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Taxonomic and functional responses of macrofaunal assemblage provide insight into ecological impacts of bottom-based Manila clam aquaculture

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ABSTRACT

Understanding ecological impacts of bottom-based clam aquaculture can improve its management. In this study, taxonomic and functional macrofaunal assemblage were evaluated for two clam farms located in Laizhou Bay, China. Beta diversity and factors potentially regulating the dissimilarity of macrofauna were estimated. Both taxonomic and functional composition of macrofauna showed large differences between the clam farm and the control area. Functional dissimilarity within the clam farms was found to be nestedness and negatively correlated to local clam abundance. Additionally, the cultured clam enhanced the functional richness but made the macrofaunal assemblage more fragile against species or function loss. This effect would increase with clam abundance, which highlights the importance of identifying optimal clam culture intensity in developing a bottom-based clam aquaculture program.

1. Introduction

Coastal benthic ecosystems are experiencing multiple natural and anthropogenic disturbances, including development of aquaculture programs which continue to rapidly expand in coastal waters in the world to meet the increasing food demand (Ferriss et al., 2016). Over the last century, intensified aquaculture industries have significantly influenced the structure and functioning of the coastal ecosystem (Price et al., 2015), altering nutrient fluxes, food webs, and service delivery in marine ecosystems (Lacoste et al., 2020). The intensified aquaculture raises questions about how to sustainably develop the aquaculture industry without having irreversible impact on the coastal ecosystems.

Bivalve aquaculture is considered sustainable marine activities since the cultured animals require no additional food inputs (Dumbauld et al., 2009; Cranford et al., 2012), particularly for bottom culture due to lower cultivation intensities compared to off-bottom culture (Kluger et al., 2016). Nevertheless, the introduction of cultured individuals increases bivalve densities above natural levels and potentially impacts the macrofaunal assemblages through enhancing local habitat complexity (Vozzo et al., 2021), changing water chemistry (Zhao et al., 2019) and attenuating water currents (Gieschen et al., 2020). Given these potential ecological impacts, an evaluation of bivalve aquaculture impacts on the adjacent assemblage is crucial for the maintenance of ecosystem health and functioning in the context of ecosystem-based management (Filgueira et al., 2016; Kluger et al., 2016).

Bottom-based cultured bivalves can directly impact other benthic macrofauna through biotic interactions. This makes macrofaunal assemblage an ideal indicator to monitor the ecological impact of bottom cultured bivalve. Many studies evaluated the response of macrofaunal assemblage to bivalve culture by comparing the differences in taxonomic and functional structure of assemblage between bivalve farms and surrounding areas (Wang et al., 2017; Liao et al., 2019). However, examinations of the responses in the composition of biological traits were less common. Additionally, the species and biological traits of macrofauna were not evenly distributed within a local farm scale, despite a homogenized environmental condition (Lacson et al., 2019). For example, Wang et al. (2017) reported a significant negative correlation between clam density and the abundance of detritivores within a bottom-based farm; Carcedo et al. (2019) found fewer filter-feeders in the places where cultured bivalves were abundant; and de Fouw et al. (2020) found that an increased abundance of cultured bivalves could attract more predators. These findings suggest that on the local farm

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scales, biotic interaction could be the dominant factor for shaping the taxonomic and functional structure of macrofaunal assemblage rather than environmental filtering (de Fouw et al., 2020).

Beta diversity, which measures dissimilarity of assemblage across space, time, or any other gradient of variation from a taxonomic, phylogenetic, and functional perspective, is a useful metric in identifying ecological processes for cross-scale biodiversity patterns (Baselga, 2010; Carlos-Júnior et al., 2019; Bevilacqua et al., 2020). Beta diversity can be divided into two components (Baselga, 2012): nestedness describing species (or functional traits) gain or loss and turnover relating to species (or functional trait) replacement without changes in richness (Baselga, 2010). The beta-diversity quantifies the degree of differentiation of biological communities in responses to different disturbances (Boyé et al., 2019; Troast et al., 2019).

To date, few studies have evaluated the taxonomic and functional differences in macrofaunal assemblage within bottom-based bivalve farms and identified potential drivers. In this study, macrofaunal assemblages were evaluated in two separated bottom-based farms for Manila clams (*Ruditapes philippinarum*) in Laizhou Bay, China. By comparing the taxonomic and functional compositions between clam farms and control area, the taxonomic and functional beta diversity within each farm along with environmental and clam abundance gradients was assessed. The overarching objective is to understand ecological impacts of bottom-based bivalve aquaculture program. Specifically, the aims of the present study are to (1) examine the overall ecological impact of bottom-based Manila clam aquaculture on taxonomic and functional composition of macrofaunal assemblage and the responses of specific biological traits and (2) identify potential drivers and mechanisms regulating the taxonomic and functional structure within the clam farms. The results derived can greatly improve the understanding on ecological impacts of bivalve aquaculture, leading to improved bottom-based clam aquaculture management.

2. Material and methods

2.1. Site description

Laizhou Bay has an average depth of 9 m (maximum ~18 m) and a total area of 700,000 ha (Zhuang et al., 2014). It is an important breeding and spawning area for many marine animals, but has been subject to disturbance from human activities such as aquaculture. Two bottom-based farms for Manila clam (*R. philippinarum*) aquaculture are in the southeast part of Laizhou Bay (37°27′ to 37°32′N, 119°50′ to 119°54′E and 37°31′ to 37°35′N, 120°02′ to 120°04′E, respectively) and were included in this study (Fig. 1). There is barely seagrass, macroalgae or reef around the study area. The two farms have typical bottom-based bivalve aquaculture operation, cover areas of approximately 36 and 27 km^2, respectively, and are approximately 5 km away from the coast. Both farms were established in July 2019 when juvenile clams (1.03 ± 0.35 cm) were cultured on the bottom. Juvenile clams were collected from another area of Laizhou Bay where large numbers of wild clams were distributed. The cultured clams were grown on the bottom to reach market sizes.

2.2. Sampling procedure

Macrofauna and environment variables were collected at 15 sampling stations in farm A and 10 sampling stations in farm B. Another 5 sampling stations were set in the control area with no aquaculture operation. Data were collected from September to October 2019. Macrofauna was collected with Van Veen Grab Sampler (0.1 m^2) and three replicates were collected randomly at each station. The collected macrofauna samples were sieved through a 0.5 mm mesh in the field and then preserved with 5% buffered formalin. The organisms were identified to the lowest possible taxonomic level (generally species) based on relevant species identification guides (Yang and Sun, 1988, 2005; Yang and Wang, 1996; Zhang et al., 2016). Nine variables were selected to describe the environmental conditions for each sampling station (Table 1). Sediment samples were preserved in -20 °C until analysis. Organic matter content of sediment was measured by the potassium dichromate-sulphuric acid ($K_2Cr_2O_7$-H_2SO_4) oxidization method (Walkley and Black, 1934). The grain size of sediment was measured by a laser particle analyzer (Master Sizer 3000). Water temperature, pH, salinity, and dissolved oxygen were determined in situ by an YSI 600XLMMulti-Parameter Water Quality Sonde (YSI Inc., USA).

