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Response of macrobenthic communities to a Spartina alterniflora management project on tidal flats in Hangzhou Bay, China

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Abstract

Spartina alterniflora is one of the most invasive plants on Chinese tidal flats. To explore the responses of macrobenthic communities to the removal of *S. alterniflora* and the restoration of tidal flats, a survey of macrobenthic and sediment environmental factors was carried out in June 2022 in the mudflat recovery area (MRA) of tidal flats on the south bank of Hangzhou Bay, China. A proximate natural mudflat area (NMA), *S. alterniflora* area (SAA) and *Scirpus mariqueter* area (SMA) were selected for comparison with the MRA. A total of 48 macrobenthic species were identified, with 13, 28, 22 and 21species in the MRA, NMA, SAA and SMA, respectively. Cluster, multidimensional scaling (MDS) and SIMPER analysis suggested that the similarity between macrobenthic communities in the MRA and the NMA was high, whereas the similarity between macrobenthic communities in the MRA and SAA and the MRA and SMA was very low. The similarity between macrobenthic communities in the SAA and the SMA was high. ANOSIM analysis suggested that the differences between regions were greater than those within regions (R = 0.881). The sediment factors in the MRA were substantially different from those in the other three habitats, while the sediment factors in the SMA and the SAA were the most similar. Macrobenthos were mainly affected by the mean diameter of the sediment particles. After 1 year of natural recovery in the MRA, the macrobenthos approached the pre-project levels, but to ensure the effectiveness of the treatment project and prevent the recurrence of *S. alterniflora*, continuous monitoring should be carried out.

1 Introduction

The biosecurity problem represented by the biological invasion of alien species has become a major global environmental concern (Haubrock et al. 2022). The intensification of globalization and the expansion of introduction channels and trade patterns have disrupted natural barriers to potential alien invasive species, leading to an increase in the rate of invasion (Seebens et al. 2018; Kaiser et al. 2021). The threat of new invasive species emerging in the 21st century is widespread, with 17% of the world's land area (excluding Antarctica and the Greenland glaciers) and 16% of global biodiversity hotspots vulnerable to invasion (Early et al. 2016). China is the third largest country in the world. Diverse climatic and environmental conditions in China enable most species, especially invasive alien plants, to find habitats that are suitable for their survival, where they can successfully establish populations and further propagate and spread, thus forming a biological invasion (Wang et al. 2021). According to the *2020 China Ecological Environment Bulletin* issued by the Ministry of Ecology and Environment of China in 2021, more than 660 invasive alien species have been found throughout the country. Among them, 71 species are or may potentially pose a threat to natural ecosystems and are included in the *list of invasive alien species in China*. A total of 219 species have invaded national nature reserves.

Spartina alterniflora is a perennial salt marsh herbaceous plant of the Gramineae family (Liu 2018). It has well-developed roots, strong adaptability, salt tolerance, flooding tolerance and a fast reproduction speed. It can be used to prevent the coast from being eroded by wind and waves and increase the land area. The species was introduced from the United States as an important ecological engineering

material in 1979 (Xie and Han 2018). Since its introduction, the ecological harm caused by *S. alterniflora* has been identified. It occupies the niche of native plants (Jie et al. 2020), destroys the habitat of offshore organisms, changes the elevation of tidal flats (Qing et al. 2006), affects the structure and function of the tidal flat ecosystem, threatens the biodiversity of tidal flats, causes the loss of habitat for migratory birds (Li et al. 2008), and causes negative effects such as the siltation of estuary channels and 'competition' with tidal flats aquaculture (Li et al. 2005). It has become the most important malignant invasive plant on China's coastal tidal flats (Jie et al. 2020). In 2015, *S. alterniflora* occurred in all coastal provinces in China except Taiwan and Hainan, with a total area of 54579.9 ha (Li 2021). China began to comprehensively control *S. alterniflora* in 2022 and aims to achieve effective management in 2025. However, whether the management process has an impact on the tidal flat ecosystem remains unknown.

