

Spatial patterns of functional diversity and composition in marine benthic ciliates along the coast of China

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ABSTRACT: Large-scale patterns of community composition and diversity along environmental gradients have been well studied for macroorganisms. However, the biogeography of microorganisms, especially ciliated protozoa, remains understudied. Here, we analyzed a comprehensive database of marine benthic ciliates found along the coast of China from 1991 to 2018 to examine the geographical patterns in species and trait composition and functional diversity. According to redundancy analysis conducted at large spatial scales, **environmental variables, i.e. habitat type and salinity, explained more variance in species composition than latitude.** In contrast, trait composition was better explained by spatial and climatic variables. At small spatial scales, both species and trait composition were probably influenced by mass effects due to the high dispersal ability of ciliates at such small scales, resulting in spatially homogenized communities. Several traits, including body size, feeding type and mobility, exhibited significant positive or negative latitudinal gradients. Functional diversity showed a significant positive correlation with latitude between 20 and 40° N, which may be caused by certain groups of ciliates possessing particular traits related to temperature. Our study is the first comprehensive evaluation of how trait composition and functional diversity of marine ciliated protozoa vary at large scales and can thus make a major contribution to the study of microbial biogeography.

KEY WORDS: Protist · Spatial ecology · Trait composition · Latitudinal gradients · Mass effects

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1. INTRODUCTION

Over the past few years, the geographic distribution of microorganisms has become a focus of biogeographic studies (e.g. Dolan et al. 2006, Martiny et al. 2006, Vyverman et al. 2007, Soininen 2012, Azovsky & Mazei 2013, Soininen et al. 2016). Due to the high dispersal rates and large population sizes of microbes, their spatial distribution patterns and underlying mechanisms may fundamentally differ from those of macroorganisms (Martiny et al. 2006, Soininen 2012). Historically, 2 opposing views about the biogeography of microorganisms have gained great attention: first is the hypothesis of Baas-Becking (1934, p. 15):

'everything is everywhere, but, the environment selects'. The rationale behind this view is the niche assembly mechanism or species sorting, i.e. species are filtered by environmental factors to occur in suitable conditions. Based on this idea, the cosmopolitan model was suggested by Finlay et al. (1996, 1999), which maintained that the distribution of microbes is fundamentally different from that of macroorganisms and not limited by geographical barriers and distance. The second view is the moderate endemism model suggested by Foissner (1998, 1999), which postulates that a proportion of microorganisms (e.g. flagship species) have restricted modern geographical distributions, which may reflect their original

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geographic ranges on ancient continents. A growing number of microbial studies have recently indicated that the relative importance of environmental and spatial variables on microbes depends on study scale and ecosystem types (Langenheder & Ragnarsson 2007, Martiny et al. 2011, Soininen et al. 2011, Chytrý et al. 2012, Soininen 2012, Heino et al. 2014).

In addition to species distributions, the latitudinal gradient of species richness is one of the most important topics in macroecology of multicellular organisms (Hillebrand 2004). However, studies on latitudinal patterns of microorganisms have been rarer (but see Rutherford et al. 1999, Dolan et al. 2006, Fierer & Jackson 2006, Vyverman et al. 2007, Fuhrman et al. 2008, Barton et al. 2010, Passy 2010, Azovsky & Mazei 2013, Soininen et al. 2016). Collectively, these studies have found various relationships between latitude and microbial diversity. For example, Passy (2010) found a U-shaped latitudinal pattern for freshwater diatom diversity in the USA, while a study on the global distribution of lake benthic diatoms revealed a hump-shaped relationship, with the highest diversity detected in a temperate zone (Vyverman et al. 2007). For microbes, the potential drivers of latitudinal diversity patterns include local environmental variables, such as salinity, pH and habitat availability, and climatic factors, especially temperature (Rutherford et al. 1999, Vyverman et al. 2007, Passy 2010, Azovsky & Mazei 2013, Soininen et al. 2016).

In addition to taxonomical species composition, there is a growing interest in the spatial patterns of trait composition and functional diversity among microbes (e.g. Green et al. 2008, Soininen et al. 2016). As traits reflect the ‘things that organisms do’, these can be more directly linked to species fitness or performance than taxonomical identity (Petchey & Gaston 2006). Moreover, functional diversity considers organisms as dynamic entities that interact with their environment (Calow 1987, Laureto et al. 2015). Therefore, investigating geographical patterns of trait composition and functional diversity as well as their potential driving factors can provide a better understanding of the relationships between communities and environmental conditions (McGill et al. 2006, Villéger et al. 2008, 2011). Consequently, trait-based approaches can make science more predictive and able to forecast ecosystem alterations occurring under rapid environmental changes.

Ciliates, a major group of unicellular eukaryotes, contribute significantly to the quantity and biodiversity of microorganisms and the energetics of microbial communities (Fenchel 1967). Ciliated protozoa

have been well characterized taxonomically and functionally compared with other groups of protozoa, which makes it possible to study their spatial patterns using both species and trait composition. However, studies on spatial patterns of ciliate communities at large scales, especially on trait composition and functional diversity, are scarce.

