



# Variable Effects on Benthic Community From Diking to Eradicate Invasive Plants in the Yangtze Estuary Salt Marsh

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The removal of invasive plants is a global concern, and ecological restoration methods have been a major research topic in recent years. In the estuarine salt marsh of the Yangtze River, dikes are typically used in ecological restoration projects to eradicate the invasive plant Spartina alterniflora. We explored ways of optimizing dike construction and of providing an effective basis for the wetland ecological control and protection of biodiversity and analyzed the effects on the macrobenthos of fully and partially dikes. The measurement of the quantitative change in macrobenthos diversity and species composition was carried out in the project area and in a control before (2013) and after (2016) dike construction. Results showed that the number of species and average density decreased significantly in the fully diked enclosed area but increased in the partially diked semi-enclosed area. Outside the project area, all site samples showed increased species richness and average density after dike construction. This study indicated that macrobenthos was negatively affected by the dike project in the inside diked area. However, when the tidewater canals were preserved to maintain the connection between the inside and outside areas, there was a positive effect on macrobenthos in the project area. We suggest that canals are preserved while diking in the salt marsh and that gates are opened regularly to maintain the water and nutrient connectedness inside and outside the dike. The diking project mostly affected mollusks and polychaetes, which are the indispensable food sources for birds and fish. The study provides valid evidence for the management of estuarine salt marsh and the protection of macrobenthos.

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#### INTRODUCTION

Coastal salt marshes provide an important interface among terrestrial, riverine, and marine ecosystems. They contain unique and irreplaceable natural resources and support key ecosystem processes (Wall et al., 2001), such as nutrient circulation, water purification, and food production (Pétillon et al., 2005). They are highly productive ecosystems and are generally regarded as a source

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of primary production to nearshore water (Lee, 1995; Kneib, 1997). A salt marsh is, however,a fragile ecosystem that is vulnerable to disturbances such as global change, pollution, plant invasions, and embankment projects (Cohen and Carlton, 1998; Grosholz, 2002; Chen et al., 2017; Christopher et al., 2021). The development and use of natural resources have led to serious damage to salt marsh wetland, the most typical of which is the dike and reclamation project. However, previous studies have mainly focused on the environmental changes, which have less impact on the ecological functions of salt marshes, and especially on the macrobenthos.

The benthic macroinvertebrate is a ubiquitous and abundant component of salt marsh ecosystems (Levin and Talley, 2002) and is functionally involved in sediment bioturbation and marsh nutrient cycling of the whole ecosystem (Bertness, 1985; Alkemade et al., 1992). Intertidal macrobenthic fauna mainly includes coelenterates, Nemertea, annelids, mollusks, crustaceans, and aquatic insects. As the important primary consumers, these organisms feed on plant detritus and associated bacteria and microflora in the sediment (Craft, 2000), serve as diet items for higher trophic-level consumers such as fish and birds, and are functionally involved in sediment bioturbation and marsh biogeochemical cycling (Bertness, 1985; Alkemade et al., 1992). The number of macrobenthos directly determines the number of birds and fish that can be supported by salt marshes. Macrobenthos has difficulty in migration, is sensitive to environmental changes, and responds quickly to human activities and environmental pressures (Naser, 2011). Therefore, macrobenthos can be used as important environmental indicators, and it is widely used to evaluate the ecological conditions of coastal and estuarine systems. Macrobenthos has been significantly affected by the exotic plant invasion in the salt marsh (Neira et al., 2007).

Spartina alterniflora (Spartina) is a widespread invader worldwide, and it not only affects the distribution and richness of macrobenthos but also changes bird habitats and threatens biodiversity and ecosystem function (Li et al., 2009; Strong and Ayres, 2009; Ma et al., 2014). During the last centuries, Spartina was intentionally or accidentally introduced outside their native ranges in numerous coastal regions of both the southern and northern hemispheres (Kriwoken and Hedge, 2000; Hedge et al., 2003; Wong et al., 2018). China is among those countries most heavily infested with Spartina. An estimate made in 2007 showed that Spartina covered 34,451 ha along the east coast of China from Guangxi (21° N) to Liaoning (40° N) (Zuo et al., 2012). In many regions, such as the Pacific Northwest of the United States and the coastal states of Australia, New Zealand, and China, efforts had been taken to control the spread of these invasive grasses (Kriwoken and Hedge, 2000; Hedge et al., 2003; Zhang et al., 2020). A wide range of control techniques, such as physical removal, mowing, and herbicide, was established, with all techniques demonstrating considerable limitations. After the repeated experiments in China, researchers have finally devised the effective methods that meet the requirements of wetland protection to eradicate this invasive plant, namely "mowing+waterlogging." In short, it is necessary to maintain a water depth of more than 40 cm of submergence, after mowing the aboveground part, to make the root die completely (Li and Zhang, 2008; Yuan et al., 2011; Zhao et al., 2019). A dike-building project was carried out to establish this waterlogging regime to control *Spartina*.