2.3. Biological traits

A total of 22 biological traits were used from the following six categories to describe the biological function of each species: feeding habit, mobility, vertical position, normal adult size, longevity, and AMBI (AZTI'S Marine Biotic Index) (Table 2). Fuzzy coding technique was applied to associate taxon with a trait category based on their affinity scores (Chevenet et al., 1994). The affinity of a taxon in each category

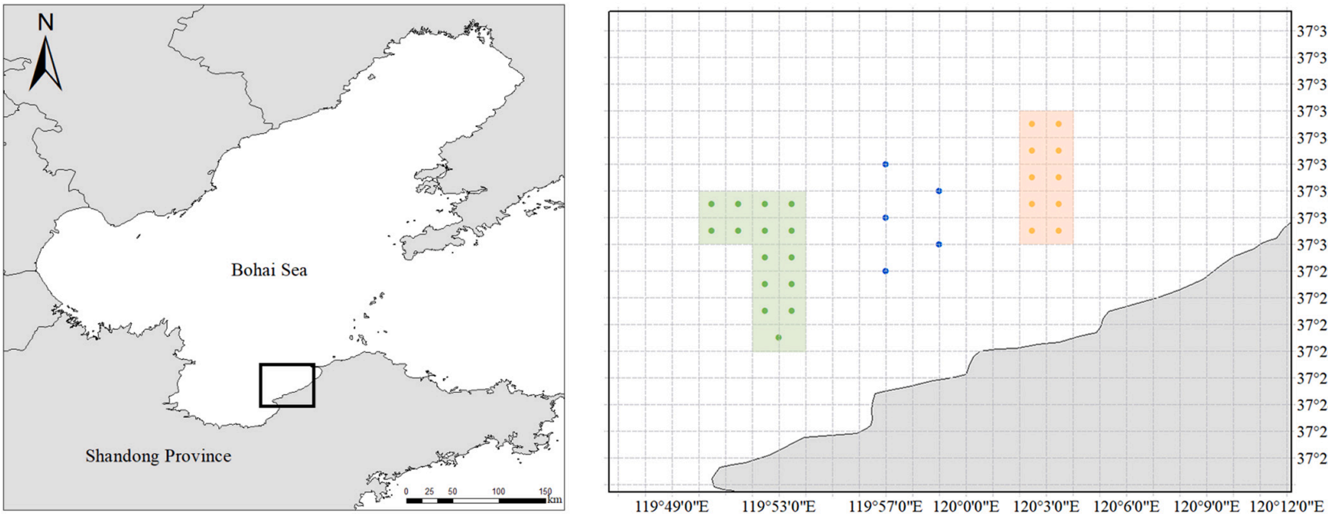

Fig. 1. Study area in the south of Bohai Sea, China, showing the sampling stations in the two farms for bottom culture of Manila clam (*R. philippinarum*) and the control area (with no aquaculture activities). Sampling stations in Farm A, Farm B, and the control area are represented in green, orange, and blue, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Environmental variables measured at sampling stations. Data are presented as mean ± SD. Clam abundance represents the total numbers of clam individual collected from the grab sampler.

	Farm A	Farm B	Control
Temperature (°C)	25.5 ± 0.3	25.5 ± 0.3	25.6 ± 0.3
Depth (m)	11.1 ± 0.3	12.4 ± 0.2	11.3 ± 0.4
pH	8.24 ± 0.02	8.26 ± 0.02	8.28 ± 0.05
Salinity	27.3 ± 0.1	27.3 ± 0.1	27.3 ± 0.1
DO (mg·L^{-1})	10.5 ± 0.3	10.6 ± 0.3	10.7 ± 0.2
Chl-a (mg·L^{-1})	2.2 ± 0.3	2.3 ± 0.4	2.4 ± 0.3
OM(%)	1.6 ± 0.1%	3.0 ± 0.4%	2.7 ± 0.4%
Sediment grain size (μm)	72.5 ± 1.3	61.5 ± 1.6	60.5 ± 1.3
Sediment type	Fine sand	Coarse silt	Coarse silt
Clam abundance	72.7 ± 41.4	35.9 ± 22.9	–
Species richness	33	32	33
Abundance per site	18.4 ± 3.7	20.2 ± 4.4	25.4 ± 3.4
Functional richness	12.5 ± 8.4	15.2 ± 7.4	1.2 ± 0.4
Functional redundancy	2.2 ± 1.2	2.3 ± 1.4	5.2 ± 0.6

Table 2
Functional traits, categories, and abbreviations in macrofaunal assemblages.

Functional traits	Trait modalities	Abbreviations
Feeding habit	Carnivore	F1
	Surface deposit feeder	F2
	Subsurface deposit feeder	F3
	Suspension feeder	F4
	Herbivorous	F5
Mobility	Sessile	M1
	Swimmer	M2
	Burrower	M3
	Crawler	M4
Vertical position	Infauna	P1
	Epifauna	P2
Normal adult size	Small (<1 cm)	B1
	Small-medium (1-3 cm)	B2
	Medium-large (3-10 cm)	B3
	Large (>10 cm)	B4
Longevity	Short (<1 year)	L1
	Medium (1-3 year)	L2
	Long (>3 year)	L3
AMBI groups	Sensitive (I)	I
	Indifferent (II)	II
	Tolerant (III)	III
	Opportunistic (IV)	IV

was assigned a score from zero (no affinity) to three (maximum affinity) based on its biology. If the information on a given trait is currently not available, it takes the trait from other taxa in the same genus. If such traits are still absent for all other taxa of the genus, a score of “0” is assigned. Affinity scores were standardized to ensure the same contribution of each trait to statistical analyses (Péru and Dolédec, 2010). Functional trait information was gathered from research papers from the mainstream web tools (i.e., Web of Science and Google Scholar) and web databases such as MarLIN BIOTIC (Biological Traits Information Catalogue: http://www.marlin.ac.uk/biotic/), WORMS (World Register of Marine Species: http://www.marinespecies.org), and Marine Species Identification Portal (http://species-identification.org/).

2.4. Data analysis

A previous investigation on the macrofaunal assemblage in the two farms reported negligible abundance of wild Manila clam at the sampling stations, suggesting that most Manila clams collected in the farms were from aquaculture. Given this, the abundances of clams and other organisms in assemblages were analyzed separately.

Correspondence analysis (CA) was used to ordinate each sampling station based on taxa abundance data to summarize the taxonomic variability of macrofaunal assemblages among Farm A, Farm B, and the control area. The continuous variables were $\log_{10}$ transformed to ensure the satisfaction of normality assumption. The significance of the axes was determined at $p < 0.05$ by testing the eigenvalues of the inertia matrix (Dedieu et al., 2015). The top 14 abundant species were plotted as representative taxa as they contributed to more than 80% of the total abundance in the study area. Biological trait structure was obtained by species' abundance in each station multiplied by their functional affinity score (van der Linden et al., 2012). The site-trait array was then processed by Fuzzy Correspondence Analysis (FCA) to obtain the functional ordination of the sampling stations (Chevenet et al., 1994). Permutational multivariate analysis of variance (PERMANOVA) was used to assess if there were significant differences in both taxonomic and functional composition among the three study areas.