China has a long coastline ranging from the tropics to the temperate zone. These coastal areas are highly heterogeneous, including divergent habitat types such as sandy beaches, rocky reefs, salt marshes and mangroves, which makes it ideal to examine how environmental and spatial factors in concert contribute to shaping ciliate distributions. Our aim was to address the following specific questions: (1) How does benthic ciliate community composition vary along the coast of China at taxonomical and trait levels, and what are the main factors driving its variation? (2) Does the functional diversity of coastal benthic ciliate communities show a latitudinal gradient?

2. MATERIALS AND METHODS

2.1. Database

The ciliate data were compiled from different sources. The data from the coastlines of the Chinese Bohai Sea, Yellow Sea and South China Sea were obtained from the marine ciliate biodiversity survey conducted by the Laboratory of Protozoology, Ocean University of China, from 1991 to 2018 (Fig. 1). We collected all species found in benthic habitats, i.e. intertidal sandy beach and mangrove, during this period. The data sources include 2 monographs (Song et al. 2009, Hu et al. 2019) and all papers published by the Laboratory of Protozoology, Ocean University of China (for a list of papers, see <http://scxy.ouc.edu.cn/lplb/13776/list.htm>). Species without specific sampling locations were excluded from the final list. The sampled habitats along the Bohai Sea and Yellow Sea coasts include only sandy beaches, while those along the South China Sea coasts include both sandy beaches and mangroves. For each site, species occurrences include data over the entire time period (1990s–2018) to reduce insufficient sampling effort in each single sample. The Yangtze River estuary data originated from Xu et al. (2018b). This data set contained 3 sampling sites in salt marsh habitat (Fig. 1). In order to facilitate taxonomic consistency between different data sets, species-level data were as-

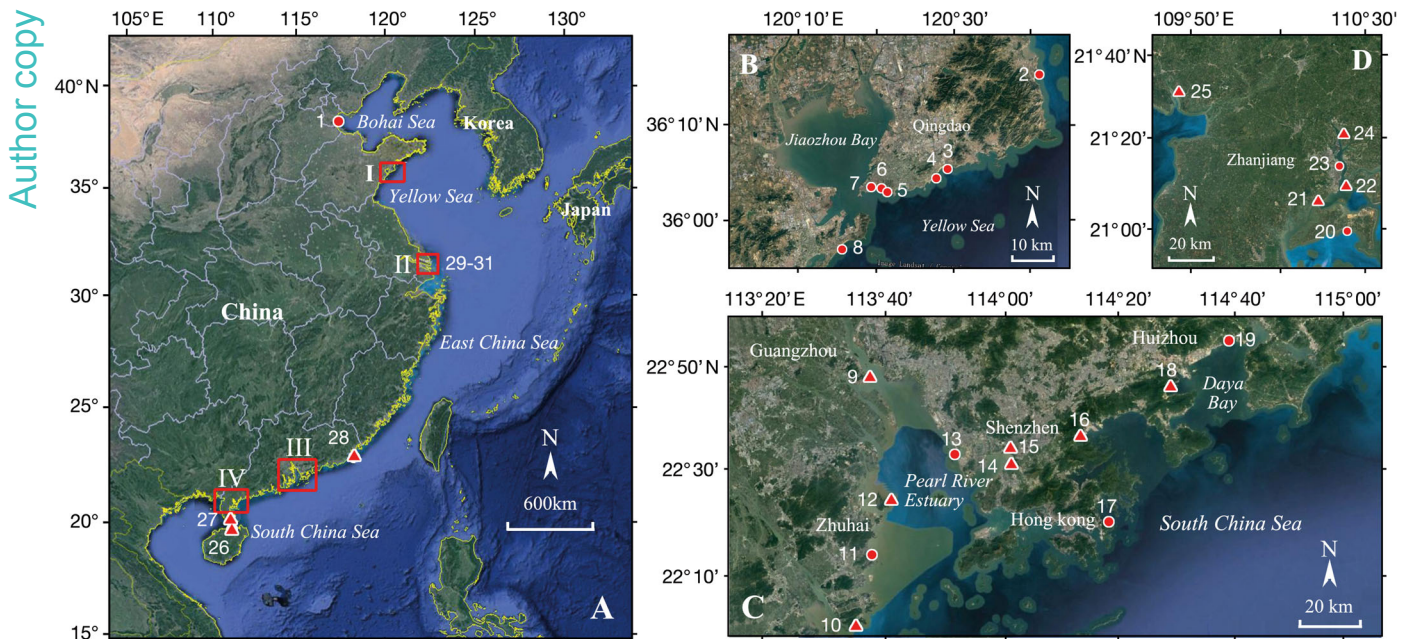


Fig. 1. (A) Locations of the study sites along the coast of China, with detailed locations of the sampling sites within squares (B) I, (C) III and (D) IV. Circles indicate sandy beach habitat and triangles refer to mangrove habitat. Site names are: 1, Tianjin; 2, Qingdao_Yangkou; 3, Qingdao_Shilaoren; 4, Qingdao_Diaosuyuan; 5, Qingdao_No. 2; 6, Qingdao_No. 1; 7, Qingdao_Zhanqiao; 8, Qingdao_Jinshatan; 9, Guangzhou; 10, Zhuhai_Hengqin; 11, Zhuhai_Haibin park; 12, Zhuhao_Qiao; 13, Shenzhen_Nanshan; 14, HK_mangrove; 15, Shenzhen_mangrove; 16, Shenzhen_Dameisha; 17, HK_Clear Water Bay; 18, Daya Bay_mangrove; 19, Daya Bay_sandy beach; 20, Zhanjiang_Donghai Island; 21, Zhangjiang_Huguang; 22, Zhanjiang_Techeng Island; 23, Zhangjiang_Haibin Park; 24, Zhanjiang_Guandu; 25, Zhanjiang_Gaoqiao; 26, Haikou; 27, Zhanjiang_Haian; 28, Shantou; 29, site 1; 30, site 2; 31, site 3