In this study, the ecological dike project was different from the previous reclamation projects. Historically, the purpose of reclamation was mainly to expand the area of cultivated land used by farms and fish ponds (Bi et al., 2012; Chen et al., 2017). This time, the dike was built as an ecological control measure, mainly to provide a guarantee of eradicating *Spartina*, and also for the restoration and optimization of bird habitats. The spatial layout of the causeway was fully intended to meet the requirement of providing adequate habitat for birds. "Enclosure without reclamation" became the core concept of ecological management and restoration in Chongming Dongtan Birds National Nature Reserve. The ultimate goal was to remove the invading *Spartina* and to provide suitable habitat and adequate food resources (e.g., macrobenthos) for birds.

The Chongming Dongtan wetland, which was an important waterfowl migration habitat in the East Asian-Australasian flyaway, has lost many native habitats due to the *Spartina* invasion. To protect this important wetland and better serve the world-class ecological Chongming Island, "The Ecological *Spartina* Control and Bird Habitat Optimization Project" was implemented in the Yangtze Estuary wetland of Chongming Dongtan Birds National Nature Reserve. This was the largest invasive plant and waterfowl habitat restoration project in the world (US\$186 million), which was started in 2013 and completed in 2016 (Zhang et al., 2020). Although the bird habitat has been reconstructed, there has been no relevant impact assessment of important food sources for birds. The impact of the dike project on the ecosystem, especially on the macrobenthos, remains unclear.

The general aim of this study was to (1) assess the effects of an ecological restoration project of an invaded salt marsh on benthic community and diversity; (2) explore the optimization of dike construction to reduce its impact on the structure and function of an estuarine wetland ecosystem; and (3) provide an effective basis for the wetland ecological control and protection of biodiversity. To do this, we used a before–after control–impact (BACI) design, comparing a restored project and a control (unmanipulated) site over 3 years in the Yangtze River estuary wetland ecosystem.

## **MATERIALS AND METHODS**

#### Study Area

The study was carried out in the Chongming Dongtan salt marsh (31°25′-31°38′ N, 121°50′-122°05′ E) in the Yangtze Estuary, the largest estuary in China (**Figure 1**). It lies within a typical semitropical monsoon region, where the climate is mild and wet, and it experiences four distinct seasons. Tides are semidiurnal and irregular, with the amplitude greatest at the river mouth, decreasing landward and seaward from there, and averaging 2.4–4.6 m within the estuarine system (Hou et al., 2013). The dominant native plants in the marsh are *Scirpus mariqueter* and *Phragmites australis*. The exotic *Spartina* has invaded the salt marsh since the mid-1990's, becoming the most dominant plant

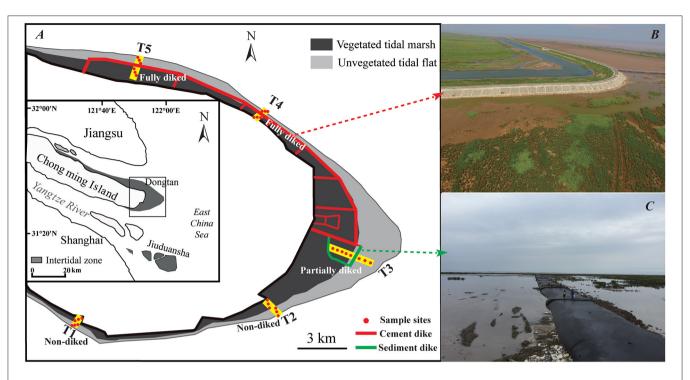


FIGURE 1 | Studyarea in the Chongming Dongtan salt marsh, Yangtze Estuary, China. The area surrounded by red and green lines was the Spartina Control and Bird Habitat Optimization Project Area. The quantitative sampling of macrobenthos was carried out before and after the construction of the diking project in 2013 and 2016, respectively. T1 and T2 were the non-diked projects as an observation area of natural changes. T3 was a partially diked project semi-enclosed by sediment dike. T4 and T5 were the fully diked projects enclosed by cement dike. In the T3–T5 from the project area, all sites inside the project are shown as treatments, while outside sites, as control areas. (A) is the overall drawing, (B) is cement dike in enclosed project area and (C) is sediment dike in semi-enclosed area.

species (over 50% of the total marsh area in 2009) in the Dongtan wetland (Li et al., 2009).

The ecological restoration project that aimed to eradicate *Spartina* began in September 2013. It comprised three parts, namely, the ecological control of *Spartina*, bird habitat optimization, and scientific research monitoring infrastructure. The total project area covered about 25 km² and was designed to promote the important ecology of the Chongming Island and the construction of the Shanghai "ecological livable city." There are two different dike-type areas in this project: the fully diked area was enclosed by cement dike, and the partially diked area was semi-enclosed by sediment dike.

This study aimed to: (1) proactively combine ecology and engineering to address the invasion and expansion of *Spartina* in Dongtan reserve; (2) restore the function of the bird habitat; (3) maintain and expand bird species and populations; (4) improve the quality of the internationally important Chongming Dongtan wetland; (5) provide a replicable control of exotic invasive species; (6) explore the protection and rational use of the coastal wetland nature reserves in China; and (7) contribute to the implementation of the International Convention on wetlands and the protection of global biodiversity.