The calculation of taxonomic beta diversity ($^{C}\beta$) and its two components (turnover, $^{C}\beta_{turn}$ & nestedness, $^{C}\beta_{nes}$) within each area follows the approach proposed by Baselga (2010). The total pairwise beta diversity was calculated with Sørensen dissimilarity index with $^{C}\beta_{turn}$ being calculated using the Simpson dissimilarity index and $^{C}\beta_{nes}$ calculated as the difference between $^{C}\beta$ and $^{C}\beta_{turn}$ (i.e., $^{C}\beta$ - $^{C}\beta_{turn}$; Baselga, 2010). The beta diversity indices were calculated with species presence/absence data. Functional β-diversity was computed using the multidimensional functional space (Villéger et al., 2008). In this method, principal coordinates analysis (PcoA) was used to generate a multidimensional functional space based on the 22 functional traits. Gower dissimilarity matrixes were created by taxa × functional traits. The Gower dissimilarity matrix was analyzed using PCoA. The PCoA axes were used as the functional space to calculate functional beta diversity ($^{F}\beta$) and its two components (i.e., turnover, $^{F}\beta_{turn}$ & nestedness, $^{F}\beta_{nes}$) (Laliberte and Legendre, 2010; Villéger et al., 2013). This procedure allows missing values in the functional trait matrix (Villéger et al., 2008; Laliberte and Legendre, 2010). Additionally, given that the calculation of $^{F}\beta$ and its components require long time when 10 functional space of the assemblage is more than four-dimension (Baselga and Orme, 2012), the first four PCoA axes of the functional space were kept for all the analysis due to their accumulative explanation of variation exceed 80% in the original functional trait matrix (Table S1).

Multiple regression on distance matrices (MRM) (Lichstein, 2007) was applied to estimate the association of taxonomic and functional beta diversity and their components with a matrix of selected explanatory variables of each sampling station based on their Euclidean distance. The significance was assessed by Mantel's tests (999 permutations). Before modeling, Spearman's correlation analysis was applied to assess the correlations of the explanatory variables to avoid multicollinearity. Variables were precluded from the analysis if Spearman's r is higher than 0.7. The explanatory variables were standardized to ensure comparable magnitudes of model coefficients in the analysis (Gelman, 2008). The relative importance of each variable contributing to the model was estimated by their absolute values of regression coefficients. Next, to obtain an overview of correlations between each component of beta diversity and their specific potential drivers, the variables were divided into two categories: environmental variable, which includes water temperature, depth, pH, salinity, DO, Chl-a, OM and sediment grain size; and clam abundance at each station.

To identify variations in beta diversity and its two components of assemblage along different levels of clam abundance, the beta diversity and its components in the two farms were plotted against the difference in standardized clam abundance from each pairwise stations within each area by a linear regression. Finally, the stations along the clam abundance gradients were divided into six levels with an equal abundance range of 20 individuals, and calculated the beta diversity and its components for each level, to analyze the beta diversity and its components of macrofaunal assemblage across clam abundance.

All the data analyses were performed in the R environment (R Core Team, 2012). Functional indices (Functional richness and functional redundancy) were calculated by package “*FD*” (Laliberte and Legendre, 2010). Package “ade4” (Dray and Dufour, 2007) was used for CA and

FCA. Package “vegan” (Dixon, 2003) was used for PCoA analysis and MRM modeling. Beta diversity and its components were assessed using “*betapart*” (Baselga, 2012). The standardization of explanatory variables used the package “*arm*” (Gelman and Hill, 2009) for environmental variables and package “MuMIn” (Bartoń, 2013) for clam abundance.

3. Results

3.1. Environmental conditions and assemblage indices

The overall water chemical conditions had no significant differences among the three areas (Table 1). Sediment organic matters (OM) in Farm B and control area were similar but were both significantly higher compared with that in Farm A (one-way ANOVA, $p < 0.05$). The average sediment grain size in Farm A (72.5 ± 1.3 μm) was significantly higher than that of Farm B (61.5 ± 1.6 μm) and the control area (60.5 ± 1.3 μm) (one-way ANOVA, $p < 0.05$). The functional richness of the regional pool of species was higher in the two farms (Fric = 12.5 ± 8.4 and 15.2 ± 7.4, respectively) than in the control area (1.2 ± 0.4), despite that the species richness was almost the same among the three areas (33 in Farm A, 32 in Farm B, and 33 in the control area, respectively). Functional redundancy in the control area (5.2 ± 0.6) was higher than that in the two farms (2.2 ± 1.2 and 2.3 ± 1.4, respectively). There was no significant correlation ($r > 0.7$) between variables according to Spearman's correlation analysis (Table S2).

3.2. Taxonomic and functional composition of macrofaunal assemblages

A total of 54 taxa were identified in this study. Detailed taxa composition and their trait codes are provided in the supplementary materials (Tables S3 and S4, respectively). The first and second CA axes explained 16.94% and 12.04%, respectively, of the total variability in the taxonomic structure of macrofaunal assemblages. The scatterplot of the taxonomic composition of sampling stations could be divided into three subsets along the first two axes when sampling stations were more specifically grouped based on their geographic origins (Fig. 2a). Three environmental variables including Chl-a, OM, and sediment grain size were significantly correlated with the CA axis1. Species abundance was identified to have an opposite trend against clam abundance. Dominant species in each area were different with Farm A being dominated by echinoderms, crustaceans, and gastropods, Farm B by echinoderms and crustaceans, and the control area by polychaetes (Fig. 2c).

The functional compositions of the three areas were analyzed using the FCA. The first and second FCA axes explained 38.21% and 25.02% of the total variability, respectively. Unlike taxonomic composition, the functional compositions for the two farms had a large overlap but was significantly different from that for the control area (PERMANOVA, $p < 0.05$) (Fig. 2b). The clam abundance for the two farms in the FCA axis