signed to genus level (presence/absence data) according to the systematic classification (Lynn 2008; see Table S1 in Supplement 1 at www.int-res.com/articles/suppl/m627p049_supp1.xls for a complete list of the genera encountered), which resulted in a data set comprising 220 ciliate genera collected from 31 sites along the Chinese coast (Fig. 1).

We included 3 local environmental variables: habitat type (4 levels, i.e. North sandy beach, South sandy beach, salt marsh and mangrove), defined as a categorical variable, and 2 water chemistry variables, i.e. salinity and pH, which were averaged for each site (Fig. S1 in Supplement 2 at www.int-res.com/articles/suppl/m627p049_supp2.pdf). We determined latitude for each site using data reported in the original publications and Google Earth. We also included 2 climatic variables (annual mean temperature and annual precipitation) from WorldClim (Hijmans et al. 2005) at 30 s resolution. Net primary productivity (NPP) was extracted from SEDAC at 0.25 decimal degrees (Imhoff et al. 2004). Salinity, climatic variables and NPP were log-transformed to better approximate normality of residuals, then all variables were standardized before analysis.

2.2. Trait analysis and functional diversity

We selected 5 traits sub-divided into 13 categories (Table 1) according to Xu et al. (2018a). These traits reflect morphological characteristics (body size, degree of flexibility and body form) and behavior (feeding and mobility). Data on traits were mainly obtained from the original sources in which the species were described, as well as from expert opinions and literature (Pratt & Cairns 1985, Lynn 2008). A fuzzy-coding procedure was used to account for an individual taxon displaying multiple trait categories according to Xu et al. (2018b). For each community, the trait values were averaged by all species present in a community using the 'FD' package in R 3.5.3 (<https://CRAN.R-project.org/package=FD>; Laliberté et al. 2014). To measure functional diversity, we used functional divergence (FDiv) since it is independent of sampling effort (Villéger et al. 2008) and has an advantage over other functional diversity indices to infer environmental status (Gusmao et al. 2016, Xu et al. 2018b). FDiv was computed using the 'FD' package in R 3.5.3 (Laliberté et al. 2014).

Table 1. Functional trait variables and categories used in the current study

Trait	Category	Description
Feeding type	Bacterivores	Feeding on bacteria
	Algivores	Feeding on algae
	Predators	Feeding on flagellates and ciliates
Body size	Small	Cell length < 50 μm
	Medium	50 μm < cell length < 200 μm
	Large	200 μm < cell length
Mobility	Attached to substrate	Non-mobile
	Swimming	Locomotion by swimming
	Crawling	Locomotion by crawling on substrate
Body form	Dorso-ventrally flattened	Ratio of thickness:width < 1: 4
	Cylindrical	Ratio of thickness:width > 1: 4
Cell flexibility	Non-flexible	Cell non-flexible and non-contractile
	Flexible	Cell either flexible or contractile or both

2.3. Data analyses

The genera presence/absence data were Hellinger transformed prior to analysis (Peres-Neto et al. 2006). We analyzed the patterns in species and trait composition with non-metric multidimensional scaling (nMDS) followed by environmental factor fitting to examine the major structure. Permutational multivariate ANOVA (PERMANOVA) was used to determine the significance of differences in species and trait compositions among different habitat types (Anderson et al. 2008). A Bray–Curtis similarity matrix based on species data and a Gowdis distance matrix based on traits data were used for nMDS and PERMANOVA. PERMANOVA was conducted on unrestricted permutation raw data using 999 random permutations. We used tests of homogeneity of dispersion (PERMDISP) to examine dispersion of species and trait composition among the 4 habitat types (Anderson et al. 2008). nMDS PERMANOVA and PERMDISP were performed in PRIMER 7 + PERMANOVA (PRIMER-E). We then used redundancy analysis (RDA) to explain the main patterns in species and trait composition. RDA was applied with forward selection, using Monte Carlo Permutation tests (999 permutations), to select only those variables that significantly explained variation in the benthic ciliate communities among sites. Forward selection and RDA were carried out using the 'packfor' and 'vegan' packages in R (R Development Core Team). Finally, we used generalized linear modeling (GLM) with Gaussian type distribution on square root transformed FDiv to examine the patterns in functional

diversity along the coast of China. We started with a full model, and the most parsimonious model was selected based on Akaike's information criterion (AIC). We provide the ANOVA results of the final model. Since the climatic variables were strongly correlated with latitude ($r > 0.85$), they were excluded from the RDA and GLM.