#### Field Sampling

The sampling design incorporated three different spatial scales: Transects (scale of kilometers), Sites (scale of hundreds of meters), and replicate cores (scale of tens of meters). Each site was defined as an area  $\sim$ 25 m<sup>2</sup> in area and was selected at random within each location on each sampling occasion.

The sampling design incorporated five sample transects located in the Chongming Dongtan salt marsh, Yangtze Estuary, China (T1-T5, Figure 1). The quantitative sampling of macrobenthos was carried out before and after the construction of the diking project in June 2013 and June 2016, respectively. As non-diked areas, transects 1 and 2 include three sites in P. australis monoculture and three sites on bare flats. Transect 3 was a partially diked project with the tidal flat enclosed by a sediment dike; five sites inside it received regular flooding to remove Spartina and three sites were on a bare flat outside the dike. Transects 4 and 5 were major projects enclosed by a cement dike; three sites inside them were completely enclosed and no longer affected by the tidal flat and three sites outside them were on bare flats. A total of 32 sites were identified by a global positioning system (GPS) in 2013 and 2016 to ensure the consistency of the sampling locations.

Each sample was mixed with three sediment cores ( $20\,\mathrm{cm}$  long  $\times$  20 width  $\times$   $15\,\mathrm{cm}$  depth), taken 3– $5\,\mathrm{m}$  from each other, and sieved through a 0.5-mm mesh. All samples retained on the sieve were fixed in 10% formaldehyde. Animals were separated carefully from the debris and then identified to the lowest possible taxonomic level under a dissecting microscope (OLYMPUS SZX9 which located in the East China Sea Fisheries Research Institute).

## **Data Analysis**

One SE of the mean is presented with the mean data unless otherwise indicated. To compare benthic assemblages affected by the construction of the project, several diversity indices were calculated using PRIMER software version 6.0 (Primer-E Ltd., Plymouth, UK). The Margalef Index  $(D = (S - 1)/(\log_2 N))$  was used to indicate species richness, giving the number of species present for a given number of individuals. The Shannon-Wiener Index  $(H' = -\sum_{i=1}^{s} P_i \log_2 P_i)$  was used for species diversity, and the Pielou's Evenness Index  $(J' = H'/\log_2 S)$  was used to express how evenly the individuals were distributed among the different species, where S is the total number of species, N is the total number of individuals, and  $P_i$  is the proportion of the total count arising from the ith species.

The density, richness, and diversity indexes of benthic communities before and after the construction of the project were tested using the Student's t-tests conducted by STATISTICA software (StatSoft Inc., 2007, version 8.0, www.statsoft.com). Differences were regarded as significant at P < 0.05. All the data were checked for normality (Shapiro–Wilk test) and homogeneity of variances (Bartlett and Levene test) prior to the parametric analyses. Where necessary, the data were  $\log(x+1)$ -transformed prior to the analysis.

Similarities and differences in macrofaunal assemblages in the project area (T3-T5) were explored using the nonmetric multidimensional scaling (n-MDS), based on the Bray-Curtis similarity indices on the log-transformed [log(x+1)], unstandardized data. The analysis of similarity (ANOSIM) was used to test the statistical significance in benthic assemblages from different sample times (2013, 2016). The ANOSIM test statistic R, in the range of 0-1, is a measure of the magnitude of dissimilarity within and between sample groups (Clarke, 1993). R-values close to 0 indicate that the dissimilarities between sample points within one group are equivalent to the dissimilarities found between different groups. R-values close to 1 indicate strong differences between two groups relative to intragroup variation among the benthic community. Statistical significances using P-values were calculated for each pairwise R-value. Both analyses were performed using PRIMER software version 6.0.

#### RESULTS

A total of 30 macrobenthic invertebrate taxa were identified, belonging to six different phyla, such as Gastropoda, Bivalvia, Polychaeta, Crustacea, Anopla, and Insecta (**Supplementary Table 1**). In the non-diked transects 1 and 2, a total of 14 species was detected (9 in 2013 and 11 in 2016). In the diked project area, 25 and 27 species were detected before and after the construction of the project, respectively. Transects 4 and 5, enclosed by the cement dike, were the most affected by the project. In the inside project, 12 macrobenthic species were identified before the project, but only 6 remained after the project (10 species disappeared and 4 new species emerged). The species that disappeared included snails (Assiminae latericea, Cerithidea largillierti, Neritina violacea,

and Pseudoringicula sinensis), bivalves (Corbicula fluminea, Sinonovacula constricta, and Morella iridescens), crustacea (Corophium sinensis and Helice tientsinensis), and polychaetes (Tylorrhynchus heterochaetus). Four new species emerged, namely, Stenothyra glabra, Potamocorbula ustulata, Chiromantes dehaani, and Gnorimosphaeroma rayi.