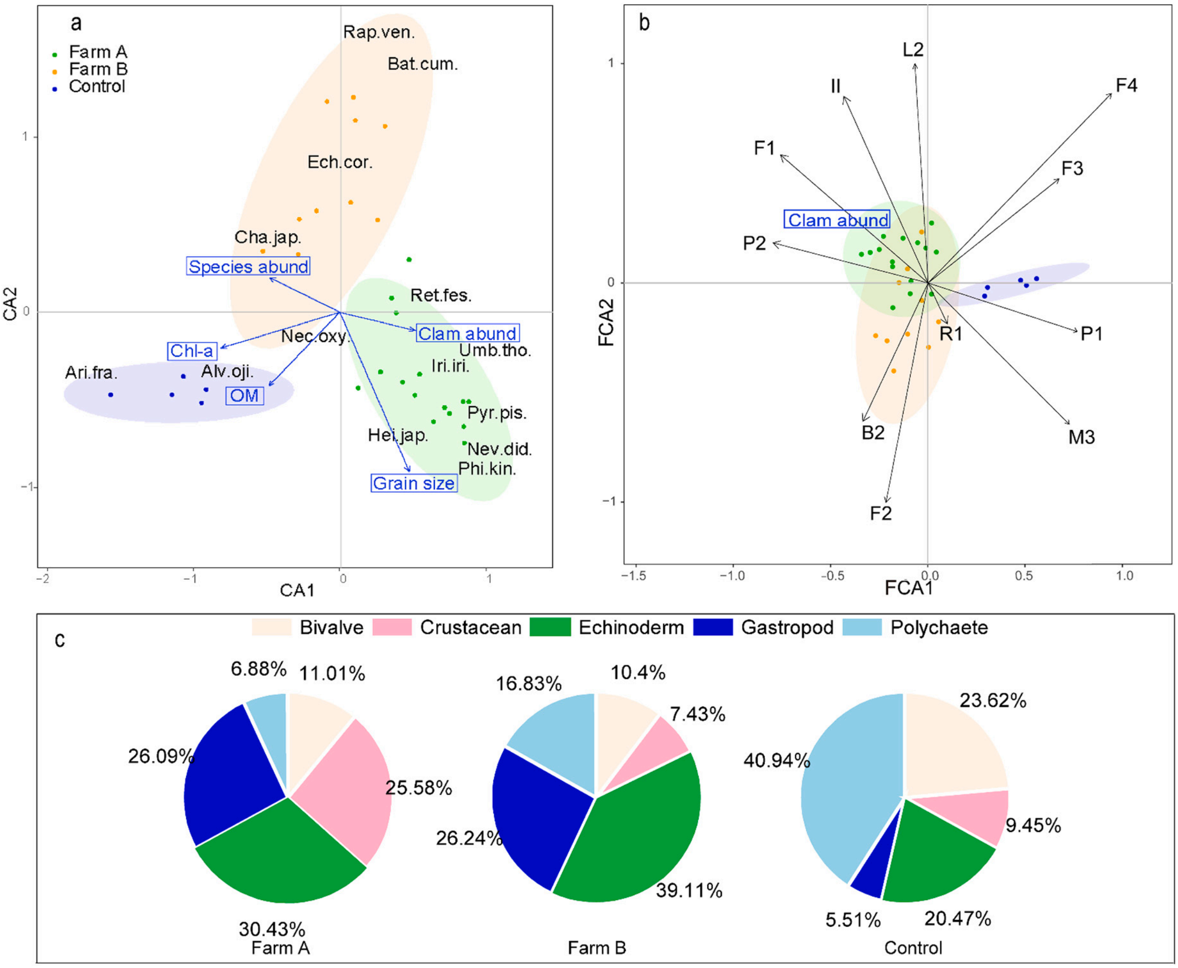

Fig. 2. Correspondence analysis (CA) of the taxonomic composition at the sampled station (a). Top 14 abundant species are illustrated as they contributed to more than 80% of the total taxa. Fuzzy correspondence analysis (FCA) of functional composition at the sampled stations (b) Green, orange, and blue dots represent the sampling stations in Farm A, Farm B, and control area, respectively. The taxonomic groups of macrofauna in each area are also shown (c). “Species abund” and “Clam abund” represent the species abundance and clam abundance, respectively. Abbreviations for traits can be found in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

corresponds biological traits including carnivore (F1), epifauna (P2), and indifferent (II). Scatterplots for the control area were generally to the right and the corresponding traits include subsurface deposit feeder (F3), filter feeder (F4), burrower (M3), and infauna (P1). The percentages of functional traits for the macrofaunal assemblages of each area were detailed in the supplementary materials (Fig. S1).

3.3. Taxonomic and functional beta diversity of macrofaunal assemblage and their potential divers

The overall taxonomic beta diversity of macrofaunal assemblage was 0.81 (${}^{C}\beta_{nes} = 0.04$, ${}^{C}\beta_{turn} = 0.77$) for Farm A, 0.77 (${}^{C}\beta_{nes} = 0.05$, ${}^{C}\beta_{turn} = 0.72$) for Farm B, and 0.55 (${}^{C}\beta_{nes} = 0.06$, ${}^{C}\beta_{turn} = 0.49$) for the control area. Overall functional beta diversity was 0.53 (${}^{F}\beta_{nes} = 0.21$, ${}^{F}\beta_{turn} = 0.33$) for Farm A, 0.67 (${}^{F}\beta_{nes} = 0.23$, ${}^{F}\beta_{turn} = 0.43$) for Farm B, and 0.13 (${}^{F}\beta_{nes} = 0.06$, ${}^{F}\beta_{turn} = 0.07$) for the control area. Both taxonomic and functional beta diversity for the control area were lower compared to those for the two farms. The partition of the ${}^{C}\beta$ (Fig. 3a) showed that differences in ${}^{C}\beta$ were mainly due to species' turnover which contributed 95.85% in Farm A and 93.06% in Farm B, respectively, compared to their low values for taxonomic nestedness. While the ${}^{F}\beta$ of the two farms were mainly contributed by ${}^{F}\beta_{turn}$, which was 60.95% and 64.92% for Farm A and Farm B, respectively (Fig. 3b).

The variation partitioning the analysis based on MRM suggests that environmental variables explained most of the variances of taxonomic beta diversity and its two components for the two farms compared to clam abundance (Fig. 4a). Nevertheless, the total contribution of the spatial, environmental, and biological variables explained less than 50% of the total variation in taxonomic beta diversity and its two components, indicating a large proportion of deviance remained unexplained by the selected variables. Functional beta diversity and its two components for the two farms were not significantly correlated to most of the selected environmental variables (Fig. 4b). However, clam abundance accounted for a relatively large proportion of the variability in the functional beta diversity and functional nestedness for the two farms, whereas functional turnover was mainly explained by the environmental variables. The regression coefficients estimated for each variable were listed in the supplementary materials (Table S5).

All the selected variables together explained less than 50% of the variations in the taxonomic beta diversity and its two components, and clam abundance contributed mostly to the variation of the functional beta diversity. Thus, only the relationships between the variance in functional compositions and clam abundance were illustrated for the two farms (Fig. 5). The statistical coefficients were documented in the supplementary materials (Table S6). For the initial Sørensen dissimilarity, the total functional beta diversity was significantly and positively correlated to the differences in clam abundance for both farms ($p < 0.05$) (Fig. 5a). Decomposing functional beta diversity revealed that the positive pattern for both farms was mainly driven by functional nestedness ($p < 0.05$) (Fig. 5b), whereas the turnover component tended to be independent of clam abundance ($p > 0.05$) (Fig. 5c). For clam abundance belts, significant negative trends were found in the total functional beta diversity and functional turnover for both farms ($p < 0.05$) (Fig. 5d, f). Functional nestedness showed non-significant differences with increased abundance ($p > 0.05$) (Fig. 5e). The points along each belt were not consistent because there were no stations in Farm A containing 20–40 clams and no stations in Farm B having more than 80 clams.

4. Discussion

The responses of macrofaunal assemblages have been widely used to measure the ecological impact of aquaculture disturbance (Liao et al., 2019; Wang et al., 2017). In the present study, both taxonomic and functional composition of macrofauna showed large differences between the clam farm and control area, which indicated strong ecological impacts of cultured clams on the ecosystem.

4.1. Responses of macrofaunal assemblage to bottom-based Manila clam aquaculture

A relatively low variability was explained by the first two CA axes. This is common when evaluating taxonomic assemblages (Céréghino et al., 2011; Dedieu et al., 2015), while the analysis of functional traits tended to provide much higher levels of explanations to the total variability in the functional composition.