3. RESULTS

3.1. Variability of water chemistry

Generally, the ranges of both salinity and pH in the Yangtze River estuary and southern coastal areas of China were wider compared to those in northern China (Fig. S1). Sites along the Bohai Sea and Yellow Sea were close to typical marine habitat, with salinity around 30 and alkaline pH values (7.5–8.0). However, sites in the Yangtze River estuary and southern China were more influenced by coastal rivers, so the salinity varied from 0.3 to 33. In these areas, 16 out of 23 sites were brackish water habitats with salinity ranging from 10 to 25, and pH values were mainly alkaline, varying from 7.0 to 8.9, with only 1 exception where the pH was 6.3.

3.2. Patterns of species composition

Overall, species composition differed significantly among different habitat types (PERMANOVA, main test, $p = 0.001$; Table 2). However, the difference in species composition between the South sandy beach and the North sandy beach or mangrove was not significant (Table 2).

The wide variation in species compositional heterogeneity was clear in the nMDS plots, which showed that salt marsh sites were closely clustered compared with sites of the other 3 habitat types (Fig. 2A). This pattern was verified by PERMDISP analysis (Table S2). Moreover, based on nMDS analysis, spatial and climatic variables had a strong relationship with species composition on nMDS axis 2, which separated mangrove from the North sandy beach, with the South sandy beach in the middle (Fig. 2A). NPP was correlated with mangrove and salt marsh while salinity was correlated with the North and the South sandy beach

Table 2. Full output from the 1-factor PERMANOVA by habitat type examining species and trait composition of benthic ciliates along the coast of China. Asterisks indicate significance at * $p < 0.05$, ** $p < 0.01$

	Species composition	Trait composition
Main test	0.001**	0.001**
Pair-wise test		
North sandy beach × Salt marsh	0.017*	0.041*
Salt marsh × South sandy beach	0.019*	0.011*
Salt marsh × Mangrove	0.002**	0.003**
North sandy beach × South sandy beach	0.122	0.022*
North sandy beach × Mangrove	0.002**	0.002**
South sandy beach × Mangrove	0.069	0.296

Sathrophilus, *Tracheloraphis*, *Hippocomos* and *Pseudocohnilembus*) were correlated with the North sandy beach; vectors for 3 genera (*Cohnilembus*, *Neurostylopsis* and *Euplotes*) were correlated with the South sandy beach and mangrove; and 1 genus (*Loxophyllum*) was correlated with salt marsh.

The results from RDA demonstrated that species composition was related significantly to both environmental variables, i.e. habitat type and salinity, and to latitude (Table 3). The total fraction of the variance explained by

(Fig. 2A). Water pH correlated notably with nMDS axis 1. A vector overlay of Pearson correlations of the typical genera ($r > 0.5$) with the axes is shown in Fig. 2B; vectors for 5 genera (i.e. *Cinetochilum*,

the separately run RDA was 8.4% (Adj R^2) and 2.4% (Adj R^2) for habitat type and salinity, respectively, while the variance explained by latitude was 2.5% (Adj R^2).

