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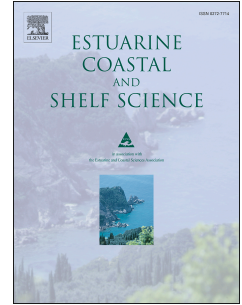
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**Microphytobenthos and an indicator of environmental quality status in intertidal flats: case study of coastal ecosystem in Pertuis Charentais, France**

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## Abstract

Microphytobenthos communities of different sediment types were investigated in intertidal flats of the coastal area around La Rochelle (Pertuis Charentais, France) in July, 2014 and January, 2015. Biotic variables of biomass, abundance and species composition of microphytobenthos were evaluated together with environmental variables, including irradiance, sediment temperature, grains size, pore water salinity, pH, nutrients, organic matter, water content and heavy metal concentrations in the sediment. The relationships between biotic and environmental parameters showed that: (1) the microphytobenthos biomass and community structures showed significant differences among different sediment types; (2) variation in the microphytobenthos communities were significantly correlated with environmental variables, especially with the heavy metals, grain size, irradiance, pore water salinity, organic matter and concentration of  $\text{PO}_4^{3-}$  and  $\text{Si(OH)}_4$ ; (3) the species number and richness were both significantly correlated with organic matter, and (4) the species *Entomoneis corrugate*, *Navicula aitchelbee* and *Gyrosigma cf. limosum* were positively correlated to heavy metals, and so were *N. phylleptosoma*, *Surirella brebissonii* to  $\text{Si(OH)}_4$  and  $\text{NH}_4^+$  concentrations, and *G. acuminatum* and *S. brebissonii* to  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$ . It is suggested that the spatial variation in biodiversity of microphytobenthos may reflect environmental quality status in coastal intertidal ecosystems.

**Keywords:** bioassessment; community; intertidal ecosystem; microphytobenthos; France, Pertuis Charentais

## Introduction

Microphytobenthos (MPB) play an important role in the functioning of food webs as primary producers, influence sediment-water nutrient flux and contribute to sediment stabilization in various aquatic ecosystems, especially intertidal ecosystems (Round, 1979; Miller et al., 1996; Serôdio and Catarino, 2000; Underwood, 2010; Underwood and Kromkamp, 1999; Wainright et al., 2000). There have been many studies on community patterns and dynamics of marine MPB (Du et al., 2010; Lundkvist et al., 2007; Mitbavkar and Anil, 2002; Paterson, 1989; Paterson et al., 2000; Propp et al., 1980; Rysgaard et al., 1995; Underwood, 1994) and the species composition of MPB communities also shows a strong relationship with environmental factors, such as sediment grain size composition, salinity, nutrients ect (Agatz et al., 1999; Cibic et al., 2007; Facca and Sfriso, 2007; Méléder et al., 2007; Du et al., 2010, 2016). With short life cycles, standardized sampling protocols, availability of user-friendly taxonomic references, MPB have been suggested as indicators to evaluate environmental conditions and anthropogenic impacts in many aquatic ecosystems, especially in freshwater systems (Admiraal and Peletier, 1980; Blanchard et al., 2001; Brotas et al., 1995; Du et al., 2009; Guarini et al., 1998; Herlory et al., 2004; Kelly and Whitton, 1995; Sabbe, 1993).

The MPB (especially diatoms) have been widely used as a useful bioindicator of environmental quality status in freshwater systems (Chen et al., 2016; Kelly and Whitton, 1995; Lavoie et al. 2006; Solimini et al. 2006; Potapova and Charles, 2007; Stevenson et al., 2008 and 2010), and also were included in the EU Water Framework Directive (Directive 2000/60/EC). It demonstrated that diatom assemblage structure metrics more

accurately assessed water quality and some species, such as *Amphora pediculus* and *Cocconeis placentula* were among the dominant species in low nutrients stream sites (Chen et al., 2016). The trophic diatom index (TDI) based on a suite of 86 taxa was highly correlated with aqueous P concentrations when tested on a dataset from 70 river sites free of significant organic pollution (Kelly and Whitton, 1995). And the Eastern Canadian Diatom Index (IDEC) successfully indicates the 'distance' from the non-impacted state under multiple stresses (Lavoie et al. 2006). Furthermore, Stevenson et al. (2008, 2010) developed robust indicators of the biological condition of diatom assemblages for streams of the western US. However, Kelly et al. (2009) also pointed out the uncertainty in ecological status assessment using diatoms owing to the spatial and temporal heterogeneity of the biological community in lakes and rivers.

Compared to the freshwater bodies, coast intertidal systems are subject to more fluctuating environmental factors (e.g. irradiance, temperature, nutrients and water content), which vary with the rise and recession of tides (Barranguet et al., 1998; Serodio and Catarino, 1999; de Brouwer et al., 2000; Christie et al., 2000; Thornton et al., 2002; Mitbavkar and Anil, 2006). Furthermore, due to complex environmental conditions in intertidal ecosystem which are directly affected by the open sea, it is more difficult to find the gradient distribution of specific factors, such as salinity, nutrient or pollutant, in coastal ecosystems than in smaller water systems as river or lake. Besides, interference by anthropogenic activities such as aquaculture and tourism, the relationship between MPB community and environmental factors is more complicated with regional characteristics, which makes the standardizing bioassessment by MPB more difficult in coastal system than in freshwater system. Therefore, there is much less information about MPB as a bioindicator in coastal ecosystems.

Nevertheless, as biotic factors, MPB not only could give an additional reference for the threshold values of which only depending on those chemical indices, but also due to their smart response, diatom assemblages could provide a more sensitive assessment of environmental quality than traditional water chemistry measurements (Katherine and Stephanie, 2005). The diatom assemblage can modify its structure to respond in a sensitive manner to the abrupt changes in multiple physical–chemical variables in a very short term (Cochero et al., 2015). However, for specific regions or stressors, a complete set of diatom species data and the coordinate environment data over a period of time is required, and the importance of spatial processes should be considered for marine coastal systems (Chen et al., 2016; Stevenson et al., 2008; Vilmi et al., 2016). Therefore, in terms of using marine MPB to assess the environmental quality status of coastal intertidal ecosystems, further studies should be carried out to enrich basic information for evaluating feasible criteria (Kelly et al., 2009; Méléder et al., 2007; Oppenheim, 1988; Smol and Stoermer, 2010).

In this study, the spatial-temporal variation of MPB and several abiotic factors were considered, in order to improve bioassessment using intertidal MPB. The main objectives of this study were: (1) to document the taxonomic composition and community structure of MPB communities in the different sediment types of intertidal flats; (2) to reveal the spatial variations in species composition of the MPB communities with

contrasting environmental conditions, and (3) to determine the feasibility of community-based and species-specific bioassessment of environmental quality status using MPBs in coastal ecosystems.

## 1. Materials and Methods

### 2.1 Study area and sampling

This study was conducted in the coastal area around La Rochelle, along the French Atlantic coast in Pertuis Charentais area. Three sites were located on Re Island (Re), Aiguillon Bay (Ag) and Aytres Bay (Ay), respectively (Fig. 1). Site Re was part of an intertidal sand flat on the east-northern of Re Island, near an oyster farm. Site Ag was part of the mudflat of Aiguillon Bay ( $47^{\circ}00' N$ ,  $1^{\circ}05' W$ ), with a mussel farm located at a distance of 1km off shore. Site Ay of Aytres Bay comprised sediments of mixed of mud and sand, and the seawater was reported to contain bacteria (and contamination by fecal coliforms) in the summer with the potential to cause human disease (C Dupuy, unpubl.).