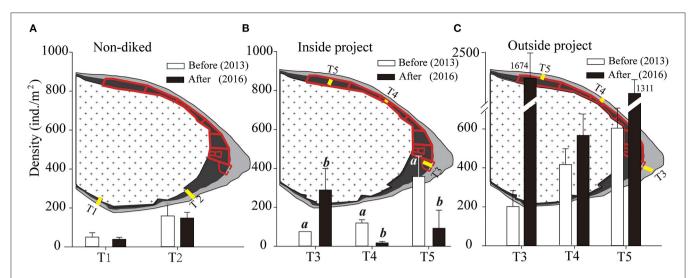
We focused on the changes in benthic fauna in the project area. Mean density changed from  $275 \pm 50$  ind./m² in 2013 to  $620 \pm 211$  ind./m² in 2016 (t-test, P = 0.118). The inside project sites showed a decrease in mean density from  $165 \pm 39$  ind./m² in 2013 to  $159 \pm 65$  ind./m² in 2016 (P = 0.082). Along transect 3 in the semi-open area, the density of the inside project sites was lower in 2013 ( $75 \pm 0$  ind./m²) than in 2016 ( $288 \pm 111$  ind./m², P = 0.091). In the fully diked project area, the density along transects 4 and 5 showed a significant decrease after the project, from  $120 \pm 17$  ind./m² to  $7 \pm 7$  ind./m² (P = 0.004) and from  $359 \pm 20$  ind./m² to  $93 \pm 93$ ind./m² (P < 0.05), respectively. The sites outside the project in the tidal flats (**Figure 2C**) showed a significant increase effect by project, and density changed from  $410 \pm 83$  ind./m² in 2013 to  $1,184 \pm 393$  ind./m² in 2016 (P = 0.072).

# Benthic Community Changes in Different Transects

There were no differences in the density of the benthic community before and after the project in the non-diked area (Table 1 and Figure 2A). Spartina was removed after the construction of the ecological project in the inside project area. There were significant differences in the benthic community before and after the project in the enclosed transects (Figure 2B). T3 showed that the significance of benthos density increased from 75  $\pm$  0.002 to 289  $\pm$  111 ind./m<sup>2</sup> after the project (P < 0.05). In contrast, T4 and T5 decreased significantly from 120  $\pm$  17 to 7  $\pm$  7 ind./m<sup>2</sup> (P < 0.01) and from 359  $\pm$  20 to 93  $\pm$  93 ind./m<sup>2</sup> (P < 0.05), respectively. The area outside the construction of the project was mainly mudflat. Although there was no statistically significant difference, the density of benthos from all transects showed increased after the construction of the project (Figure 2C). The density of benthic fauna increased most along T3 (from 201  $\pm$  82 to 1,674  $\pm$  920 ind./m<sup>2</sup>), followed by T5 (from  $604 \pm 173$  to  $1{,}311 \pm 822$  ind./m<sup>2</sup>) and T4 (from  $423 \pm 68$ to  $567 \pm 110 \text{ ind./m}^2$ ).

Species richness showed similar changes to density. In the non-diked area, before and after the project, the number of species along T1 and T2 changed from 4 to 5 and from 7 to 9, respectively (**Table 1** and **Figure 3A**). In the inside project sites, the number of species along T3 increased from 7 to 12, while the number of species along T4 and T5 decreased from 5 and 7 to 2 and 6, respectively (**Figure 3B**). In the outside project sites, the species richness of benthic fauna clearly increased, from 7 to 17 along T3; remaining unchanged along T4; and increasing slightly from 9 to 10 along T5 (**Figure 3C**).

In the area affected by the project (T3–T5), the MDS ordination of the benthic community data was clearly separated, into "before" (2013) and "after" (2016) construction (**Figure 4B**). The benthic community structure in 2013 showed significant



**FIGURE 2** | Benthic macroinvertebrate density in different transects before and after diking in the Chongming Dongtan salt marsh, Yangtze Estuary, China. Mean values with one SE (error bars) are shown, and different characters (a, b) indicate that values were significantly different before and after diking (t-test, P < 0.05). (A) Non-diked area, (B) inside the dike project, and (C) outside the dike project area.

differences from that in 2016 (ANOSIM, P = 0.001; Global tests R = 0.234). There was no significant difference in the community structure of benthic fauna between inside and outside the project area in 2013 (ANOSIM, P = 0.712; pairwise tests R = 0.057). After the completion of the project in 2016, however, there was a significant difference between inside and outside the project area (ANOSIM, P = 0.001; pairwise tests R = 0.654). In the non-diked area, the MDS results showed no significant difference in the macroinvertebrate assemblages recorded from 2013 and 2016 (ANOSIM, P = 0.139; Global tests R = 0.055, **Figure 4A**).

## **Changes in Benthic Groups**

In non-diked project area, the density of benthic groups showed non-obviously changes between 2013 and 2016 (**Figure 5A**). After the completion of the project in 2016, an examination of benthic fauna groups in the project area showed that only the total density of Anopla and Polychaeta had decreased, while the density of other groups (i.e., Gastropoda, Bivalvia, Crustacea, and Insecta) had increased (**Figure 5B**). There was, however, no statistically significant difference. Gastropoda and Bivalvia were the two densest benthic faunae in both 2013 and 2016. Their density was clearly increased, changing from 177  $\pm$  49 and 56  $\pm$  18 ind./m<sup>2</sup> to 468  $\pm$  189 and 97  $\pm$  40 ind./m<sup>2</sup>, respectively.