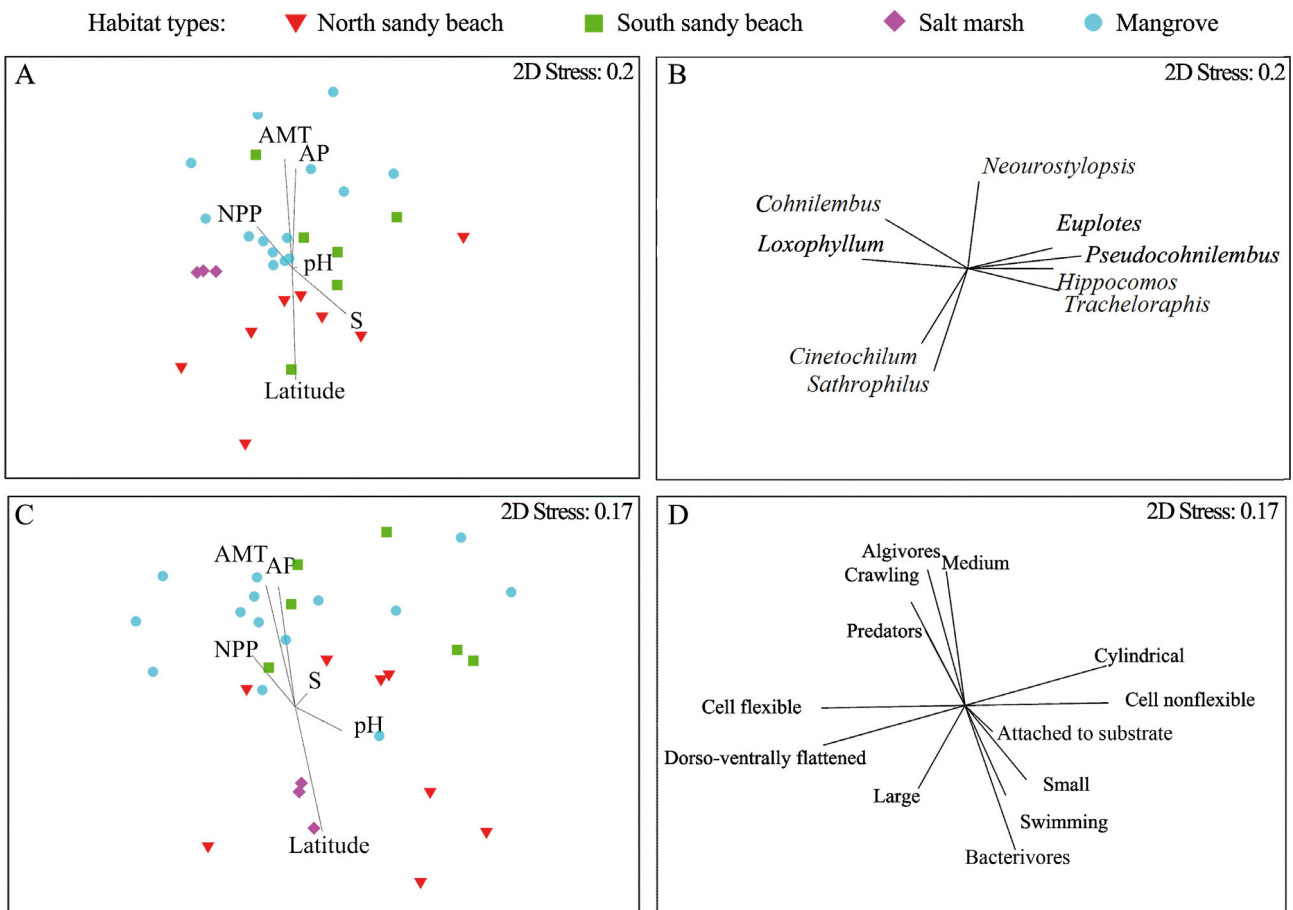


Fig. 2. Non-metric multidimensional scaling (nMDS) ordination plots of benthic ciliate communities along the coast of China based on (A,B) species composition and (C,D) trait composition. Panels A and C also show environmental variables. The length and direction of each vector in B and D indicate the strength and significance of the relationship between typical genera with correlation coefficients > 0.5 and axes (B) or traits and axes (D). AMT: annual mean temperature; AP: annual precipitation; NPP: net primary productivity; S: salinity

Table 3. Effects of spatial and environmental variables on the species or trait composition of benthic ciliates along the coast of China analyzed by redundancy analysis (RDA). The p-values were calculated from a Monte Carlo test with 999 permutations ($p < 0.01$). (-): Variables not selected by forward selection; NPP: net primary productivity

	Species composition		Trait composition	
	R ² (%)	Adj R ² (%)	R ² (%)	Adj R ² (%)
Habitat type	17.6	8.4	-	-
Salinity	5.7	2.4	-	-
pH	-	-	-	-
NPP	-	-	-	-
Latitude	5.7	2.5	21.3	18.5

3.3. Patterns of trait composition

Similar to species composition, trait composition mainly differed significantly among different habitat types (PERMANOVA, main test, $p = 0.001$; Fig. 2C, Table 2), while only the difference between the South sandy beach and mangrove was not significant (Table 2).

The nMDS plot showed clear heterogeneity in dispersions for sites from different habitat types, with salt marsh having markedly smaller dispersion than the other 3 habitat types (Fig. 2C). The test for heterogeneity was also statistically significant in the PERMDISP analysis (Table S2 in Supplement 2). In addition, spatial and climate variables correlated strongly with trait composition on nMDS axis 2, which separated mangrove and South sandy beach from North sandy beach and salt marsh (Fig. 2C). A vector overlay of Pearson correlations of functional traits with the axes is shown in Fig. 2D. The trait categories for the small, swimming and bacterivore taxa were positively correlated with North sandy beach and salt marsh, while medium, crawling, algivores and predators were positively correlated with mangrove and South sandy beach (Fig. 2D).

The results from RDA revealed that the trait composition was only related significantly to latitude among all the variables (Table 3). The fraction of the variance explained by latitude on trait composition was 18.5% (Adj R²).

For body size, medium size was negatively correlated with latitude ($R^2 = 0.36$, $p < 0.01$) while large size was positively correlated with latitude ($R^2 = 0.28$, $p < 0.01$; Fig. 3). For feeding type, bacterivores were positively correlated with latitude ($R^2 = 0.44$, $p < 0.01$) while algivores were negatively correlated with latitude ($R^2 = 0.31$, $p < 0.01$; Fig. 3). For mobility, swimming taxa were positively correlated with latitude

($R^2 = 0.36$, $p < 0.01$) while crawling taxa were negatively correlated with latitude ($R^2 = 0.41$, $p < 0.01$; Fig. 3). For body form and cell flexibility, we found no significant correlations with latitude.

3.4. Patterns in functional diversity (FDiv)

According to GLM, FDiv of benthic ciliates was significantly ($p < 0.05$) related only to latitude, but the most parsimonious model also included pH and habitat type (Table 4). FDiv showed a significant positive correlation with latitude ($R^2 = 0.27$, $p < 0.01$; Fig. 4).

4. DISCUSSION

4.1. Species and trait composition

Based on PERMDISP analysis, the heterogeneity of both species and trait composition varied considerably between salt marsh and the other 3 habitat types (Table S2). Due to our unbalanced study design, i.e. different numbers of study sites among the 4 habitat types, the detection of differences in centroids among habitat types can be affected by the differences in within-group dispersions (Anderson & Walsh 2013). However, Anderson & Walsh (2013) pointed out that if the group with the larger dispersions also has a larger number of samples, the results of a PERMANOVA can be conservative. In our study, salt marsh had a smaller number of samples and smaller dispersion compared with the other 3 habitat types. Moreover, a clear visual pattern of the separation of these sites can be observed in the nMDS plot (Fig. 2A,C). Thus, the difference in centroids of these habitat types can be verified.