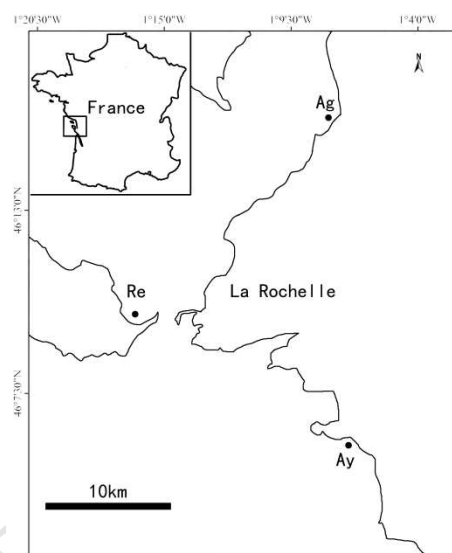


Fig.1. Sampling sites in the coast of La Rochelle, France, Re in Re Island, Ag in Aiguillon Bay; Ay in Aytres Bay.

Samples were collected during low tide on 12<sup>th</sup>, 16<sup>th</sup> and 17<sup>th</sup> in July, 2014 and on 26<sup>th</sup>, 28<sup>th</sup> and 29<sup>th</sup> of January 2015 for site Re, Ag and Ay, respectively. Sediment cores (12cm width) were collected on the basis of a systematic random sampling. The upper 0.5 cm or so sediment was immediately sectioned off. Three cores were mixed together as one sample (or replicate) and stored in a plastic box. Three samples were taken for each site or sub-site and kept in the dark at 4°C until processed in the laboratory for further analysis as cited below. The temperature of the upper 0.5 cm sediment and light irradiance on the sediment surface were monitored at 5 min intervals during the sampling process, using HOBO pro v2 sensor and WALZ ULM-500 light meter, respectively. The porewater of the upper 0.5 cm sediment was collected for each site or sub-site (Rhizon with crystal polystyrene syringes and Rhizon tube, Rhizosphere Research Products, NL-6706, Wageningen) (Seeberg-Elverfeldt et al. 2005).

## 2.2 Samples treatment and environmental factors measurements

### 2.2.1 Abiotic variables

In the laboratory, subsamples were taken for measurement of physic-chemical variables. Nutrient concentrations (nitrates, nitrites, ammonium, phosphates and silicates) were determined with an autoanalyzer (Seal Analytical, GmbH Norderstedt, Germany) equipped with an XY-2 sampler according to Aminot and K erouel (2007). Fifteen heavy metals (Ag, Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, Zn) in the sediments were analyzed by ICP-MS (X series 2 - Thermofisher Scientific). The pH was estimated by pH test paper (6-8), and the salinity was measured using a refractometer (ATAGO). Water content was determined as the percentage of water in relation to the fresh sediment weight by freeze-drying. Organic matter of sediment was estimated by weight loss of frozen-dried sediment at 450°C for 24 h. Grain size of sediment was determined by a laser granulometer Mastersizer 2000 (Malvern Instruments Ltd, Worcs, U.K.).

### 2.2.2 Microphytobenthic biomass, abundance and taxonomic composition

Chlorophyll *a* (chl *a*) was extracted by 90 % acetone from freeze-dried sediments in darkness overnight at 4°C, measured using fluorimetry (Turner TD 700, Turner Designs, USA) after centrifugation (10 min, 3500 g, 8°C), and corrected for phaeopigments according to the method of Lorenzen (1967). Chl *a* concentration was expressed in  $\mu\text{g chl } a \text{ mL}^{-1}$  fresh sediment using volumetric mass as a proxy for MPB biomass. MPB was separated from sediment by the isopycnic separation technique using the silica sol Ludox<sup>®</sup> (HS-40) (Ribeiro, 2010). Identification of benthic diatoms was performed to the species level by light microscopy and assisted by scanning electron microscopy (SEM), in accordance with Hustedt and Jensen (1985), Round et al. (1990), Tomas (1996), Witkowski et al. (2000), Ribeiro (2010). For the SEM, the diatom valves were cleaned in 30 % (v/v) H<sub>2</sub>O<sub>2</sub> at 90 °C for 1 to 3 hours and washed 5 times with distilled water (gentle centrifugation 2500 g, 10 min; Taylor et al., 2005). Diatoms abundance was estimated under light microscope (400 × and 1000 × magnification, Zeiss Axioskop). The diatom valves were counted for 400 valves of diatoms/each sample of at least 50 random fields, up to 100 random fields. Abundance (cell density) of each species was calculated by multiplying the number of cells by the dilution factor, while adjusting the value to 3 ml volume of subsample. The relative abundance of a species was the proportion of the particular species in the total abundance of one sample.

## 2.3 Data analysis

Differences in abundance, biomass (chlorophyll *a* concentration) and community indices of the sampling months and sites were tested through univariate analysis of variance followed by Fisher's least significant difference (LSD) tests. The correlations between biomass, abundance, species and environmental factors were determined by multivariate correlation analyses. These analyses were performed using SPSS 21.0. Ordination analysis was carried out using CANOCO for Windows 5.0 to assess the relationship of species composition with environmental factors. Analysis included a total of 30 samples (all samples for two seasons), 61 species, and 28 environmental factors. Species data were  $\log(1*Y+1)$  transformed. The nonmetric multidimensional scaling

(nMDS) analysis was used to classify the samples based on the species composition. Detrended correspondence analyses (DCA) (detrended by segments) was used to determine gradient lengths, so as to assess whether a linear or unimodal multivariate method should be used. Because the longest length was 3.1 ( $3 < X < 4$ , both methods could be used, Ter Braak and Šmilauer, 2002), a unimodal ordination method was chosen. Principal component analysis (PCA) was used to explore the major variation patterns in the data set (with centering of the species data). The ordination scores in the PCA diagram were focused on 'inter-species correlation', and the species scores were post-transformed. The PCA biplot based on the species composition of every sample was used to illustrate the spatial distribution of MPB. Based on the variance inflation factors (VIF) and Monte Substitute test, top 11 environmental factors were used for further analysis. Finally, detrended canonical correspondence analyses (DCCA) and an unrestricted Monte Carlo permutation test (MCP-test) were applied to test for statistical significance between environmental factors and their effects on species composition variability.

### 3. Results

#### 3.1 Environmental factors

Environmental factors for the sampling sites in summer and winter are summarized in Table 1. Temperature, irradiance and salinity had notable seasonal difference for all sampling sites between summer and winter. Less difference of temperature and irradiance existed among three sampling stations except for site Ay when it was raining during the sampling in winter, e.g. the salinity and irradiance were extremely low as 10 and  $97.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  respectively, and temperature was  $11^\circ\text{C}$ . In addition, site Ay also had larger difference in grain size ( $D_{50}$ ,  $< 63 \mu\text{m}$  %), organic matter (OM), water content (WC, %) and nutrients of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  between the two seasons than the other two sites. Generally, the sediment characteristics of the three sites were clearly characterized by the diameter medium  $D_{50}$  and the percentage of grain size  $< 63 \mu\text{m}$  (Table 1). Details of sediment composition were presented by the grain size proportions of mud ( $< 63 \mu\text{m}$ ), very fine sand ( $63\text{-}125 \mu\text{m}$ ), fine sand ( $125\text{-}250 \mu\text{m}$ ), medium sand ( $250\text{-}500 \mu\text{m}$ ), and coarse sand ( $> 500 \mu\text{m}$ ) (Fig. 2). Sites Re and Ag had little difference in OM and WC between summer and winter. The pH value was relatively stable ( $P > 0.05$ ) except slightly higher at stations Ag and Ay in winter. Dissolved inorganic nutrient concentrations in pore water oscillated dramatically across sampling sites and different nutrients. In details, the species of nitrogen ( $\text{NO}_2^-$  and  $\text{NO}_3^-$ ) increased during winter, especially  $\text{NO}_3^-$  increased more than 10 times. In contrast, the concentration of  $\text{PO}_4^{3-}$  decreased in winter, and the  $\text{NH}_4^+$  and  $\text{Si(OH)}_4$  values were slightly higher at site Re but were lower at site Ag and Ay in winter than in summer. The highest  $\text{NH}_4^+$  and  $\text{Si(OH)}_4$  value was observed at site Ag in summer, and the lowest at station Re in summer. The extremely highest  $\text{NO}_3^-$  value (average  $395.9 \mu\text{mol L}^{-1}$ ) was measured at site Ay in winter, but ranged from 3.7 to 5.2 without significant difference among the three sites in summer ( $P > 0.05$ ), and varied from 41.4 to 73.3 (no significant difference) between site Re and Ag in winter ( $P > 0.05$ ).