There were no artificial barrier or strong current isolating the study areas, which confirmed the connection and free distribution of organisms in the areas. However, the variations in taxonomic and functional composition among the three study areas indicated that the macrofauna were not randomly distributed. Echinoderms and gastropods were observed much higher in percentage in the two farms, compared to those in the control area. These two taxonomic groups include clam predators (e.g., *Rapana venosa* and *Asterias amurensis*) that were commonly observed in bivalve farms (Kluger et al., 2017; Wang et al., 2017). Polychaetes and other bivalves were abundant in the control area but their dominance were replaced in the two farms. This could be explained by the competition for habitat among burrower species (Leventon,

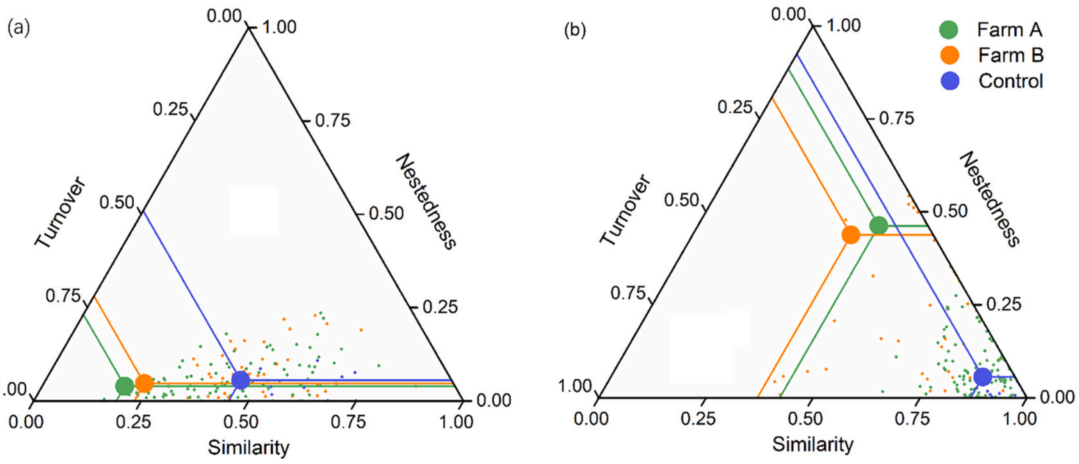

Fig. 3. Ternary plots of the composition of taxonomic beta diversity (a) and functional beta diversity (b) of the two farms. The points represent triplets of value of Similarity (Similarity = 1 – Sørensen dissimilarity), Turnover, and Nestedness and the lines extending to each axis. Each triplet sums to 1. The big dots represent the total dissimilarity of each area, while the small dots represent the pairwise between-site values. Manila clams were excluded from the analysis.

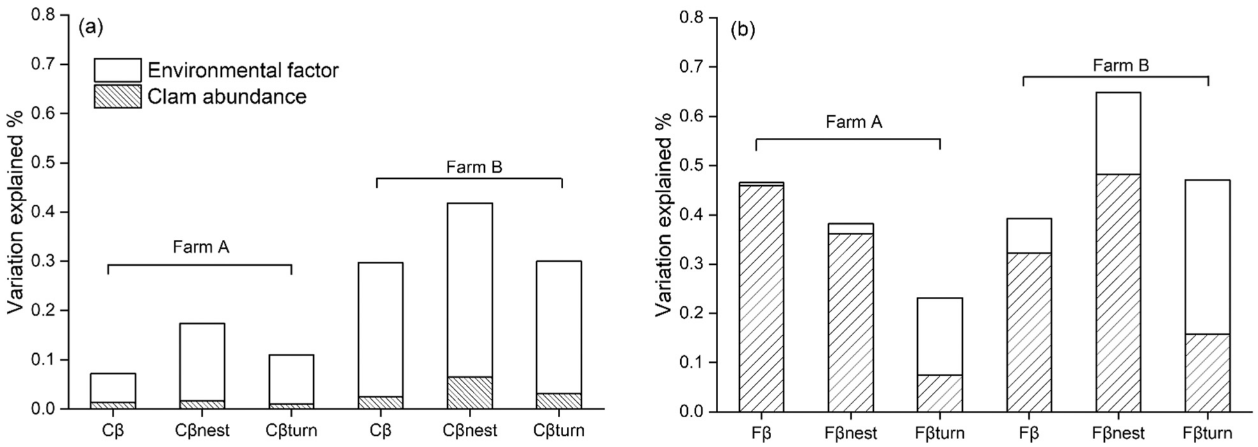

Fig. 4. The relative importance of environmental factors and clam abundance in explaining the variance in taxonomic (a) and functional (b) composition of macrofaunal assemblage for the two clam farms. Specific correlations for each variable are listed in Table S5 in the supplement materials.

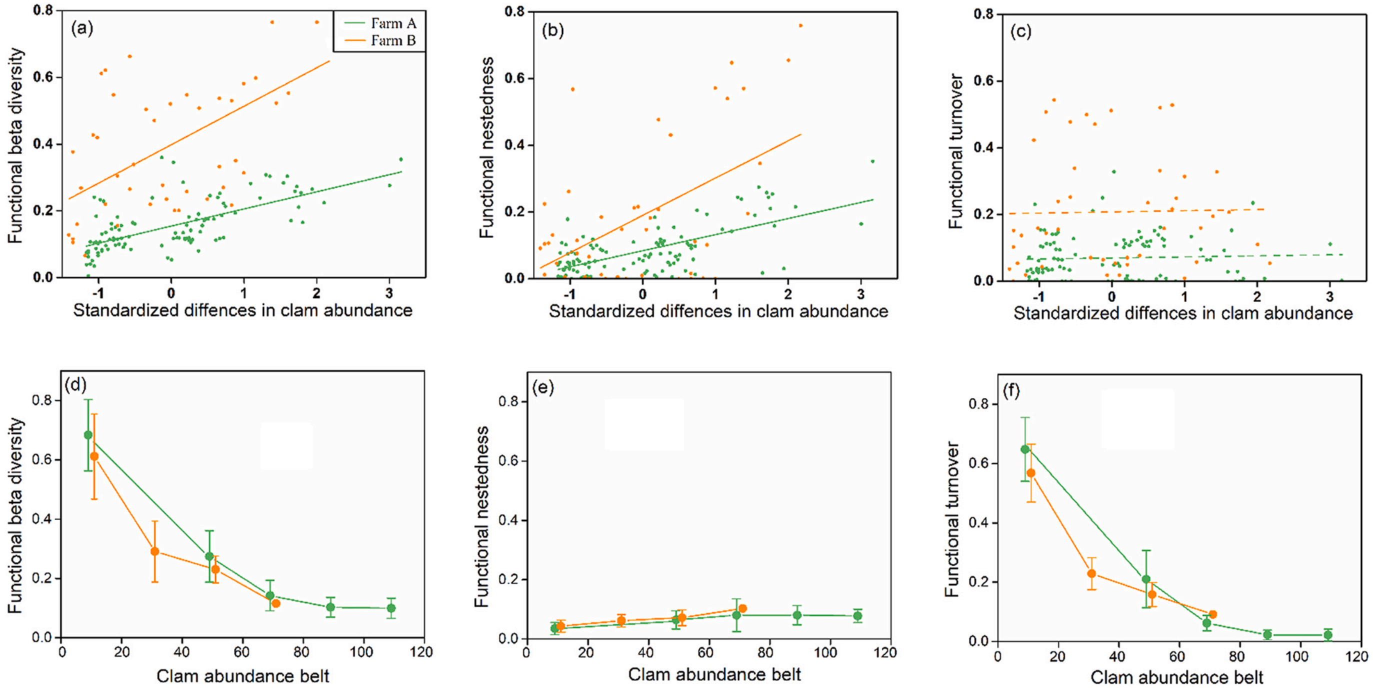

Fig. 5. The relationships between the variance in functional compositions and clam abundance for the two farms. The total functional β diversity (a), functional nestedness (b), and functional turnover (c) for the two farms were linearly modelled against the standardized differences of clam abundance. The total functional β diversity (d), functional nestedness (e), and functional turnover (f) for the two farms were modelled against clam abundance. The non-significant ($p > 0.05$) relations are shown as dash lines (a–c). The statistical coefficients were illustrated in the supplementary materials (Table S6).