Furthermore, we evaluated the relative importance of environmental and spatial factors for variation in benthic ciliate community composition along the coast of China, by adopting both trait-based and more traditional morphological data. RDA showed that 2 environmental variables, i.e. habitat type and salinity, and latitude were the main drivers of species composition, with environmental variables explaining more variation than latitude. Our finding agrees with the notion that microbial species composition is jointly driven by local environmental factors and spatial or climatic variables, but local environment explains more variance due to the high dispersal capacity of microbes and strong species sorting (Langenheder & Ragnarsson 2007, Soininen et al. 2007, Verleyen et al. 2009, Jyrkänkallio Mikkola et al.

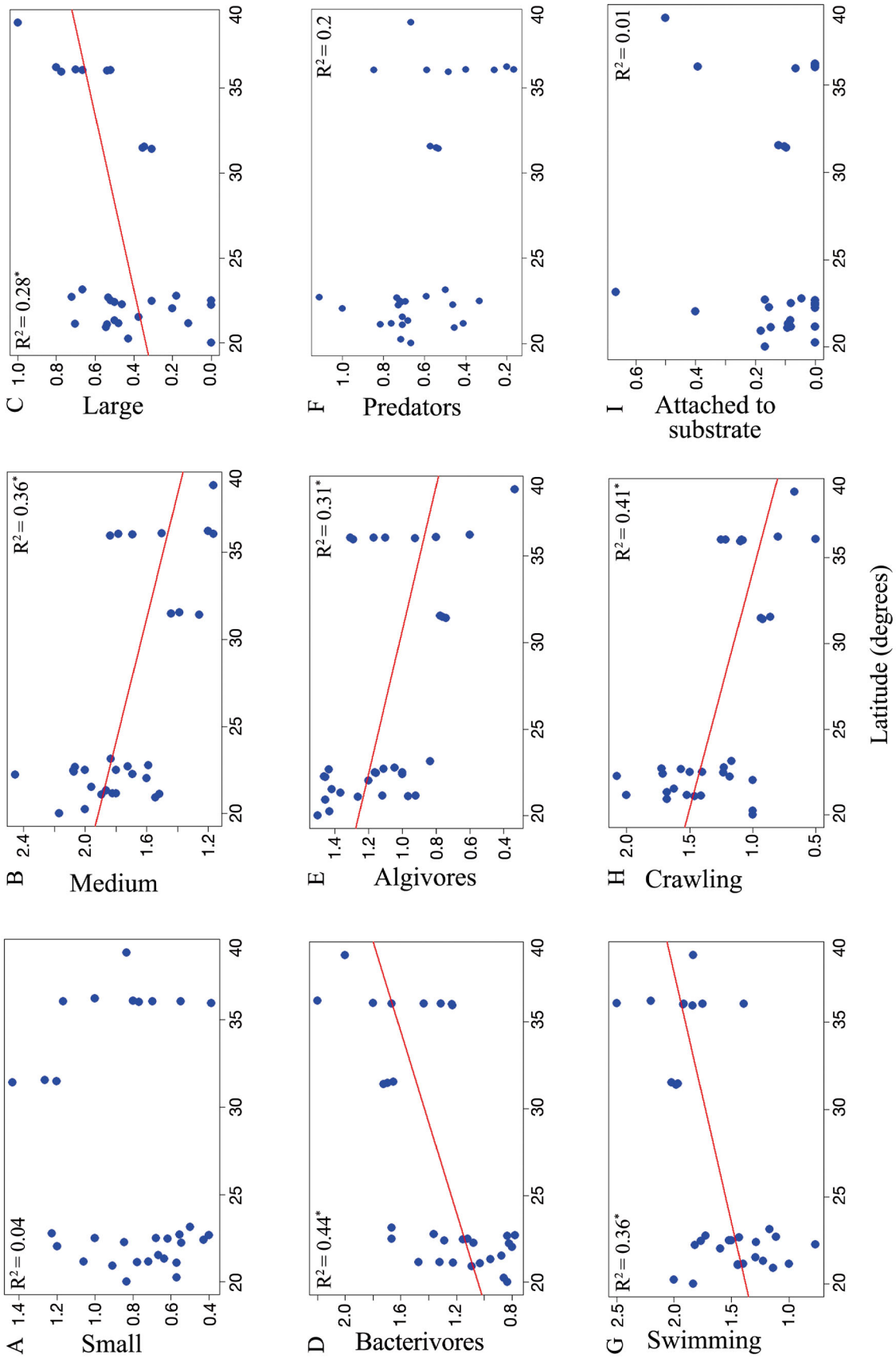


Fig. 3. Relationship between latitude and functional traits of benthic ciliates along the coast of China; * $p < 0.01$. See Table 1 for full descriptions of the trait variables

Table 4. ANOVA output of the minimal adequate model showing how environmental and spatial variables affect the functional diversity (FDiv) of benthic ciliates along the coast of China. Significant effect is marked in **bold** ($p < 0.01$)