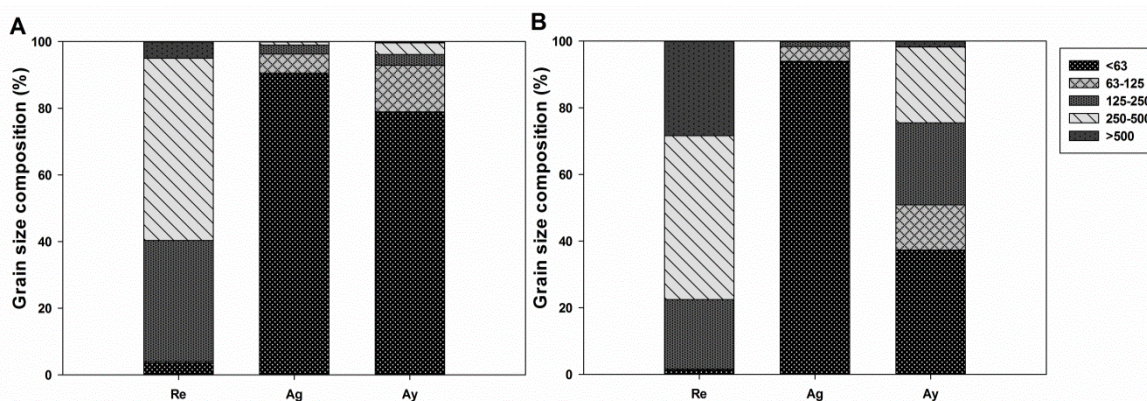


Fig.2. Grain size distribution in the studied sites. Grain size fractions expressed in percentage and consisted of mud (<63 $\mu\text{m}$ ), very fine sand (63-125 $\mu\text{m}$ ), fine sand (125-250 $\mu\text{m}$ ), medium sand (250-500 $\mu\text{m}$ ), and coarse sand (>500 $\mu\text{m}$ ); A: summer, B: winter.

Strongly multicollinearity was found among the variability of 15 heavy metals ions in sediments, indicated by high variance inflation factors ( $VIF > 20$ ). Therefore,  $\text{Zn}^{2+}$  concentration was used as a proxy to represent the 15 measured heavy metals based on its minimum  $p$  value of the  $F$ -statistic (Table 1). Statistical analyses showed significant differences ( $P < 0.05$ ) in these environmental variables among/between three sampling sites. The general trends of heavy metals concentration in three sites was  $\text{Ag} > \text{Ay} > \text{Re}$  whether in summer or winter.

### 3.2 Biomass, abundance and taxonomic composition of MPB

MPB biomass indicated by Chlorophyll  $a$  concentrations of all samples varied from 5.96 to 42.21  $\mu\text{g mL}^{-1}$  fresh sediment in summer and from 5.39 to 51.22  $\mu\text{g mL}^{-1}$  in winter (Table 2). Among three sampling stations, the highest biomass and chl  $a$ /pheo-pigments ratio were found at site Re with sandy sediments, but the lowest in both seasons at site Ag with muddy sediment (Table 2). Comparing to other two sites, the biomass ( $p$ ) and chl  $a$ /pheo-pigments ratio varied significantly ( $p < 0.05$ ) at site Ay with mixed sandy and muddy sediment between summer and winter. Although the highest species number was founded at site Ag, the Shannon diversity index ( $H'$ ) indicated relatively higher species diversity at site Ay, slightly lower at site Ag, and lowest at site Re both in summer and winter (Table 2). The cell abundance of MPB showed higher values in winter for all sites, and the difference between seasons was significant in sandy sediment ( $P < 0.05$ ), and extremely significant in mixed sandy and muddy sediment at site Ay ( $P < 0.01$ ), but not in muddy sediment at site Ag. The correlation analysis also showed low coefficient as 0.567 but significantly positive relationship between biomass and abundance ( $P \leq 0.01$ ). There were no significant difference for Shannon index and Pielou Evenness Index for all sites and seasons. Significant difference of species richness was found between the two seasons at site Ay.

In summer, a total of 111 species of 46 genera were recorded. Among them, 35 species of 18 genera presented in the sand sediment of site Re with highest abundance of  $10.7 \times 10^5$  cells  $\text{mL}^{-1}$  fresh sediment; 70 species of 35 genera were observed in mud sediment of site Ag with lower abundance of  $4.03 \times 10^5$  cells  $\text{mL}^{-1}$ , and 58 species of 33 genera were identified in sand and mud mixing sediment of site Ay, with lowest abundance of  $0.928 \times 10^5$  cells  $\text{mL}^{-1}$ . In winter, only 75 species of 30 genera were observed in three sites, among them, 30 species of

14 genera, 60 species of 25 genera and 28 species of 13 genera presented in site Re, site Ag and site Ay, respectively with  $25.4 \times 10^5$  cells mL<sup>-1</sup>,  $4.30 \times 10^5$  cells mL<sup>-1</sup> and  $6.30 \times 10^5$  cells mL<sup>-1</sup>.

The MPB species with relative abundance > 5 % observed in summer and winter are illustrated respectively for each sample (Figure 3A and B). In summer, the most dominant species *Navicula consentanea* was distributed over all different types of sampled sediment, reaching highest relative abundance as 34.5 %, 25.7 % and 15.9 % of the diatom assemblage in site Re, Ag and Ay, respectively. In addition, *Amphora hassiaca* (1.8-7.3 %), *Cocconeis hauniensis* (1.3-8.1 %), *N. germanopolnica* (9.5-14 %), *N. perminuta* (8.5-20.1 %), *Fallacia scaldensis* (1.0-10 %), were abundant in the sand sediment of site Re; *N. gregaria* (10.6-33.5 %), *N. phylleptosoma* (8.4-25.8%), *Gyrosigma acuminatum* (0.0-12.4 %), *N. dilucida* (0.8-7.8 %) occurred more frequently in mud sediment of site Ag; and *Planothidium aff. engelbrechtii* (10.3-13 %), *N. gregaria* (26.1-30.2 %), *P. deperditum* (3.5-6.7 %) thrived in the sand-mud mix of sediment at site Ay. In winter, dominant species varied little in site Re, where *N. consentanea* was still the most abundant (24.0-34.3 %), *F. scaldensis* abundance was similar to that of summer (3.5-11.8 %), but more *N. germanopolnica* (11.3-32.7 %), *N. gregaria sippe* (5.5-10.8 %) and *N. perminuta* (5.5-25.1 %) were present. In site Ag, except *N. dilucida* (0.8-7.8 %) was similar to that of summer, *Gyrosigma fasciola* became the most abundant in some samples (8.0-69.5 %), and *Skeletonema costatum* appeared frequently (0-24.4 %). In site Ay, the *N. consentanea* kept a consistent relative abundance with that of summer (3.5-12.2 %), *P. engelbrechtii* (7.75-16.04 %) varied slightly, and *N. dilucida* (15.3-39.5 %) and *N. perminuta* (13.3-16 %) became more abundant.

