou et al., 2018). To the best of our knowledge, our work is the first study investigating the effects of HABs on the ecosystem functions from the perspective of resource use efficiency and thus the productivity in a natural marine plankton system. The significant negative correlation between species richness (OTUs) and the
abundance of *P. donghaiense* indicates that the species richness was markedly decreased by the blooming of *P. donghaiense*. Since different species of phytoplankton also differ in their affinity to a certain form of nutrients (e.g. phosphorus) (Guedes et al., 2019; Marinho et al., 2013; Olsen, 1989; Olsen et al., 1989; Wu et al., 2009) understandably, the decrease of species richness results in less occupancy of trophic niches and thus less overall RUE (i.e. consumption of the same amount of nutrients led to less primary production). Our results are supported by the theoretical prediction that greater numbers of coexisting species with different and complementary niches will more thoroughly utilize limiting resources than communities with fewer species (Tilman et al., 1997). Effects of niche differentiation on nutrient uptake have been either assumed to occur based on theoretical arguments (Chesson, 2000; Tilman et al., 2012) or inferred from post hoc statistical analyses of experiments where different biological mechanisms were not able to be separated (Loreau and Hector, 2001). Because of the lack of direct evidence confirming a biological mechanism, there has been considerable debate about why diverse ecosystems tend to be more efficient at sequestering dissolved nutrients (Hooper et al., 2005; Huston, 1997; Loreau et al., 2001). Cardinale (2011) reported that niche partitioning could allow ecosystems to capture a greater proportion of biologically available resources (nitrate) using artificial phytoplankton communities in the laboratory, and Ye et al. (2019) demonstrated that niche partitioning enhances resource use efficiency. The term niche has been used as an explanans of diversity since 1910s (i.e. diverse species coexist because each occupies its own niche;
Pocheville, 2015). In our study, the observed positive correlation between $\text{RUE}_{\text{chl}}$ and plankton species richness, and the negative correlation between $\text{RUE}_{\text{chl}}$ and the cell density of *P. donghaiense* together indicate that it was mainly the decrease of species richness (thus less occupancy of the trophic niches) that accounted for the decrease of $\text{RUE}_{\text{chl}}$ during the bloom of *P. donghaiense*. Given that the decrease of richness would lead to lower niche partitioning, our results that HABs decreased the richness and $\text{RUE}_{\text{chl}}$ support the previously documented notion that niche partitioning enhances RUE. It is noteworthy that Ptacnik et al. (2008) studied the effects of species diversity on the $\text{RUE}_{\text{chl}}$ based on the richness of microscopically identified genera, from which we created our hypothesis and adopted the definition of $\text{RUE}_{\text{chl}}$, while our study is based on a much more comprehensive inventory of species obtained via high throughput sequencing and focuses on the negative effects of HABs on the ecosystem functions.

### 5. Conclusions

In summary, the results fully support our initial hypothesis that HABs can significantly reduce the RUE of plankton community. Our results also suggest that HABs reduce $\text{RUE}_{\text{chl}}$ by reducing the species richness and thus leading to less occupancy of trophic niches. We believe this work provides important insights into the ecological consequences of HABs at the ecosystem level and supports the previously documented notion that niche partitioning (or higher niche occupancy) enhances resource use efficiency, and more generally, provide striking evidence for
the relationship between species diversity and ecosystem functions.

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Declaration of competing interest

The authors declare no competing interests.

Data access statement

The raw sequence data have been deposited to the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/)(accession No. PRJNA498163).
Appendix A. Supplementary Information

Supplementary information includes 6 figures (Fig. S1-S6) and 1 table (Table S1).
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Figure legends

Fig. 1. Sampling locations in Sansha Bay, Ningde, China.

Fig. 2. Principal coordinates analysis (PCoA) plot and correlation between the reads number of *P. donghaiense* and richness (OTUs). The “reads number” means the read numbers out of the normalized total numbers of sequences (i.e. 10,000 reads). (a) The PCoA of all the samples. The PCoA based on the Bray–Curtis distance matrix generated from rarefied taxon abundance and depicting patterns of beta diversity for eukaryotic plankton communities. Correlation between reads number of *P. donghaiense* and richness (OTUs) of all samples (b), of samples during blooming period (c), and of each sample time during blooming period (d). The horizontal box plots show the reads number of *P. donghaiense* for each dataset. Color codes refer to the single dataset.

Fig. 3. Resource use efficiency (RUE$_{chl}$) as a function of diversity (species richness, as expressed with the number of OTUs). The horizontal box plots show the reads number of *P. donghaiense* for each dataset. Color codes refer to the single dataset.

Fig. 4. *P. donghaiense* cell density of all the samples and the correlation between *P. donghaiense* cell density and RUE$_{chl}$. (a) Based on the cell density of *P. donghaiense*, all the samples (n=20) were divided into blooming and non-blooming groups. Correlation between RUE$_{chl}$ and *P. donghaiense* cell density of all the samples (n=20) (b), of all the blooming samples (c), of the non-blooming samples belonging to pre- and after-blooming periods (d), of all the blooming samples and the single control sample (red dot) (e), and of the non-blooming samples and the single control sample
(red) (f). The dashed line on (b) indicates the threshold of blooming cell density of \( P.\)
donghainense.

**Highlights**

- HABs decreased OTU richness of eukaryotic plankton community
- HABs changed community structure of eukaryotic plankton community
- HABs reduced resource use efficiency of phytoplankton community
- Species richness exhibited to be a predictor of resource use efficiency
- The work revealed a profound consequence of HABs to the community function

**Conflict of interest statement**

The authors declare no conflicts of interest to this work.

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2\textsuperscript{nd} November 2019

Dear Professors Damià Barceló, Jay Gan and Sergi Sabater,
TITLE: Harmful algal blooms significantly reduce the resource use efficiency in a coastal plankton community

AUTHORS: Zhao Yang Chai, Huan Wang, Yunyan Deng, Zhangxi Hu, Ying Zhong Tang

We now re-submit the revised version of the above titled Full Paper for publication in Science of the Total Environment. We would like to thank you very much for your efforts and giving us the opportunity to revise the manuscript. We are also highly grateful of the two reviewers' careful and constructive reviews. From the "Response to Reviewers" attached to the submission, you will see we have carefully considered and addressed all questions, concerns, and suggestions raised by the two reviewers. For instance, we cited more than 25 new references to clarify key concepts (e.g. RUE, species diversity, harmful algal blooms, etc.) and other issues concerned the reviewers. We made changes in accordance to the reviewers' suggestions in almost all cases, but in a few cases we decided not to make the suggested changes based on the reasons that you will see in our Response. While we itemized the two reviewers' comments in our Responses according to their original order, we also highlighted our modifications and insertions in the main text of the revision (the document named as “Revised manuscript with changes marked”). We believe that by addressing the issues raised by these reviewers, the quality of the manuscript has been significantly improved, which we hope will satisfy your expectation.

Thank you again for considering this manuscript for publication in Science of the Total Environment.

Sincerely yours,

Ying-Zhong Tang