In the non-diked area of T1 and T2 (**Figure 6A**), there were no significant differences in any benthic fauna groups between 2013 and 2016.

In the partially diked area (**Figure 6B**), the mean density of benthic fauna showed a clear increase along T3 and a significant increase from  $8\pm 5$  to  $29\pm 9$  ind./m² for Bivalvia (t-test, P<0.05) and from  $53\pm 14$  and  $4\pm 4$  ind./m² to  $209\pm 117$  and  $44\pm 36$  ind./m² for Gastropoda and Insecta, respectively. Fully diked T4 and T5 showed opposite change characteristics, and the density of main benthic groups was decreased. The Gastropoda along T4 and the Bivalvia along T5 decreased significantly from

113  $\pm$  22 and 229  $\pm$  59 ind./m<sup>2</sup> to 0 and 4  $\pm$  4 ind./m<sup>2</sup>, respectively (*t*-test, *P* < 0.05).

In the outside of the project area, the mean density of main groups showed an increase after the construction of the project (**Figure 6C**). Although there were no statistical differences between 2013 and 2016, the mean density of Gastropoda showed a clear increase in T3 (from 0 to 1,500  $\pm$  957 ind./m²), T4 (from 342  $\pm$  61 to 415  $\pm$  85 ind./m²), and T5 (from 516  $\pm$  166 ind./m² to 856  $\pm$  656 ind./m²). The mean density of Bivalvia showed a significant increase from 6  $\pm$  6 to 433  $\pm$  161 ind./m² (*t*-test, *P* < 0.05) in T5 and increased from 99  $\pm$  12 to 152  $\pm$  47 ind./m² in T4. However, the opposite changes appeared in T3, where the density of Bivalvia decreased from 157  $\pm$  87 to 37  $\pm$  13 ind./m². The mean density of Crustacea showed an increase in T3 and decreased in T5. Anopla and Polychaeta also showed some decrease in density after the project was completed in 2016 compared with their status in 2013.

#### DISCUSSION

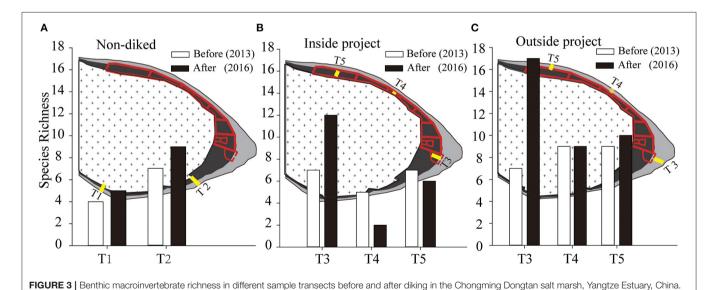
The ecological restoration project in the Yangtze Estuary had a significant environmental impact on the populations and assemblages of macrobenthic invertebrates, but its impacts varied according to the type of dike area examined. The total abundance of bivalves and gastropods showed a general long-term decline (from before to after diking) in the fully diked area (T4 and T5) but not in the non-diked (T1 and T2) or partially diked area (T3).

The distribution of macrobenthos is related to the physical, chemical, and geological processes in a salt marsh (Mucha et al., 2004; Bavestrello et al., 2018). The changes in the environment are all reflected in the community structure and diversity index of benthic macroinvertebrates (Borja et al., 2000). Due to the characteristics of limited mobility and migration, as well as sensitivity to environmental change, the community change in

TABLE 1 | Macrobenthos diversity in five transects before (2013) and after (2016) the diking project in the Chongming Dongtan salt marsh in the Yangtze Estuary, China.