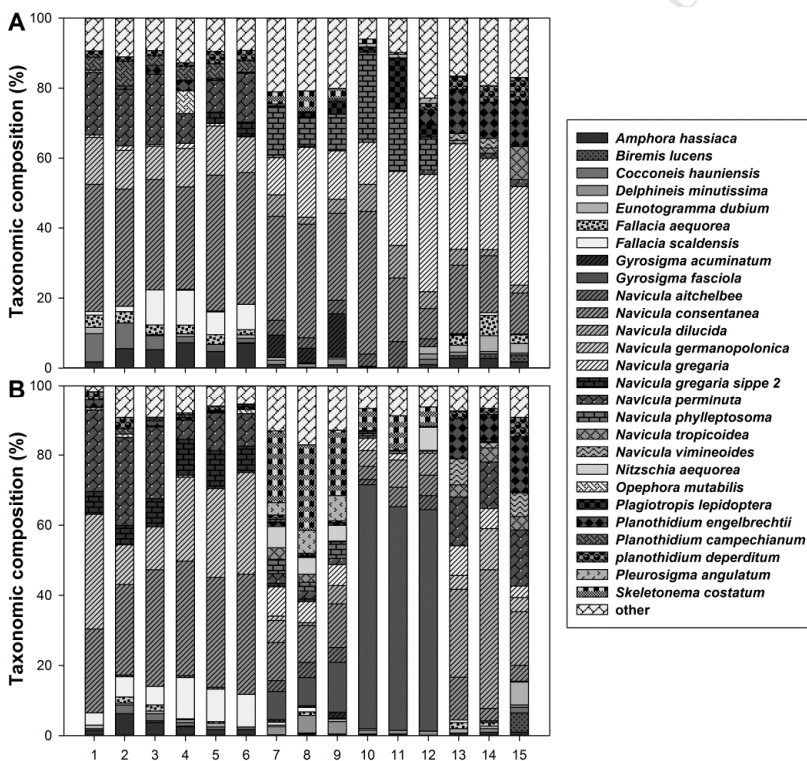


Fig.3 Taxonomic composition of microphytobenthic communities of individual samples collected from site Re (1-6), Ag (7-12) and Ay (13-15), A: summer; B: winter. (The species with relative abundance above 5% are presented, with the remaining species

grouped as 'others'. The relative abundance is presented as the percentage of the total cells counted).

NMDS analysis on species composition pooled for all samples clearly separated the assemblages of the three individual sampling sites (Fig. 4). The summer and winter groups of site Ay were easily distinguished from each other, and the seasonal groups of site Ag also could be separated. However, in site Re, the summer and winter assemblages were overlapping due to their closer similarity. Different structural community types could be recognized according to representative species: (1) those featured by *Navicula germanopolnica*, *N. perminuta* and *Fallacia scaldensis* (at site Re with sand sediment); (2) those by *N. aitchelbee*, *Skelethonema costatum* and *Entomoneis corrugate* (at site Ag with mud sediment), and (3) those by *Planothidium septentrionalis*, *P.aff. engelbrechtii* (site Ay with sand mixing mud sediment) (Fig.3 and Fig.4).

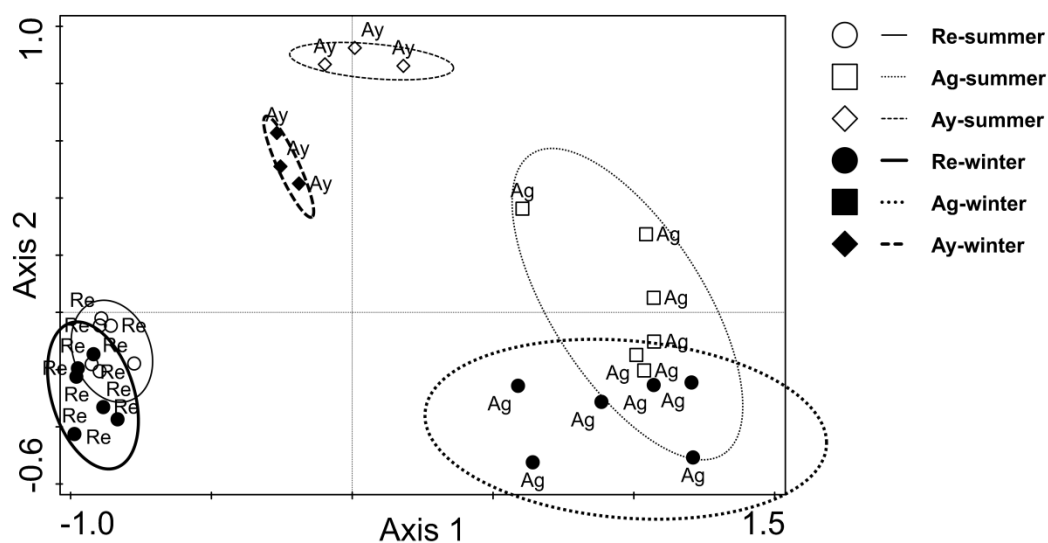


Fig.4. Classification of samples based on species composition by nonmetric multidimensional scaling (nMDS) in CANOCO 5.0.

### 3.3 Relationship between MPB communities and environmental conditions

Multivariate correlation analysis revealed that MPB biomass was significant positively correlated with D50 ( $r = 0.803$ ,  $P < 0.01$ ), and significantly ( $P < 0.01$ ) negatively related to  $< 63 \mu\text{m}$  (mud,  $r = 0.845$ ), and heavy metals (proxy of  $[\text{Zn}^{2+}]$ ), water content, organic matter,  $\text{Si}(\text{OH})_4$ ,  $\text{NH}_4^+$  ( $r = -0.907$ ;  $-0.903$ ,  $-0.753$ ,  $-0.615$ ,  $-0.514$ , respectively). Other environmental factors, such as nutrients of  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , sediment temperature, salinity, irradiance and pH had no significant ( $P > 0.05$ ) relationship with MPB biomass. The DCCA revealed the relationships of species composition with environmental conditions for all samples. It was shown that 56 % of the total variance of species composition could be represented by the first two axes (Fig. 5).

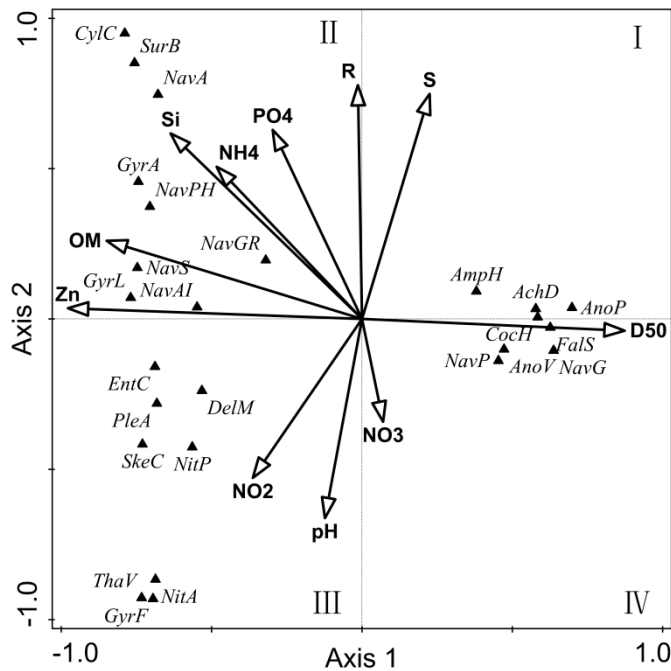


Fig.5. Detrended canonical correspondence analysis (DCCA) based on square-root transform of species abundance. The solid arrowheads and italic labels indicate the species: AchD: *Achnantheiopsis delicatula*; AmpH: *Amphora hassiaca*; AnoP: *Anorthoneis pulex*; AnoV: *Anorthoneis vortex*; CocH: *Cocconeis hauniensis*; CylC: *Cylindrotheca closterium*; DelM: *Delphineis minutissima*; EntC: *Entomoneis corrugate*; FalS: *Fallacia scaldensis*; GyrA: *Gyrosigma acuminatum*; GyrL: *Gyrosigma cf. limosum*; GyrF: *Gyrosigma fasciola*; NavA: *Navicula abscondita*; NavAI: *Navicula aitchelbee*; NavG: *Navicula germanopolonica*; NavGR: *Navicula gregaria*; NavP: *Navicula perminuta*; NavPH: *Navicula phylleptosoma*; NavS: *Navicula* sp.1; NitA: *Nitzschia aequorea*; NitP: *Nitzschia panduriformis var. continua*; PleA: *Pleurosigma angulatum*; SkeC: *Skeletonema costatum*; SurB: *Surirella brebissonii*; ThaV: *Thalassiosira cf. visurgis*; The lines with empty arrowhead and bold labels indicate the environmental factors: D50: diameter of 50% of the sediment grains; OM: organic matter content; R: Irradiance; S: salinity; pH; NO<sub>2</sub>: NO<sub>2</sub><sup>-</sup>; NO<sub>3</sub>: NO<sub>3</sub><sup>-</sup>; NH<sub>4</sub>: NH<sub>4</sub><sup>+</sup>; PO<sub>4</sub>: PO<sub>4</sub><sup>3+</sup>; Zn: Zn<sup>2+</sup>; Si: Si(OH)<sub>4</sub>.