Response variable	Predictor	df	SS	MS	F	p
FDiv	Latitude	1	0.0034	0.0034	12.416	0.002
	pH	1	0.0003	0.0003	0.948	0.340
	Habitat type	3	0.0020	0.0007	2.511	0.082
	Residuals	25	0.0067	0.0003		

2017). We also found that habitat type had the strongest effect on shaping benthic ciliate species composition. Previous studies on diatoms, bacteria and fish also showed that habitat type explained a larger amount of variation in community composition than local environmental, mainly chemical variables (Drenovsky et al. 2010, Erős et al. 2012, Jyrkänkallio Mikkola et al. 2017). Jyrkänkallio Mikkola et al. (2017) suggested that habitat type might reflect the effects of some unmeasured variables. For example, the sediment grain size is typically one of the most important factors in shaping benthic ciliate community structure (Azovsky & Mazei 2005, 2018, Hamels et al. 2005, Burkovsky & Mazei 2010). Unfortunately, we lacked grain size data for most of the original data sources that we collected. Therefore, we use the habitat type as proxies for the unmeasured variables, including sediment grain size, in explaining community variation.

Salinity was also an important factor in determining coastal benthic ciliate species composition. This is congruent with previous studies, which reported the importance of salinity in shaping coastal and estuarine ciliate communities (Kchaou et al. 2009, Sun et al. 2017, Xu et al. 2018a). Therefore, in general, our

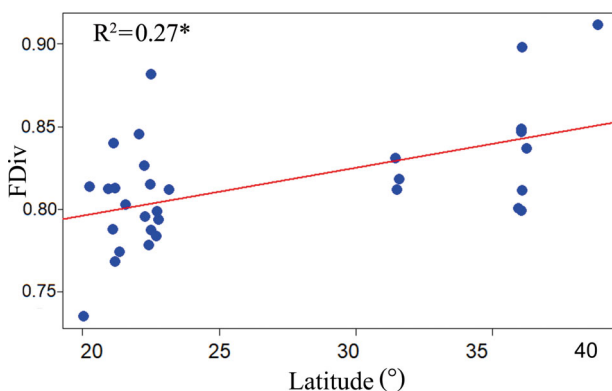


Fig. 4. Relationships between latitude and functional diversity (FDiv) of benthic ciliates along the coast of China; * $p < 0.01$

findings are consistent with the idea that local environmental conditions have important effects on taxonomical composition (Hawkins et al. 2000, Tolonen et al. 2017).

However, we found that the trait composition of ciliate communities was mainly driven by spatial or climatic variables at this regional study scale. Similar findings were reported in a study on trait composition of testate

amoebae, with a stronger correlation with spatial variables compared to environmental factors (Fournier et al. 2016). However, a study on diatom communities found that local environmental variables had stronger effects on trait composition compared to spatial factors (Soininen et al. 2016). We think this relationship depends on the traits chosen in the analyses. The traits of the diatoms used in the analyses by Soininen et al. (2016) included acid tolerance and nitrogen-fixing ability, which could be closely related to local environmental conditions, such as pH and the form and concentration of nitrogen. However, the traits of the ciliates analyzed in our study include morphological characteristics (body size, degree of flexibility and body form) and behavior (feeding and mobility), which may not be as directly linked to local environmental factors, but also to spatial or climatic variables. A study on copepod trait distributions also revealed clear spatial patterns of body size, which exhibited a strong positive trend with latitude (Brun et al. 2016). Kissling et al. (2012) revealed a latitudinal gradient in species richness for different feeding preferences of birds due to the distribution of food resources. Here, we detected significant correlations between latitude and several traits of marine benthic ciliates (Fig. 3). Based on our findings, the latitudinal gradient of body size showed a decreasing trend in medium sized species towards high latitudes, and the opposite trend in large-sized species (Fig. 3), leading to a positive correlation between body size and latitude. Considering the strong covariation between temperature and latitude, we suggest that temperature plays an important role in driving this spatial pattern. In fact, Horne et al. (2016) also pointed out that temperature is the dominant explanatory variable of the spatial patterns in body size of copepods. Moreover, Atkinson et al. (2003) found a significant negative trend between temperature and body size across all protist taxa through a meta-analysis, which agrees with our findings here.

Spatial patterns of feeding type can be more directly linked to the distribution of food resources.

Since chlorophyll concentrations correlate positively with temperature, it is not surprising to find a change in feeding type composition from bacterivorous to herbivorous ciliates towards lower latitudes (Fig. 3). The mobility trait also exhibited a changing trend from swimming to crawling types with decreasing latitude (Fig. 3). We think this is because the swimming type co-varied with bacterivores, while the crawling type co-varied with algivores. The typical bacterivores found at the North sandy beach included *Cinetochilum*, *Sathrophilus* and *Hippocomos*, which are all scuticociliates and are classified as being the swimming movement type, while the typical algivores from the South sandy beach and mangroves included genera belonging to Spirotrichea and Phyllopharyngea (crawling types).

We further found that benthic ciliate communities from the South sandy beach and mangrove habitats shared a large number of species and traits, although these 2 habitat types differed fundamentally. Since all of the South sandy beach sampling sites were located close to mangrove habitat, we suggest the reason for this is the high dispersal ability of ciliates in a marine system. It is likely that their community structure at adjacent localities is highly influenced by mass effects, which means that within a certain geographic distance, individuals could also occur temporarily in less favorable habitats, resulting in spatially homogenized communities (Pulliam 1988, Leibold et al. 2004). In fact, Heino et al. (2015) suggested that compared with isolated ponds and lakes, the importance of mass effects may be higher in coastal areas and offshore marine systems due to the high physical connection. Based on our findings, mass effects could override the effect of species sorting on shaping ciliate communities in marine coastal systems, especially at smaller spatial extents.