	Transect	Year	s	Density	D	J'	H'
Non-diked	T1	2013	4	50.33 ± 22.68	0.181 ± 0.062	0.821 ± 0.141	0.410 ± 0.194
	T1	2016	5	$38.89 \pm 10.64$	$0.195 \pm 0.090$	$0.820 \pm 0.101$	$0.508 \pm 0.261$
	T2	2013	7	$155.29 \pm 76.11$	$0.152 \pm 0.053^a$	$0.804 \pm 0.044$	$0.545 \pm 0.185$
	T2	2016	9	$148.14 \pm 29.39$	$0.521 \pm 0.054^{b}$	$0.839 \pm 0.034$	$0.148 \pm 0.134$
Inside (Partially diked)	Т3	2013	7	$75.48 \pm 0.002^a$	$0.277 \pm 0.086^a$	$0.740 \pm 0.040$	$0.962 \pm 0.276^a$
	T3	2016	12	$288.88 \pm 110.9^{b}$	$0.787 \pm 0.103^{b}$	$0.873 \pm 0.049$	$2.029 \pm 0.131^{b}$
Inside (Fully diked)	T4	2013	5	$119.52 \pm 16.64^{a}$	$0.283 \pm 0.078$	$0.775 \pm 0.117$	$0.944 \pm 0.239$
	T4	2016	2	$7.41 \pm 7.41^{b}$	$0.107 \pm 0.107$	$0.333 \pm 0.333$	$0.333 \pm 0.333$
	T5	2013	7	$358.90 \pm 20.47^a$	$0.510 \pm 0.097$	$0.806 \pm 0.043^{a}$	$1.578 \pm 0.146$
	T5	2016	6	$92.59 \pm 92.59^{b}$	$0.296 \pm 0.296$	$0.247 \pm 0.247^{b}$	$0.640 \pm 0.640$
Inside (all transects)		2013	16	$164.7 \pm 38.52$	$0.342 \pm 0.057$	$0.768 \pm 0.085$	$1.125 \pm 0.160$
		2016	14	$158.5 \pm 64.98$	$0.468 \pm 0.127$	$0.555 \pm 0.135$	$1.188 \pm 0.303$
Outside (Partially diked)	Т3	2013	7	$201.3 \pm 81.78$	$0.399 \pm 0.106^{a}$	$0.713 \pm 0.157$	$1.061 \pm 0.270$
	Т3	2016	17	$1674. \pm 920.4$	$1.424 \pm 0.194^{b}$	$0.510 \pm 0.183$	$1.759 \pm 0.640$
Outside (Fully diked)	T4	2013	9	$423.4 \pm 68.34$	$0.832 \pm 0.024$	$0.686 \pm 0.070$	$1.773 \pm 0.182$
	T4	2016	9	$566.6 \pm 109.6$	$0.694 \pm 0.121$	$0.637 \pm 0.050$	$1.534 \pm 0.211$
	T5	2013	9	$603.91 \pm 172.9$	$0.470 \pm 0.077^a$	$0.474 \pm 0.097$	$0.933 \pm 0.211^{a}$
	T5	2016	10	$1311.11 \pm 822.4$	$1.139 \pm 0.149^{b}$	$0.703 \pm 0.079$	$2.177 \pm 0.258^{b}$
Outside (all transects)		2013	21	$409.5 \pm 82.63^a$	$0.567 \pm 0.077^a$	$0.624 \pm 0.068$	$1.255 \pm 0.172$ a
		2016	24	$1183. \pm 393.0^{b}$	$1.086 \pm 0.132^{b}$	$0.617 \pm 0.065$	$1.823 \pm 0.228^{b}$
Project all transects		2013	25	$274.9 \pm 50.03$	$0.443 \pm 0.052^a$	$0.703 \pm 0.057$	$1.184 \pm 0.115$
		2016	27	$619.9 \pm 210.2$	$0.746 \pm 0.114^{b}$	$0.583 \pm 0.078$	$1.474 \pm 0.204$

Different bold lowercase superscript letters (a, b) refer to significant differences before and after the diking project, respectively.



macrobenthos can reflect the degree of impact on the habitat (Naser, 2011). It is important to examine the benthic community across a project area and to compare information from before and after a project, when working toward a comprehensive understanding of the role of an ecological restoration project.

(A) Non-diked area, (B) inside the dike project, and (C) outside the dike project area.

We found 12 species before and 6 species after the completion of the dikes. Only two (*Heteromastus filiformis* and *Glauconome* 

chinensis) of the original species were preserved. In the diked project sites, the mean density of many groups showed significant differences, such as Crustacea in T3, Bivalvia in T4, and Gastropoda in T5. However, in the outside project area sites, only Bivalvia in T5 showed a significant difference. The results indicated that the dike construction had a greater impact on the habitat inside than outside of the dike. This may also explain

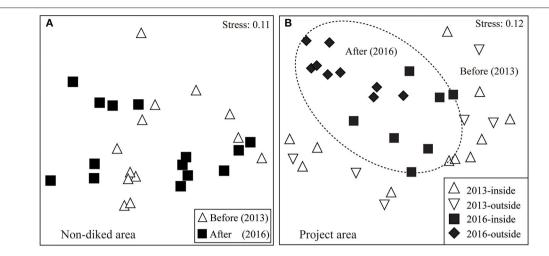
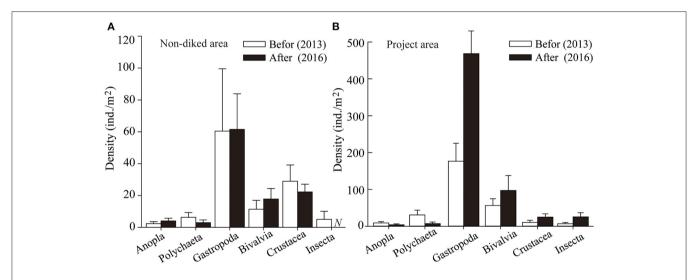


FIGURE 4 | A multidimensional scaling plot of benthic macroinvertebrate communities in the non-diked area (A) and project areas (B) before (2013) and after (2016) the project, in the Chongming Dongtan salt marsh, Yangtze Estuary, China.