The DCCA axis1 explained 40.5 % of the total variance, and was correlated with environmental data at  $r = 0.991$ , and most importantly with environment variables of heavy metals (Zn<sup>2+</sup>,  $r = -0.969$ ), D50 (average grain size of sediment,  $r = 0.866$ ), OM (organic matter,  $r = -0.841$ ) and Si(OH)<sub>4</sub> ( $r = -0.630$ ). The DCCA axis2 accounts for 15.5 % of total variance, and was correlated with environmental data at  $r = 0.901$ , and the individual variables were R (irradiance,  $r = 0.701$ ), S (salinity,  $r = 0.674$ ), pH ( $r = -0.596$ ), and the nutrient of PO<sub>4</sub><sup>3+</sup> ( $r = 0.566$ ) and Si(OH)<sub>4</sub> ( $r = 0.556$ ). Among the displayed top 11 environmental variables, heavy metals (Zn<sup>2+</sup>) had positive correlation with organic matter ( $r = 0.888$ ) and a negative relation with D50 ( $r = -0.850$ ); salinity positively related to irradiance ( $r = 0.901$ ) and negatively to pH and NO<sub>n</sub><sup>-</sup> ( $r = -0.714$  and  $-0.747$ ); pH was positively related to NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> ( $r = 0.581$  and  $0.749$ ); and NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> were closely related to each other ( $r = 0.703$ ), PO<sub>4</sub><sup>3+</sup>, NH<sub>4</sub><sup>+</sup> and Si(OH)<sub>4</sub> were also positively correlated with each other ( $r = 0.900$ ,  $0.815$ ,  $0.703$ , respectively).

The DCCA biplot and based on multivariate correlation analysis, indicated that D50 was positively correlated with some species abundant in sand sediments of site Re, especially significantly for 7 species *Achnantheiopsis*

*delicatulum*, *Amphora hassiaca*, *Anorthoneis pulex*, *A. vortex*, *Fallacia scldensis*, *Navicula germallopolnica* and *N. Perminuta* ( $p < 0.01$ ). Heavy metals (represented by  $Zn^{2+}$ ) were significant positively related to most of species, especially very closely with *Entomoneis corrugate*, *Gyrosigma cf.limosum*, *N. aitchelbee* and *Navicula* sp.1 ( $p < 0.01$ ). In addition to species closely related to heavy metals, species *N. gregaria* were more strongly correlated to organic matter ( $r = 0.729$ ,  $p < 0.01$ ). Salinity contributed to variation of whole community but was only significantly related to a few species of *Amphora hassiaca* ( $r = 0.520$ ,  $p < 0.01$ ) and *Navicula abscondita* ( $r = -0.764$ ,  $p < 0.01$ ). Species *Cylindrotheca closterium*, *Navicula abscondita*, *N. phylleptosoma* and *Surirella brebissonii* were positively correlated to high  $Si(OH)_4$  and  $NH_4^+$  nutrient ( $p < 0.05$ ). The species *Gyrosigma acuminatum* and *Surirella brebissonii* were also closely related to  $PO_4^{3+}$  and  $NH_4^+$  ( $p < 0.01$ ). Although lacking high Pearson correlation values, *Gyrosigma fasciola*, *N. germanopolonica* and *N. perminuta* were significantly negative related to  $Si(OH)_4$  ( $r = -0.514 \sim -0.547$ ,  $p < 0.01$ ), and *Amphora hassiaca* and *Anorthoneis pulex* were significantly negatively related to  $NO_2^-$  ( $r = -0.489, -0.484$ ,  $p < 0.01$ ). However, species such as the most abundant species *Navicula consentanea* were excluded from the biplot because they had little effect on the variation of MPB community. Factors such as sediment temperature that had strong relationship with irradiance ( $r=0.925$ ,  $p < 0.01$ ),  $< 63 \mu m$  % sediment that was significantly negative to D50 ( $r = -0.920^{**}$ ,  $p < 0.01$ ) and water content that was significantly positive related to  $63 \mu m$  % ( $r = 0.968^{**}$ ,  $p < 0.01$ ) were not selected in the biplot due to their high multicollinearity with other variable by VIF  $> 40$ .

Univariate correlations revealed the relationships between community structural parameters with the 10 most influential environmental variables. The species diversity and evenness had no significant correlation with these environmental factors. The species number were strongly positively correlated with organic matter (0.542,  $p < 0.01$ ), and species richness were significantly correlated with organic matter, heavy metals (proxy of  $Zn^{2+}$ ) and  $NH_4^+$  (0.656, 0.587, 0.536, respectively,  $p < 0.01$ ).

## 4. Discussion

### 4.1 Distribution characteristics of MPB community

The temporal/spatial variations in MPB species distribution are commonly related to a complex set of interactions with environmental conditions (Kromkamp et al., 2006; MacIntyre et al., 1996; Underwood and Kromkamp, 1999; Underwood et al., 1998). Among those variables, compared to other rapidly fluctuating abiotic factors, such as nutrients, salinity and water contents, sediment grain size composition remains relatively stable, and previous studies have identified close relationships between sediment composition and the MPB community (e.g. Cahoon et al., 1999; Facca and Sfriso, 2007; Mitbavkar and Anil, 2002; Oh and Koh, 1995; Du et al., 2010, 2012). In the present study, close relationships of MPB community not only were revealed with sediment grain size (D50), but also with other main environmental factors, such as irradiance, salinity, organic matter, heavy metals concentrations and nutrient of  $NH_4^+$ ,  $PO_4^{3+}$ , and  $Si(OH)_4$ . On the other hand, the absolute predominance of the genus *Navicula* (species as *N. consentanea* and *N. gregaria*) was consistent with studies in most coastal area of temperate to subtropical zone (Oh and Koh, 1995; Du et al.,

2009; Montani et al., 2003; Easley et al., 2005; Haubois et al., 2005). MPB assemblages at study sites could also be characterized according to the sediment types and other main environmental conditions, with respectively typical species as demonstrated in our previous studies (Du et al., 2009 and 2016).

In this study, the MPB biomass was strongly related to the sediment composition and positively related to grain size D50 and negatively correlated to sediment of  $< 63 \mu\text{m}$ , which is contrary to most previous studies (Du et al., 2010; Lundkvist et al., 2007; Miller et al., 1996; Paterson, 1989). It implied that sediment with larger grain size, such as site Re, had higher MPB biomass than muddy sediment, although a typical golden-brown biofilm was visible by naked eyes at site Ag in winter but in neither seasons at site Re (observed personally). In addition to the difference of sampling periods and study regions, one reason might due to the method for the calculation chlorophyll *a* concentration which was transferred from freeze dried sediment methodology to that of fresh sediment, where MPB biomass was relatively diluted by interstitial water in volumetric mass ( $\mu\text{g chl } a \text{ mL}^{-1}$ ). On the other hand, sandy sediments could supply more tridimensional space for MPB (Du et al., 2010). Another reason might be stronger grazing pressure by benthic animals in muddy sediment of site Ag, which was indicated by the higher pheo-pigments and lower chl *a*/peo-pigments.