1972). As the abundance of cultured clams is much higher than that of natural species, they competed the habitats with polychaetes and other burrowers bivalves, resulting in a large decrease in these species (Sun et al., 2021). Additionally, the opposite trend of macrofauna abundance and clam abundance also support that there might be intensive habitat competition of macrofauna within clam farms. Although the two clam farms had similar ratios in taxonomic groups, the large variations in macrofaunal composition were observed. This might result from different sediment types between the two farms, since sediment types are commonly considered as key factor driving the composition of macrofauna or dominant groups (Macdonald et al., 2012).

The functional compositions of the macrofaunal assemblage were significantly different between clam farms and the control area. The macrofaunal assemblage in the two clam farms had large functional overlaps according to the FCA, suggesting the macrofaunal functions had similar responses on bottom-based clam aquaculture. More carnivores and epifaunas were identified in the clam farms. The clams cultured on the bottom might attract their predators so that carnivore organisms became more abundant compared to the control area (Sardenne et al., 2019). Predators of Manila clam including *P. kinglipini, N. didyma,* and *C. japonica* were epifaunal species, resulting in epifauna being proportionally increased. On the other hand, clam farms were negatively correlated to suspension-feeder, infauna, and burrower. These traits are consistent with Manila clam, suggesting that species having functional overlapping with cultured bivalve species suffered most because of competition (Kluger et al., 2016; Lacson et al., 2019). Moreover, an increase in clam predators might also raise the predation pressure on these species and therefore put even higher pressure on these organisms.

Burial bivalve species increase the deposition of organic matters by their metabolism and thus benefit the surrounding detritivores (Huang et al., 2018). However, the subsurface deposit feeder showed a similar negative trend against clam abundance. These might suggest that clam-caused biotic interactions, rather than organic matters, were major factors in regulating the abundances of these species. In general, subsurface deposit feeders prefer to inhabit in sediment, which accordingly resulted in niches overlapping and competing suitable habitats with cultured Manila clam (Carcedo et al., 2019). This also explained the negative correlation between clam abundance and infauna found in the present study.

4.2. Taxonomic and functional beta diversity and their potential drivers

In the present study, the increased taxonomic beta diversity in clam farms were mainly due to species turnover. Higher contributions of species turnover to beta diversity hinted that the natural environmental parameters were the major factors in shaping the macrofaunal composition (Baselga, 2010; Socolar et al., 2016). Indeed, the compositional variation driven by environmental sorting could be abrupt even on small spatial scales (Heino et al., 2015). The cultured clam could increase the environmental complexity (Jones et al., 1994) and accordingly caused the increased taxonomic beta diversity through turnover.

Macrofaunal assemblages were more functionally different in clam farms compared to the control area where macrofaunal functions turned to be homogenized (Fig. 3b). Unlike taxonomic dissimilarity, the raised functional dissimilarity was mainly caused by turnover rather than nestedness. High species turnover may not always correspond to high functional turnover when replaced species have functional overlaps (Villéger et al., 2013). This suggested that species turnover in the clam farms was mainly concentrated on the functionally overlapped group. Thus, their replacement did not involve functional replacement because their functional traits could be compensated by other species with similar traits (Bevilacqua et al., 2020).

The cultured organisms commonly act as keystone species as their abundance was higher above natural levels in an aquaculture system (Forde et al., 2015). For Manila clams, even though limited, their mobility enables them to aggregate so that bottom cultured clams commonly showed high variance in abundance within a farm (de Fouw et al., 2020). The examination of functional categories showed that traits were related to the clam abundance. Such traits, either overlapping in niches with cultured Manila clam (e.g., suspension-feeder, epifauna) or directly attracted by them (e.g., carnivore), were strongly linked to biotic interactions between the cultured clams and surrounding organisms. Therefore, the distribution pattern of Manila clam shaped macrofaunal assemblage differently against their abundance and changed the functional similarity through functional nestedness. On the other hand, functional homogenization was identified in high clam abundance stations. Given that the environmental factors explained more variations of functional turnover (Fig. 4b), for low clam abundance stations, the functional composition of macrofaunal assemblages might be mostly shaped by environmental sorting rather than biotic interaction as there was no fierce competitions for ecological niches, namely food and living space between the cultured clams and surrounding organisms (Wang et al., 2017; Bevilacqua et al., 2020). However, for the stations with high clam abundances, clam-caused biotic interactions became the dominated factors for shaping the functional composition and resulted in the functional homogenization in the macrofaunal assemblage.

4.3. Insights of functional assessment and management of bottom-based clam aquaculture

It is important to note that the ecological impacts in the present study arisen in a short period of time (approximately three months after seeded) in relatively less-abundant macrofaunal assemblage. Although taxonomic and functional composition had significantly changed due to clam culture, these changes were mainly caused by short-term effects such as bio-competition and predator attraction. The macrofaunal assemblages were subsequently structured by the accumulative effects of cultured clams such as the continuous load of organic matter through sedimentation, or resuspension of organic matters in sediments through bioturbation. Such effects might lead to gradual successions of macrofaunal assemblage, e.g., an increased appearance of opportunistic species (Wilding and Nickell, 2013). Therefore, subsequent variations of macrofaunal assemblage remain to be investigated to evaluate the chronic impacts of bottom-based clam aquaculture.

The responses of the taxonomic and functional structure of macrofaunal assemblage to certain environmental stresses allow the evaluation of disturbance level (Dong et al., 2021; Lacson et al., 2019). In this study, species that are indifferent to environmental stress (AMBI-II) were identified in clam farms, suggesting that mild disturbance of clam culture to the macrofaunal assemblages. On the other hand, given a common knowledge that higher diversity indicates a better status for the ecosystem (Delpech et al., 2010), the macrofaunal assemblage seemed to benefit from bottom-based clam aquaculture, from the perspective of the enhancement of functional diversity. Nevertheless, such a conclusion should not simply be drawn. Previous studies described bivalve aquaculture could either enhance biodiversity by providing more niches. For example, organic load from sedimentation (Huang et al., 2018) or negatively impact an assemblage by threatening niches from the competition (Stenton-Dozey et al., 2001). In this study, both patterns were expected to happen, given the replacement of functional traits that occurred in both farms. Moreover, the positive effect (functional diversity benefited from the niches created by the introduction of clam) exceed the negative effect (functional diversity reduced by bio-competition) so that functional richness in the two farms was significantly higher compared to the control area. The domination of the positive effect is more likely to occur when functional compositions of macrofaunal assemblage were relatively simple. This is confirmed by Kluger et al. (2017) who used trophic modeling approach to indicate that the functional diversity could benefit from the cultured scallops (*Argopecten purpuratus*) when the original functional diversity was in low levels, but would decrease in functionally abundant assemblage. Therefore, positive effects of bottom-based clam aquaculture on functional diversity are more likely to occur in the less functionally-abundant macrofaunal assemblages.