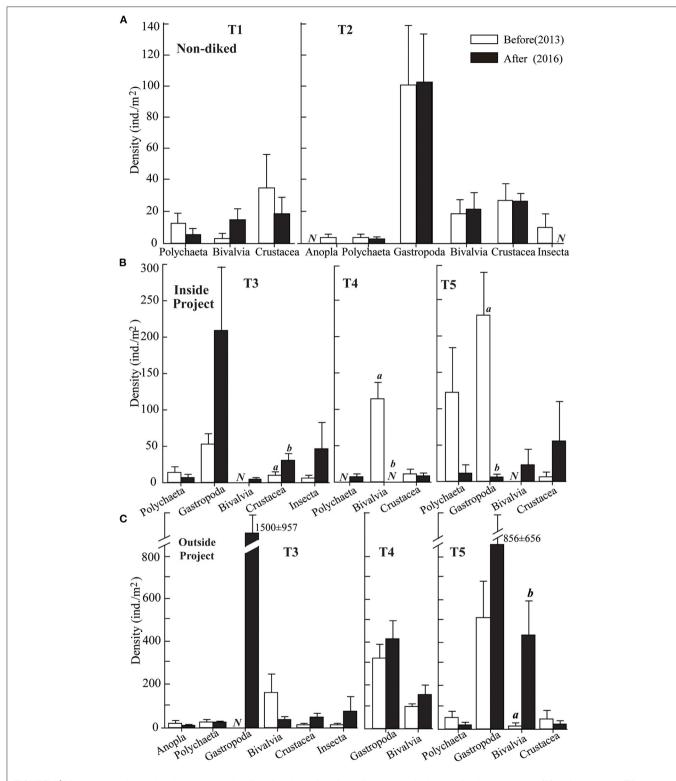
**FIGURE 5** | Density change in different macrobenthic groups before and after the diking project in non-diked **(A)** and project **(B)** areas in the Chongming Dongtan salt marsh, Yangtze Estuary, China. Error bars indicate the SE of the mean. There were no significant differences before and after the diking project in each group (*t*-test, *P* < 0.05).

why macrobenthos can act as an indicator of habitat change in an estuarine wetland (Zeppilli et al., 2015).

There is a very close relationship between the benthos and the tide in a salt marsh wetland. Tides are semidiurnal and irregular in the Yangtze Estuary, bringing rich nutrients from estuarine waters to the salt marsh every day, but this connectivity was blocked by dikes. The dike project changed the tidal power in the salt marsh and hindered tidal circulation, both being important reasons for the decrease in benthic species and quantity in the inside project area after the diking was complete. The fully diked wetland of the project gradually evolved from being a natural ecosystem to an artificial ecosystem, because "mowing+waterlogging" measures changed the original habitat. Some benthos disappeared because they did not adapt to the

change in habitat, and the benthic community structure also changed significantly. The changes we observed were consistent with similar studies and also with changes in the physical environment (Skilleter et al., 2006; Dolbeth et al., 2013).

The changes in the benthic community structure were varied due to the different types of dike construction. In this study, the results show that the density and diversity of benthos in the fully diked area clearly decreased but increased significantly in the partially diked area. During the "mowing+waterlogging" treatment in the partially diked area, the floodgate was opened to introduce tidewater. This maintained the communication between the inside area and the external natural tidewaters, which provided exogenous nutrient input. The tide also brought in some individuals that supplemented biological species and



**FIGURE 6** | Mean density of macrobenthos groups before (2013) and after (2016) the diking project in the non-diked area transects **(A)**, inside transects **(B)**, and outside transects **(C)** of the project in the Chongming Dongtan salt marsh, Yangtze Estuary, China. Error bars indicate the SE of the mean. Different lowercase superscript letters (a, b) refer to significant differences before and after the diking project, respectively (t-test, P < 0.05).

quantity, especially gastropods such as *Assimima latericea*, *A. violacea*, and *S. glabra* (Warren et al., 2002; Dolbeth et al., 2013; Dou et al., 2016). In contrast, the number of species and individuals was significantly reduced in the fully diked area, perhaps because the area lacked communication with the natural resources of the outside tidal flat. This kind of ecological restoration project is similar to the violent disturbance caused by blown sand and deposition, which will cause a very significant disturbance to macrobenthos and a significant change in the community structure (Lv et al., 2016; Yang et al., 2016).

In the outside project area, the mean density of the benthos increased after diking, mainly due to the significant increase in gastropods such as S. glabra and Bullacta exarata. Before the diking project, the outside sampling sites far from the dike and vegetated areas had very few snails. After the dike was constructed, however, snails were found to be the most abundant group present in this area. The change in water flow may be the main reason for the increases in density and diversity at the sampling sites outside the dike. Generally, snails that are small and capable of only limited movement live on the sediment surface and are easily affected by tidal flow (Leonard et al., 1998). The outside project area is a low-elevation mudflat and clearly affected by tidewater, and the fine-grained sediment is rare due to the impact of the tidal current. Many benthic species, especially snails, settle in the vegetation as a result of the tidal current. However, the water flow slowed after the project was completed as it was barred by the dike. On the one hand, the migration of species was prevented by the dike. On the other hand, suspended sediment in the water was deposited, accumulating in this habitat and causing the benthos (especially the snails) to collect near