The negative correlation between MPB biomass and heavy metals in the present study, and significant influence of heavy metals on MPB species composition suggested further concern for pollution effects on coastal systems. Negative correlation between MPB biomass and nutrient such as concentrations of  $\text{Si(OH)}_4$  and  $\text{NH}_4^+$  in porewater were similar to previous studies with significant but low coefficients (Welker et al., 2002; Skinner et al., 2006; Facca and Sfriso, 2007). A clear inverse relation was observed between  $\text{NO}_n^-$  and MPB biomass in sublittoral sediments of the Gulf of Trieste, northern Adriatic Sea (Cibic et al, 2007). Nevertheless,  $\text{NO}_n^-$ ,  $\text{PO}_4^{3+}$  had no significant correlation to MPB biomass in the present study, similarly four nutrients ( $\text{Si(OH)}_4$  and  $\text{NH}_4^+$ ,  $\text{NO}_n^-$ ,  $\text{PO}_4^{3+}$ ) in porewater had no significant relations with MPB biomass in intertidal flats of Nakdong estuary, South Korea (Du et al., 2009). The large oscillations of nutrient concentrations in intertidal sediment (Kuwae et al., 2003; Sakamaki et al., 2006) and MPB themselves influencing the sediment-water interface nutrient fluxes (Rysgaard et al., 1995) may contribute to these phenomena.

#### 4.2 MPB community and environmental quality status

Multivariate analyses were applied in the present study for assessing the relationship of species composition with environmental factors. Multivariate approaches are more effective than univariate analyses for analyzing temporal/spatial variations in community structure and relationships to environmental variables (Clarke and Ainsworth, 1993; Jiang et al., 2014; Xu et al., 2012 a, b). The differences between communities or species distributions on spatial and temporal scales and their variation along gradients of environmental conditions were well illustrated through the multivariate approaches. In coastal intertidal ecosystems, which are notably affected by anthropogenic impacts, multivariate analysis of MPB was a suitable tool to assess the environmental quality status.

At the community level, based on the variation in species composition and environmental variables, the separation of MPB assemblages could contribute understanding the divergence on environmental status. In the present study, the assemblages of site Re and Ag were mainly distinguished by the grain size of sediment, heavy metal concentration and organic matter. The assemblage of site Ay was more affected by irradiance, pH and  $\text{PO}_4^{3-}$ . The seasonal differences in MPB communities of summer and winter groups at site Ay could be explained by the variation in environmental variables of grain size of sediment, heavy metal concentration, salinity and  $\text{NO}_3^-$ . The separation of seasonal groups of site Ag could be attributed to differences in  $\text{Si(OH)}_4$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . In general, the MPB communities presented clear spatial and temporal distribution of 61 MPB species, and especially the 25 most influenced species, reflecting the differences of environmental factors in coastal area of Pertuis Charentais (France). Facca and Sfriso (2007) also pointed out that the community structure, particularly the abundance of opportunistic species, offered the possibility to distinguish the anthropic pressures on the ecosystem of shallow coastal areas. On the other hand, the community-based ecological parameters (e.g., species richness, diversity, and evenness) have also been employed in field investigations and used to assess environmental quality status (Huston, 1979; Ismael and Dorgham, 2003; Xu et al., 2014). In the present study, the indices of diversity and evenness showed no difference among all samples, however, the species richness was relative higher in muddy sites with higher nutrient of  $\text{NH}_4^+$ , organic matter and heavy metals ( $\text{Zn}^{2+}$ ), the species number was also significantly positively related to organic matter. This implied that higher species number and richness might indicate relatively higher nutrient levels in the coastal area. Therefore, the distribution characteristics of MPB communities combining with community-based ecological index could reflect environmental quality status and have the potential for use in bioassessment of coastal intertidal ecosystems.

At the species level, distinctive species which significantly correlated to specific environmental variable may be considered as potential bioindicators. Firstly, with regard to certain sediment type of grain size composition, three of 7 species (*Navicula germanopolnica*, *N. perminuta* and *Fallacia scaldensis*) were closely related to D50 characterized sandy sediment. The relatively larger and longer species *Gyrosigma fasciola*, *G. acuminatum*, *N. gregaria* and *N. phylleptosoma* which were negatively related to D50 but positively to  $< 63 \mu\text{m}$  were dominant in muddy sediment. In a study of the intertidal MPB community in Nakdong estuary, the small diatom *N. ramosissium* (ca.  $35 \times 8 \mu\text{m}$ ) linearly correlated to medium and fine sand (125 - 500  $\mu\text{m}$ ) (Du et al., 2009). In study of Skinner et al. (2006), species *Cyclotella* sp. and *Entomoneis alata* were associated with the percentage of very fine sand (63-125 $\mu\text{m}$ ). Four species (*Amphora coffeaeformis*, *Gyrosigma acuminatum*, *Cymbella turgidula* and *Thalassiosira eccentric*) were proved significantly positive correlated to the percentage of grain size  $< 63 \mu\text{m}$  %, but *Hantzschia amphioxys* and *Nizshia* sp2. were negatively related (Du et al., 2016). Although the characteristic species for sediment types varied among difference study regions, the general relationship was consistent, e.g. large and long epipellic diatom indicate finer sediment  $< 125 \mu\text{m}$ , and small species represent coarser sediments  $> 125 \mu\text{m}$  (Oh and Koh, 1995; Mitbavkar and Anil, 2002; Skinner et al., 2006; Du et al., 2009).

With respect to nutrients, there is considerable literature demonstrating the strong preferences of certain taxa for particular levels of specific nutrients (Underwood et al., 1998; Sullivan, 1999), although few studies determined an explicit indicator function for diatom species in open coastal areas due to the complicated environmental conditions. The centric diatom *Thalassiosira* sp., which represented one of the most abundant genus and was dominant close to the mainland where the pollutant discharge is high, was suggested as important bioindicator for assessing the trophic status (Facca and Sfriso, 2007). A study carried on the Ems-Dollard estuary showed increases in the relative abundance of *Navicula phyllepta*, *N. flanicata* and *Pleurosigma angulatum* with decreasing ammonium ( $\text{NH}_4^+$ ) concentrations due to the installation of waste treatment processes by industry (Peletier, 1996). In the oligotrophic Van Stadens Estuary, South Africa, *Planothidium delicatulum* and *Petronella humerosa* were closely associated with porewater  $\text{NH}_4^+$  concentrations (Skinner et al., 2006). In the study of Du et al. (2016), *Navicula lacustris* and *Fragilaria* sp.1 were significantly negatively correlated to  $\text{NH}_4^+$  concentration. In the present study, without obvious pollution of waste water, *Navicula abscondita*, *Surirella brebissonii* and *Cylindrotheca closterium* were significantly positive related to  $[\text{NH}_4^+]$ , and *Gyrosigma acuminatum* and *Surirella brebissonii* also closely related to  $\text{NH}_4^+$  concentration. N compounds of  $\text{NO}_3^-$  showed negative relationships with many species, such as *Nitzschia sigma* and *N. dissipata* and other species of *Navicula*, *Nitzschia* (Kotsedi, 2011) and *Pleurosigma angulatum* (Du et al., 2016). In the present study, it was *Amphora hassiaca* and *Anorthoneis pulex* negative to  $\text{NO}_2^-$ . With regards to Si ( $\text{OH}$ )<sub>4</sub>, which is usually abundant in sediment, Du et al. (2016) showed *Pleurosigma angulatum* was negatively correlated to Si ( $\text{OH}$ )<sub>4</sub>, however, in this study, it was *Gyrosigma fasciola*, *N. germanopolonica* and *N. perminuta* significantly negatively but *Navicula abscondita*, *Surirella brebissonii* and *Cylindrotheca closterium* significantly positively related to Si ( $\text{OH}$ )<sub>4</sub>. The species *Gyrosigma acuminatum* and *Surirella brebissonii* were closely related to  $\text{PO}_4^{3-}$ . This demonstrates that various species have specific relationships with certain nutrients under different environmental conditions.