On the other hand, higher functional richness in the clam farms compared to the control area (Table 1) indicated that clam farms were more functionally abundant. However, the increased functional richness was not due to the increases of the species number because the species richness was basically the same (33 and 32 vs 33). Accordingly, macrofaunal assemblages in clam farms became less representative of the functional richness, thus less functionally redundant (Bevilacqua et al., 2020). This raised concerns in those intensified culture farms. Although most traits are likely to be compensated, the increased abundance of cultured clam can further increase the functional sensitivity so that macrofaunal assemblages become more fragile against species or function loss since such traits are supported by a simplified species group (Micheli and Halpern, 2005). Accordingly, the elimination of species that have niche overlapping with cultured clam, such as suspension-feeder and burrower, represents a potential threat for the ecosystem because such functional traits are maintained only through the external input of cultured clam that artificially seeded into the ecosystem, and an interruption of this supply (e.g., harvest) would thus greatly destabilize the ecosystem (Kluger et al., 2017). Moreover, this effect would exacerbate with an increased farmed density, since the highly functional similarity was identified in those stations with high clam abundance (Fig. 5f). Additionally, although the introduction of cultured clams may initially benefit functional diversity, these benefits are likely to be lost as their abundance increases beyond a certain threshold (Kluger et al., 2016). Therefore, it is essential to identify an appropriate carrying capacity for the bottom-based clam aquaculture.

CRediT authorship contribution statement

Xin Sun: Conceptualization, Methodology, Software, Formal analysis, writing original draft.
Jianyu Dong: Software, Data Curation.
Yuyang Zhang: Software.
Qipeng Zhan: Investigation, Resources.
Xiayang Ding: Investigation.
Yong Chen: Writing - Review & Editing.
Xiumei Zhang: Supervision, Project administration.

Declaration of competing interest

The authors declare that there is no conflict of interests, we do not have any possible conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.113270.

References

Bartoń, K., 2013. MuMIn: Multi-model Inference. R Package Version 1.9. 13. The Comprehensive R Archive Network (CRAN), Vienna, Austria.

Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. Glob. Ecol. Biogeogr. 19, 134–143.

Baselga, A., 2012. The relationship between species replacement, dissimilarity derived from nestedness, and nestedness. Glob. Ecol. Biogeogr. 21, 1223–1232.

Baselga, A., Orme, C.D.L., 2012. Betapart: an R package for the study of beta diversity. Methods Ecol. Evol. 3, 808–812.

Bevilacqua, S., Terlizzi, A., Barnes, A., 2020. Nestedness and turnover unveil inverse spatial patterns of compositional and functional β-diversity at varying depth in marine benthos. Divers. Distrib. 26, 743–757.

Boyé, A., Thiébaut, É., Grall, J., Legendre, P., Broudin, C., Houbin, C., Le Garrec, V., Maguer, M., Droual, G., Gauthier, O., 2019. Trait-based approach to monitoring marine benthic data along 500 km of coastline. Divers. Distrib. 25, 1879–1896.

Carcedo, M.C., Fiori, S.M., Scotti, M., Ito, M., Dutto, M.S., Carbone, M.E., 2019. Dominant bivalve in an exposed Sandy Beach regulates community structure through spatial competition. Estuar. Coast 42, 1912–1923.

Carlos-Júnior, L.A., Spencer, M., Neves, D.M., Moulton, T.P., Pires, D.D.O., e Castro, C.B., Ventura, C.R.R., Ferreira, C.E.L., Serejo, C.S., Oigman-Pszczol, S., Casares, F.A., Mantelatto, M.C., Creed, J.C., Sorte, C., 2019. Rarity and beta diversity assessment as tools for guiding conservation strategies in marine tropical subtidal communities. Divers. Distrib. 25, 743–757.

Céréghino, R., Oertli, B., Bazzanti, M., Coccia, C., Compin, A., Biggs, J., Bressi, N., Grillas, P., Hull, A., Kalettka, T., Scher, O., 2011. Biological traits of european pond macroinvertebrates. Hydrobiologia 689, 51–61.

Chevenet, F., Doleadec, S., Chessel, D., 1994. A fuzzy coding approach for the analysis of long-term ecological data. Freshw. Biol. 31, 295–309.

Cranford, P.J., Kamermans, P., Krause, G., Mazurié, J., Buck, B.H., Dolmer, P., Fraser, D., Van Nieuwenhove, K., O'Beirn, F.X., Sanchez-Mata, A., Thorarinsdóttir, G.G., Strand, Ø., 2012. An ecosystem-based approach and management framework for the integrated evaluation of bivalve aquaculture impacts. Aquac. Environ. Interact. 2, 193–213.

de Fouw, J., van der Zee, E.M., van Gils, J.A., Eriksson, B.K., Weerman, E.J., Donadi, S., van der Veer, H.W., Olff, H., Piersma, T., van der Heide, T., 2020. The interactive role of predation, competition and habitat conditions in structuring an intertidal bivalve population. J. Exp. Mar. Biol. Ecol. 523.

Dedieu, N., Rhone, M., Vigouroux, R., Céréghino, R., 2015. Assessing the impact of gold mining in headwater streams of eastern Amazonia using ephemeroptera assemblages and biological traits. Ecol. Indic. 52, 332–340.

Delpech, C., Courrat, A., Pasquaud, S., Lobry, J., Le Pape, O., Nicolas, D., Boet, P., Girardin, M., Lepage, M., 2010. Development of a fish-based index to assess the ecological quality of transitional waters: the case of french estuaries. Mar. Pollut. Bull. 60, 908–918.

Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930.

Dong, J.Y., Zhao, L., Sun, X., Hu, C., Wang, Y., Li, W.T., Zhang, P.D., Zhang, X., 2021. Response of macrobenthic communities to heavy metal pollution in Laoshan Bay, China: a trait-based method. Mar. Pollut. Bull. 167, 112292.

Dray, S., Dufour, A.B., 2007. The ade4 package implementing the duality diagram for ecologists. J. Stat. Softw. 22, 1–20.

Dumbauld, B.R., Ruesink, J.L., Rumrill, S.S., 2009. The ecological role of bivalve shellfish aquaculture in the estuarine environment: a review with application to oyster and clam culture in west coast (USA) estuaries. Aquaculture 290, 196–223.

Ferriss, B.E., Reum, J.C.P., McDonald, P.S., Farrell, D.M., Harvey, C.J., 2016. Evaluating trophic and non-trophic effects of shellfish aquaculture in a coastal estuarine foodweb. ICES J. Mar. Sci. 73, 429–440.

Filgueira, R., Guyondet, T., Comeau, L.A., Tremblay, R., 2016. Bivalve aquaculture-environment interactions in the context of climate change. Glob. Chang. Biol. 22, 3901–3913.

Forde, J., O'Beirn, F.X., O'Carroll, J.P., Patterson, A., Kennedy, R., 2015. Impact of intertidal oyster trestle cultivation on the ecological status of benthic habitats. Mar. Pollut. Bull. 95, 223–233.