4.2. Latitudinal gradients in functional diversity

The functional diversity of ciliates was only significantly correlated with latitude and showed a positive trend. This contradicts the general pattern found in macroorganisms, where species richness and functional diversity peak in (sub)tropical regions (e.g. Stevens et al. 2003, Hillebrand 2004, Kissling et al. 2012, Pease et al. 2012). However, Hillebrand (2004) noted that both the strength and slope of this negative relationship decreased with organism body size, suggesting that such a pattern is weaker in smaller taxa. Soininen (2012) further emphasized that a high dispersal ability and sensitivity to environmental fluctua-

tions could lead to substantially different underlying mechanisms for the latitudinal diversity gradients among micro- and macroorganisms. In fact, several exceptions to this classic relationship have been noted in microorganisms. For example, Soininen et al. (2016) found species richness of diatoms to scale positively with latitude, while Passy (2010) reported a U-shaped latitudinal distribution of freshwater diatom richness in the USA, with the peaks located in both subtropical and temperate regions. Furthermore, studies on pelagic tintinnid ciliates and foraminifers revealed a hump-shaped relationship, with the maximum species number at 20–30° north or south rather than at the equator (Rutherford et al. 1999, Dolan et al. 2006), while Vyverman et al. (2007) showed a hump-shaped relationship between latitude and lake diatom richness in the northern hemisphere with the peak located between 55° and 70° N. Finally, studies on the global distribution of marine benthic ciliates and heterotrophic flagellates detected no significant correlation between diversity and latitude (Azovsky & Mazei 2013, Azovsky et al. 2016). In sum, such high variation among study results points to high context-dependency (e.g. in diversity metrics or ecosystem types) in latitudinal richness gradients in microbial taxa.

The potential drivers behind these deviations are manifold. Studies on freshwater diatoms suggested that habitat availability, including lake density and wetland areas, matched closely with species richness gradients (Vyverman et al. 2007, Passy 2010, Soininen et al. 2016), whereas in the present study, no significant correlation between functional diversity and habitat types was detected. Moreover, we found that salinity was not an important factor affecting the functional diversity of ciliates. A study on global marine benthic ciliates found a significant negative relationship between salinity and species richness (Azovsky & Mazei 2013); however, the study did not consider functional diversity. As a result of functional redundancy, the patterns in species richness and functional diversity do not always follow the same trend (Vil- léger et al. 2010, Fournier et al. 2012, Xu et al. 2018a). In the present study, climatic variables strongly covaried with latitude; thus, climate is a potential predictor of benthic ciliate functional diversity along the coast of China. The correlations of species richness and functional diversity with climatic variables have been widely reported from macro- to microorganisms, such as birds, soil microbes and foraminifera (Rutherford et al. 1999, Fuhrman et al. 2008, Kissling et al. 2012). For example, Fernández et al. (2009) reported that temperature was the most important environmental variable showing a clear and con-

sistent relationship with species richness, but with opposite effects on different taxa. They found a positive relationship for planktotrophic marine species, while a negative relationship was found for directly developing marine benthic species (Fernández et al. 2009). Species-specific temperature adaptation among different ciliate groups affects their reproduction and growth rates at different temperatures (Müller & Geller 1993, Weisse et al. 2001), and therefore, higher temperatures at lower latitudes may confine the distribution of certain groups of ciliates. We found 11 genera of the karyorelictean ciliates in the North sandy beach habitat, but only 2 genera in the South sandy beach and 3 genera in mangrove habitats. Most species within Karyorelictea possess special traits such as giant body size (200–2000 µm body length), e.g. *Geleia*, *Kentrophoros* and *Trachelocercids*, and an extremely flattened body form, e.g. *Remanella*, *Cryptopharynx* and *Apocryptopharynx*. Since the FDiv index measures functional divergence, we think that the reduction of certain groups of ciliates in southern China, such as Karyorelictea, would have an impact on FDiv, leading to a decreasing trend of FDiv towards lower latitudes. Similar findings were also reported in a study of the global distribution of marine benthic heterotrophic flagellates, with lower taxonomic diversity in warm regions compared with temperate and polar zones (Azovsky et al. 2016). Therefore, whether this positive relationship between functional or taxonomical diversity and latitude exists in other groups of microbial eukaryotes needs to be verified in future studies.

5. CONCLUSIONS

Collectively, our results indicate that benthic ciliate community composition varies substantially along the coast of China in response to environmental and spatial or climate variables. Species composition was driven by both environmental factors and latitude, with the former explaining more variation than the latter, while trait composition was only significantly correlated with latitude. Moreover, functional diversity also exhibited a significant trend of increasing towards higher latitudes. Since climatic variables strongly covary with latitude, we postulated that temperature is the main factor driving the trait composition and functional diversity of benthic ciliates along the coast of China. Our findings support that combining analyses of taxonomical and functional trait data can give more insights into microbial biogeography in marine ecosystems.

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