Furthermore, using the gradient of salinity and nutrient concentration along river estuary to the fully marine system, several studies explore the relationship of diatom function to environmental status. Nodine and Gaiser (2014) indicated that diatom assemblages of 18 indicator taxa were strongly related to environmental variables such as total phosphorus (TP), and total nitrogen (TN) within the subregions of the investigated three rivers and a harbor. Salinity was the predominant driver of difference among diatom assemblages across the catchment. These relationships were evaluated for predicting the environmental status by the diatom assemblages (Nodine and Gaiser, 2014). Along with gradient in eutrophication on a tidal flat, *Navicula gregaria*, *Nitzschia sigma*, and *Nitzschia tryblionella* proved to be tolerant of pollution, even nutrient-loving for the former two species, while the genera *Achnanthes* and *Amphora* were typical in the nutrient-poor regions (Agatz et al., 1999). Along a saltmarsh creek, diatoms *Nitzschia sigma* and *Gyrosigma limosum* and the cyanobacteria *Oscillatoria limosa* and *O. princeps* had significantly higher population densities near the sewage outfall, and *Navicula phyllepta*, *N. pargemina*, *Nitzschia frustulum*, *Cylindrotheca signata* and *Pleurosigma angulatum* were significantly more abundant at the seaward end of the gradient. Laboratory experiments



supported the field observation and emphasized the importance of ammonium concentration for trophic preferences (Underwood et al., 1998). In the present study, although without obvious gradients, salinity created significant difference among three sites and two seasons except for site Re and Ag in summer, and contributed to the main variation of MPB community revealed by DCCA and was also significantly related to species of *Amphora hassiaca* and *Navicula abscondita*. In the study of Du et al. (2016), *Navicula lacustris* and *Pleurosigma anglulatum* were significantly positive, and *Leptocylindrys* sp.1 was significantly negatively correlated to salinity.

In general, these potential indicator species had site specific variation among the different study areas, but with accumulating and categorizing more data, general indicators of diatom assemblages might be designated. For instance, comparing with the above studies, the negative relationship of *Pleurosigma anglulatum* with ammonium or  $\text{NO}_3^-$ , and its positive relationship with salinity was shown, and *Nitzschia sigma* was pollutant-tolerant (Agatz et al., 1999; Underwood et al., 1998). These findings suggest that the presence/dominance of these species may be considered as a potential bioindicator for determining the environmental status of coastal intertidal ecosystems. However, to designate a bioindicator, further field and laboratory studies need to be carried out, especially for coastal areas where many factors fluctuate such as hydrodynamic conditions (tides and currents). It requires frequent observations before the sensitivity to pollution of each taxon (genus or species) is measured and its role as water quality indicator recognized. This is why existing methodologies for diatom bioindicators are mainly limited in freshwater systems, representing by the Trophic Diatom Index (TDI; Kelly, 1995) which is recognized by United Kingdom legislation as a reliable tool to assess river quality. On the contrary, only a very few studies used the transitions of estuary and wetland to marine system for exploring bioindicator systems as discussed above (Underwood et al., 1998; Agatz et al., 1999; Nodine and Gaiser, 2014). Therefore, using benthic diatoms as water quality indicator in coastal area routinely needs further standardized investigation methodology.

In the present study, fifteen heavy metals were analyzed their correlations with MPB assemblages. The positive correlation of species *Entomoneis corrugate*, *Gyrosigma cf. limosum*, *Navicula aitchelbee* with heavy metals (represented by  $\text{Zn}^{2+}$ ) implied that at least these species tolerate heavy metals and could be focused further as potential indicators for higher heavy metals concentration. Previous studies on heavy metals pollution with high excess concentration caused the deformation of diatoms. It proved that the deformity frequency of *Navicula rhyncocephala*, *Achnanthes hauckiana*, *Fragilaria capucina*, and *Diatoma vulgare* was significantly lower at unpolluted sites than at polluted sites (Dickman, 1998). In addition, several publications showed that the diatoms had the ability to adapt/resist environmental heavy metals, not only requiring certain metals as essential elements or showing tolerance through physiology, but also by the restructuring of diatom communities under metal stress (Masmoudi et al., 2013). In the present study, the heavy metals concentrations were almost lower by an order of magnitude than those of in study of Dickman (1998) and review of Masmoudi et al.(2013) , and the strongly multi-collinearity among the variations of 15 heavy metal ions handicapped finding the relationship between individual heavy metal and species. However, it provided a base on the

influence of heavy metals on MPB species composition and a possibility of potential species indicating the level of heavy metals concentrations.

In general, based on our study, there is potential for using MPB to assess the environmental status at a community level, i.e. discriminating environmental quality status using community pattern of MPB. The MPB species that were significantly correlated with individual environmental factors may be used as a potential bioindicator. However, it should be noted that the identification of MPB requires skilled taxonomic experts, and the numeration of MPB is time-consuming, especially for environmental agencies that are often dealing with large spatial/temporal scales in a limited time span. Molecular methods have been tried in this area (Jahn et al., 2007; Kaczmarcka et al., 2007; McGregor, 2010; Moniz and Kaczmarcka, 2010; Trobajo et al., 2010; Hamsher et al., 2011), however, due to the low universal and quantification efficiency based on amplification of environmental samples, there is still a handicap for the practical situation (Regine et al., 2007; Guo et al., 2016). Accompanying the development of new ideas and techniques, further research will promote the application of bioassessment of environmental quality status using MPB community for coastal ecosystems

## 5. Conclusions

The MPB community structures showed spatial and temporal variation patterns in the coastal area of Pertuis Charentais (La Rochelle, France). The variation in MPB communities significantly correlated with environmental variables, especially sediment grain size composition, heavy metals, irradiance, salinity and  $\text{PO}_4^{3-}$  and  $\text{Si(OH)}_4$ , indicating the difference in environmental quality status. The species number and richness might be used as a community level index for high organic matter and ammonium concentration in assessing the quality of environmental status. Particular species could be considered as potential bioindicator for specific environmental factor, such as *Anorthoneis pulex*, *Fallacia scldensis*, *Navicula germalopolnica* for larger grain size, *Entomoneis corrugate*, *Navicula aitchelbee* and *Gyrosigma cf. limosum* for heavy metals, *Navicula phylleptosoma* and *Surirella brebissonii* for  $\text{Si(OH)}_4$  and  $\text{NH}_4^+$ , as well as *G. acuminatum* and *S. brebissonii* for  $\text{PO}_4^{3-}$  and  $\text{NH}_4^+$ . It suggested that MPB communities may be used as a potential bioindicator for assessing environmental quality status in coastal intertidal ecosystems.

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**Table 1.** Environmental factors (mean  $\pm$  SD) at three sampling sites Re, Ag and Ay during the study period