Gelman, A., 2008. Scaling regression inputs by dividing by two standard deviations. Stat. Med. 27, 2865–2873.

Gelman, A., Hill, J., 2009. arm: data analysis using regression and multilevel hierarchical models. J. Stat. Softw. 30.

Gieschen, R., Schwartpaul, C., Landmann, J., Fröhling, L., Hildebrandt, A., Goseberg, N., 2020. Large-scale laboratory experiments on mussel dropper lines in ocean surface waves. J. Mar. Sci. Eng. 9.

Heino, J., Melo, A.S., Siqueira, T., Soininen, J., Valanko, S., Bini, L.M., 2015. Metacommunity organisation, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. Freshw. Biol. 60, 845–869.

Huang, Q., Olenin, S., Sun, S., De Troch, M., 2018. Impact of farming non-indigenous scallop Argopecten irradians on benthic ecosystem functioning: a case-study in Laizhou Bay, China. Aquac. Environ. Interact. 10, 227–241.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as eco-system engineers. Oikos 69, 373–386.

Kluger, L.C., Taylor, M.H., Barriga Rivera, E., Torres Silva, E., Wolff, M., 2016. Assessing the ecosystem impact of scallop bottom culture through a community analysis and trophic modelling approach. Mar. Ecol. Prog. Ser. 547, 121–135.

Kluger, L.C., Filgueira, R., Wolff, M., 2017. Integrating the concept of resilience into an ecosystem approach to bivalve aquaculture management. Ecosystem 20, 1364–1382.

Lacoste, É., McKindsey, C.W., Archambault, P., 2020. Biodiversity-ecosystem functioning (BEF) approach to further understanding aquaculture–environment interactions with application to bivalve culture and benthic ecosystems. Rev. Aquacult. 12, 2027–2041.

Lacson, A.Z., Piló, D., Pereira, F., Carvalho, A.N., Cúrdia, J., Caetano, M., Drago, T., Santos, M.N., Gaspar, M.B., 2019. A multimetric approach to evaluate offshore mussel aquaculture effects on the taxonomical and functional diversity of macrobenthic communities. Mar. Environ. Res. 151, 104774.

Laliberte, E., Legendre, P., 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology 91, 299–305.

Levinton, J., 1972. Stability and trophic structure in deposit-feeding and suspension-feeding communities. Am. Nat. 106, 472–486.

Liao, Y., Shou, L., Jiang, Z., Tang, Y., Du, P., Zeng, J., Chen, Q., Yan, X., Chen, J., 2019. Effects of fish cage culture and suspended oyster culture on macrobenthic communities in Xiangshan Bay, a semi-enclosed subtropical bay in eastern China. Mar. Pollut. Bull. 142, 475–483.

Lichstein, J.W., 2007. Multiple regression on distance matrices: a multivariate spatial analysis tool. Plant Ecol. 188, 117–131.

Macdonald, T.A., Burd, B.J., van Roodselaar, A., 2012. Facultative feeding and consistency of trophic structure in marine soft-bottom macrobenthic communities. Mar. Ecol. Prog. Ser. 445, 129–140.

Micheli, F., Halpern, B.S., 2005. Low functional redundancy in coastal marine assemblages. Ecol. Lett. 8, 391–400.

Péru, N., Dolédec, S., 2010. From compositional to functional biodiversity metrics in bioassessment: a case study using stream macroinvertebrate communities. Ecol. Indic. 10, 1025–1036.

Price, C., Black, K.D., Hargrave, B.T., Morris, J.A., 2015. Marine cage culture and the environment: effects on water quality and primary production. Aquac. Environ. Interact. 6, 151–174.

R Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Sardenne, F., Forget, N., McKindsey, C.W., 2019. Contribution of mussel fall-off from aquaculture to wild lobster Homarus americanus diets. Mar. Environ. Res. 149, 126–136.

Socolar, J.B., Gilroy, J.J., Kunin, W.E., Edwards, D.P., 2016. How should beta-diversity inform biodiversity conservation? Trends Ecol. Evol. 31, 67–80.

Stenton-Dozey, J., Probyn, T., Busby, A., 2001. Impact of mussel (Mytilus galloprovincialis) raft-culture on benthic macrofauna, in situ oxygen uptake, and nutrient fluxes in Saldanha Bay, South Africa. Can. J. Fish. Aquat. Sci. 58, 1021–1031.

Sun, X., Dong, J., Hu, C., Zhang, Y., Chen, Y., Zhang, X., 2021. Use of macrofaunal assemblage indices and biological trait analysis to assess the ecological impacts of coastal bivalve aquaculture. Ecol. Indic. 127.

Troast, B., Paperno, R., Cook, G.S., 2019. Multidecadal shifts in fish community diversity across a dynamic biogeographic transition zone. Divers. Distrib. 26, 93–107.

van der Linden, P., Patrício, J., Marchini, A., Cid, N., Neto, J.M., Marques, J.C., 2012. A biological trait approach to assess the functional composition of subtidal benthic communities in an estuarine ecosystem. Ecol. Indic. 20, 121–133.

Villéger, S., Mason, N.W., Mouillot, D., 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. Ecology 89, 2290–2301.

Villéger, S., Grenouillet, G., Brosse, S., 2013. Decomposing functional β-diversity reveals that low functional β-diversity is driven by low functional turnover in european fish assemblages. Glob. Ecol. Biogeogr. 22, 671–681.

Vozzo, M.L., Mayer-Pinto, M., Bishop, M.J., Cumbo, V.R., Bugnot, A.B., Dafforn, K.A., Johnston, E.L., Steinberg, P.D., Strain, E.M.A., 2021. Making seawalls multifunctional: the positive effects of seeded bivalves and habitat structure on species diversity and filtration rates. Mar. Environ. Res. 165, 105243.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38.

Wang, L., Fan, Y., Yan, C., Gao, C., Xu, Z., Liu, X., 2017. Assessing benthic ecological impacts of bottom aquaculture using macrofaunal assemblages. Mar. Pollut. Bull. 114, 258–268.

Wilding, T.A., Nickell, T.D., 2013. Changes in benthos associated with mussel (Mytilus edulis L.) farms on the west-coast of Scotland. PLoS One 8, e68313.

Yang, D., Sun, P., 1988. The Polychaetes in CoastalWaters of China. Agriculture Press, Beijing (in Chinese).

Yang, D., Sun, S., 2005. Marine Invertebrate Zoology. China Ocean University Press, Qingdao (in Chinese).

Yang, D., Wang, Y., 1996. Marine Invertebrates in Northern China. Higher Education Press, Beijing (in Chinese).

Zhang, S., Zhang, L., Chen, Z., Xu, F., 2016. Mollusks of the Yellow Sea and Bohai Sea. Science Press (in Chinese).

Zhao, Y., Zhang, J., Lin, F., Ren, J.S., Sun, K., Liu, Y., Wu, W., Wang, W., 2019. An ecosystem model for estimating shellfish production carrying capacity in bottom culture systems. Ecol. Model. 393, 1–11.

Zhuang, W., Gao, X., Zhang, Y., Xing, Q., Tosi, L., Qin, S., 2014. Geochemical characteristics of phosphorus in surface sediments of two major chinese mariculture areas: the Laizhou Bay and the coastal waters of the Zhangzi Island. Mar. Pollut. Bull. 83, 343–351.