A previous study found that the diking project reduced the number of benthic species, particularly crustaceans and polychaete worms, while the proportion of mollusk and insect larval species increased (Yuan and Lu, 2001). The density of benthos inside the project area was significantly lower than that outside, and the mollusks, polychaetes, and arthropods were mainly affected by the diking project (Ma et al., 2017; Yin et al., 2019). When we made a comparison before and after the diking project, we found that the species and density of crustaceans with strong mobility increased significantly, while that of mollusks and polychaetes with limited mobility decreased significantly. In addition, since mollusks and polychaetes are also important food organisms for birds or fish (Schwemmer et al., 2012; Touhami et al., 2019), it is necessary to take measures to restore the population of these benthic species and to maintain the balance and stability of an ecosystem when the presence of a dike is unavoidable.

The purpose of the dike project in Chongming Dongtan salt marsh was to remove the invasive plant *Spartina* and to carry out the ecological restoration. The dike can quickly inhibit the diffusion of alien invasive plants. After the removal of *Spartina*, shallow water ponds, bare land, or reed community habitats suitable for bird foraging and breeding were constructed. However, the density and diversity of benthos, which is a food source for birds, decreased significantly in the enclosed area. Moreover, the salt marsh was an important transit station and

food supply base for migratory birds (Li et al., 2009). The degradation of the benthos will result in adverse effects on the diet of birds (Ma et al., 2003; Pedro et al., 2018). This result was mainly due to the excessive short-term human interference to the ecosystem during dike construction (Vieira et al., 2012). We continue to monitor the changes and hope to provide more long-term data about the ecological project.

The increased density and richness of the benthos in the semiopened area indicates that preserving the tidewater canals of a dike should be adopted in other similar projects. The ecological restoration after the biological invasion has received considerable attention worldwide (Bakker and Wilson, 2004; Neira et al., 2006; Feng et al., 2018). In China, the ecological management of Spartina is also being carried out along the coast. This study provides some ecological management methods for Spartina invasion in other salt marsh ecosystems. We found that, during the ecological dike construction, retaining the water canals and semi-enclosed diking was the best way to maintain the balance and stability of the salt marsh ecosystem. Tidewater should be introduced regularly by the construction of the canals to maintain water and nutrient connectedness inside and outside the dike. In fact, changes in the diking approach have the same effect on Spartina eradication, and building water inlet and outlet will increase the construction cost, which may be an additional economic cost to be paid. However, we think that the cost is acceptable compared with the ecological benefits. The diking project mostly affected mollusks and polychaetes, which are the indispensable food sources for birds and fish. The study provides valid evidence for the management of salt marsh and the protection of birds and fish.

#### CONCLUSION

Removing the *Spartina*, which was the most serious invasive plant in the world, has become a great challenge in the global coastal (Li et al., 2009; Tang et al., 2009). To control *Spartina*, scholars have adopted a variety of methods such as physical, chemical, and biological control (Grevstad et al., 2003; Hedge et al., 2003). The method of "mowing+waterlogging" in this study in the Chongming Island has achieved good control results and has become a classic case of *Spartina* control (Li and Zhang, 2008; Yuan et al., 2011; Zhao et al., 2019). Hence, the dikebuilding project was carried out to establish this waterlogging regime to control *Spartina*.

The dike project has been documented in numerous studies to strongly affect macrobenthos in a broad range of ecosystems (Xue et al., 2019; Wu et al., 2021). But, the effects of different dike types, which were built as the ecological restoration, on benthos have been neglected previously. The benthic community, however, is recognized to be of critical important food organism for birds or fish (Schwemmer et al., 2012; Touhami et al., 2019). Our study examines the changes of macrobenthos by the ecological restoration project to remove exotic *Spartina* from a salt marsh in China. This will fill a gap in knowledge about the macrobenthic responses to the restoration projects of diking.

The result showed that the number of species and average density decreased significantly in the fully diked enclosed area but increased in the partially diked area. Macrobenthos was negatively affected by the dike project in the inside area, but, where the tidal connection between inside and outside was maintained in the project area, it had a positive effect on macrobenthos. Focusing on the tidewater connection efforts is likely to be the most ecologically beneficial and sustainable way to conserve ecological function, by promoting and increasing the connectivity between an ecological project area and the natural habitats. The connective conservation in the ecological project of the salt marsh may be best served by the combination of diking and the creation of new canals. We suggested the preservation of canals and the incorporation of more detail into diking projects in salt marshes, and that gates are opened regularly to maintain water and nutrient connectedness inside and outside a dike. For dikes that have been built in the estuary and coastal areas, the ecological restoration measures may need new waterways to reintroduce the tidal hydrology. When the presence of a dike is unavoidable, however, it is necessary to take measures to restore the population of these benthic species and to maintain the balance and stability of an ecosystem.

#### DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

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## **AUTHOR CONTRIBUTIONS**

SW conceived and designed the research, wrote, and edited the manuscript. QS and FZ performed the experiments. TZ and PZ analyzed the data. All authors contributed to the article and approved the submitted version.

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## SUPPLEMENTARY MATERIAL

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