Variables	summer			winter		
	Re	Ag	Ay	Re	Ag	Ay
T (°C)	21.86 $\pm$ 0.38	29.82 $\pm$ 0.50	28.20 $\pm$ 0.27	9.34 $\pm$ 0.09	9.83 $\pm$ 0.31	11.03 $\pm$ 1.31
R ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	1533.32 $\pm$ 359.86	1347.11 $\pm$ 388.52	1613.18 $\pm$ 23.83	222.60 $\pm$ 62.68	235.22 $\pm$ 81.56	97.07 $\pm$ 11.63
Pore water salinity	46.00 $\pm$ 2.53a	40.00 $\pm$ 0.89b	47.67 $\pm$ 0.58a	31.00 $\pm$ 1.00c	18.25 $\pm$ 1.50d	10.00 $\pm$ 0.00e
D50	276.62 $\pm$ 25.20b	8.85 $\pm$ 0.27d	25.64 $\pm$ 0.87d	394.27 $\pm$ 114.57a	9.15 $\pm$ 0.18d	120.21 $\pm$ 1.41c
<63 $\mu\text{m}$ (%)	3.51 $\pm$ 1.21e	90.57 $\pm$ 2.88b	78.92 $\pm$ 1.09c	1.23 $\pm$ 2.15e	93.95 $\pm$ 0.88a	37.33 $\pm$ 0.61d
pH	7.1 $\pm$ 0.16b	7.0 $\pm$ 0.15b	7.1 $\pm$ 0.12b	7.10 $\pm$ 0.11b	7.33 $\pm$ 0.10a	7.47 $\pm$ 0.23a
OM ( $\text{gmL}^{-1}$ )	1.49 $\pm$ 0.08c	4.16 $\pm$ 0.81a	4.36 $\pm$ 0.18a	1.15 $\pm$ 0.11c	3.53 $\pm$ 0.32b	1.80 $\pm$ 0.06c
WC (%)	21.61 $\pm$ 1.24d	69.93 $\pm$ 6.79a	53.73 $\pm$ 3.00b	21.58 $\pm$ 1.68d	68.97 $\pm$ 1.98a	27.89 $\pm$ 0.49c
NO <sub>3</sub> <sup>-</sup> ( $\mu\text{molL}^{-1}$ )	3.70 $\pm$ 4.30c	5.19 $\pm$ 2.01c	3.99 $\pm$ 1.31c	41.64 $\pm$ 28.45b	73.33 $\pm$ 73.07b	395.89 $\pm$ 113.89a
NO <sub>2</sub> <sup>-</sup> ( $\mu\text{molL}^{-1}$ )	0.21 $\pm$ 0.10e	1.54 $\pm$ 0.62c	0.61 $\pm$ 0.26c	0.32 $\pm$ 0.16d	4.31 $\pm$ 2.80b	6.51 $\pm$ 2.07a
NH <sub>4</sub> <sup>+</sup> ( $\mu\text{molL}^{-1}$ )	5.18 $\pm$ 4.17b	70.56 $\pm$ 61.79a	41.70 $\pm$ 31.13ab	11.51 $\pm$ 6.38b	21.93 $\pm$ 9.81ab	8.95 $\pm$ 1.08b
PO <sub>4</sub> <sup>3+</sup> ( $\mu\text{molL}^{-1}$ )	4.57 $\pm$ 1.25a	10.60 $\pm$ 9.72a	6.51 $\pm$ 5.29a	3.42 $\pm$ 0.35a	2.50 $\pm$ 0.56a	1.37 $\pm$ 0.14a
Si(OH) <sub>4</sub> ( $\mu\text{molL}^{-1}$ )	5.83 $\pm$ 3.80c	307.82 $\pm$ 162.30a	138.70 $\pm$ 15.16ab	6.89 $\pm$ 6.64c	69.80 $\pm$ 28.75b	65.36 $\pm$ 9.33b
Zn <sup>2+</sup> ( $\mu\text{g g}^{-1}$ )	10.58 $\pm$ 1.93	114.82 $\pm$ 5.75	75.62 $\pm$ 3.70	8.15 $\pm$ 1.44	113.70 $\pm$ 3.23	24.77 $\pm$ 1.54

R: Irradiance; D50: diameter of 50% of the sediment grains; <63 $\mu\text{m}$ : percentage of sediment grain size < 63  $\mu\text{m}$ ; OM: organic matter content; WC: water content; Same letter denotes no significant difference between different sites or seasons, and different letter denotes significant difference by <0.05 as determined by multiple comparisons in univariate analysis.

**Table 2.** Species number, diversity, evenness, richness, abundance and pigments of microphytonbenthos at three sampling sites during the study period, presented by mean  $\pm$ SD, unit  $\text{mL}^{-1}$  means for fresh sediment.

Season	Site	Species number	Species diversity (Shannon Index)	Species evenness (Pielou Index)	Species richness (Margalef Index)	Abundance ( $10^5$ cells $\text{mL}^{-1}$ )	Chl <i>a</i> ( $\mu\text{g mL}^{-1}$ )	Pheo-pigment ( $\mu\text{g mL}^{-1}$ )	Chl <i>a</i> /pheo
summer	Re	35	2.25 $\pm$ 0.14a	0.71 $\pm$ 0.02a	3.73 $\pm$ 0.45b	10.73 $\pm$ 9.12b	38.79 $\pm$ 2.78a	3.21 $\pm$ 0.26d	12.14 $\pm$ 1.12b
	Ag	70	2.39 $\pm$ 0.37a	0.70 $\pm$ 0.05a	5.22 $\pm$ 1.94ab	4.03 $\pm$ 2.65bc	8.59 $\pm$ 2.04c	11.77 $\pm$ 3.29b	0.75 $\pm$ 0.20d
	Ay	58	2.59 $\pm$ 0.14a	0.72 $\pm$ 0.03a	6.12 $\pm$ 0.68a	0.93 $\pm$ 0.11c	29.06 $\pm$ 1.78b	21.82 $\pm$ 1.52a	1.33 $\pm$ 0.02cd
winter	Re	30	2.01 $\pm$ 0.17a	0.67 $\pm$ 0.04a	3.14 $\pm$ 0.40b	25.39 $\pm$ 9.38a	38.71 $\pm$ 12.14a	2.70 $\pm$ 1.17 d	16.09 $\pm$ 6.59a
	Ag	60	2.16 $\pm$ 0.76a	0.63 $\pm$ 0.17a	4.87 $\pm$ 1.49ab	4.30 $\pm$ 1.90bc	11.57 $\pm$ 4.66c	10.57 $\pm$ 1.18b	1.08 $\pm$ 0.38d
	Ay	28	2.38 $\pm$ 0.24a	0.77 $\pm$ 0.08a	3.50 $\pm$ 0.29b	6.30 $\pm$ 1.39bc	41.76 $\pm$ 3.58a	7.39 $\pm$ 1.03c	5.69 $\pm$ 0.51d

\* Same letter denotes no significant difference between different sites or seasons, and different letter denotes significant difference by  $<0.05$  as determined by multiple comparisons in univariate analysis.

## Figure Captions

Fig.1. Sampling sites in the coast of La Rochelle, France, Re in Re Island, Ag in Aiguillon Bay; Ay in Aytre Bay.

Fig.2. Grain size distribution in the studied sites. Grain size fractions expressed in percentage and consisted of mud (<63µm), very fine sand (63-125µm), fine sand (125-250µm), medium sand (250-500µm), and coarse sand (>500µm); A: summer, B: winter.

Fig.3 Taxonomic composition of microphytobenthic communities of individual samples collected from site Re (1-6), Ag (7-12) and Ay (13-15), A: summer; B: winter. (The species with relative abundance above 5% are presented, with the remaining species grouped as 'others'. The relative abundance is presented as the percentage of the total cells counted).

Fig.4. Classification of samples based on species composition by nonmetric multidimensional scaling (nMDS) in CANOCO 5.0.

Fig.5. Detrended canonical correspondence analysis (DCCA) based on square-root transform of species abundance. The solid arrowheads and italic labels indicate the species: AchD: *Achnantheiopsis delicatula*; AmpH: *Amphora hassiaca*; AnoP: *Anorthoneis pulex*; AnoV: *Anorthoneis vortex*; CocH: *Cocconeis hauniensis*; CylC: *Cylindrotheca closterium*; DelM: *Delphineis minutissima*; EntC: *Entomoneis corrugate*; FalS: *Fallacia scaldensis*; GyrA: *Gyrosigma acuminatum*; GyrL: *Gyrosigma cf. limosum*; GyrF: *Gyrosigma fasciola*; NavA: *Navicula abscondita*; NavAl: *Navicula aitchelbee*; NavG: *Navicula germanopolonica*; NavGR: *Navicula gregaria*; NavP: *Navicula perminuta*; NavPH: *Navicula phylleptosoma*; NavS: *Navicula sp.1*; NitA: *Nitzschia aequorea*; NitP: *Nitzschia panduriformis var.continua*; PleA: *Pleurosigma angulatum*; SkeC: *Skeletonema costatum*; SurB: *Surirella brebissonii*; ThaV: *Thalassiosira cf. visurgis*; The lines with empty arrowhead and bold labels indicate the environmental factors: D50: diameter of 50% of the sediment grains; OM: organic matter content; R: Irradiance; S: salinity; pH; NO<sub>2</sub>: NO<sub>2</sub><sup>-</sup>; NO<sub>3</sub>: NO<sub>3</sub><sup>-</sup>; NH<sub>4</sub>: NH<sub>4</sub><sup>+</sup>; PO<sub>4</sub>: PO<sub>4</sub><sup>3+</sup>; Zn: Zn<sup>2+</sup>; Si: Si(OH)<sub>4</sub>.

## Highlights:

1. The variation in microphytobenthos community was spatio-temporal different
2. The microphytobenthos community closely relate to environmental factors
3. The community level index could assess the environmental quality status
4. Particular species can be bioindicator for specific environmental factor

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