At present, studies on the control of *S. alterniflora* have been reported in Asia, North America, Europe and Africa, especially in China and the United States (Li et al. 2008; Adams et al. 2016; Taylor and Hastings 2004; Gao et al. 2014). Chemical control is the main method outside China, while physical control is the main method inside China (Cheng et al. 2020). In recent years, China has explored a variety of methods to control *S. alterniflora*. The chemical method uses herbicides for treatment, but the use of herbicides usually leaves residual toxicity, which can cause harm to the ecological environment (Paveglio et al. 1996; Kilbride and Paveglio 2001). Chemical methods have not been widely used in China, and the impact of herbicides on ecosystems needs to be further studied (Xie and Han 2018). Physical methods include manual removal, cutting, flooding and other measures, usually as a pre-treatment to biological alternatives (Xie and Han 2018). Biological replacement is an ecological control technology that replaces invasive plants with native plants according to the principles of plant community succession (Taylor and Hastings 2004; Gao et al. 2014; Knott et al. 2013; Tan et al. 2022; Wang et al. 2017). However, effective and safe alternative species in specific areas are still difficult to find. At present, most of the research uses reeds and mangrove plants as substitutes. The aim of the various control methods is to limit the growth and reproduction of *S. alterniflora* to control diffusion or completely remove the species (Xie and Han 2018). However, owing to the successful invasive and competitive properties of *S. alterniflora*, economical and effective ways to control its diffusion are still lacking. China has carried out much research on *S. alterniflora*, but this has mostly focused on the analysis and prediction of regional and general data, and it lacks the basic data from field investigations and field tests. There is no detailed research on the impact of *S. alterniflora* on the local biological and environmental conditions, nor on the response of organisms in the recovery process.

As an important part of the tidal flat ecosystem, macrobenthos plays a key role in material circulation and energy flows, and helps to maintain the health and integrity of marine ecosystem (Lv et al. 2018). The biodiversity, community structure and species composition of macrobenthos can indicate the status of the ecological environment of the region (Harrel and Smith 2002). Through a comprehensive investigation of the macrobenthic community, we can better understand their response to environmental changes. In turn, their response will reflect the effect of restoration on the beach ecosystem, and provide a basis for judging the implementation success of the restoration project. Some scholars have studied the community composition and ecological characteristics of macrobenthos in the intertidal zone of

Hangzhou Bay (Fan et al. 1996; Shou et al. 2012; Yan et al. 2021), especially the resources and population changes of macrobenthos after the implementation of several large-scale marine projects in Hangzhou Bay (Zhou et al. 2009; Li et al. 2007; Luo et al. 2007). However, a study comparing specific habitat types has not been undertaken. Different habitats have different effects on the composition of macrobenthic communities owing to the differences in hydrodynamic conditions (Zheng et al. 2011), physical factors such as water depth (Cai et al. 2012), physical and chemical factors such as water quality (Wang et al. 2005) and biological factors such as competition and predation (Holland et al. 1980).

To explore the impact of *S. alterniflora* management on organisms and the restoration of the tidal flat ecosystem, this study carried out a survey of macrobenthos in the mudflat recovery area (MRA), natural mudflat area (NMA), *S. alterniflora* area (SAA) and *Scirpus mariqueter* area (SMA) in June 2022, 1 year after *S. alterniflora* management measures were undertaken in the ecological restoration area on the south bank of Hangzhou Bay. A large amount of *S. alterniflora* originally grew in the MRA, which gradually recovered to mudflats after the implementation of the control project. By comparing the species composition, quantity and distribution of macrobenthic communities in different habitats and the differences in biodiversity, the study identified the distribution changes of macrobenthic communities and the impact of ecological restoration projects on macrobenthic communities. The findings provide a theoretical basis and practical experience for the prevention and control of *S. alterniflora* in the future.

2 Materials and methods

2.1 Study area

Hangzhou Bay is located in the northeastern part of Zhejiang Province, China. It is a trumpet-shaped estuary formed by the Qiantang River. It is also the world's strongest tidal estuary (Li et al. 2019). The southern bank of Hangzhou Bay is the largest tidal flat in Zhejiang Province. It is also the most concentrated area of waterbirds in Zhejiang Province and it is a transit station and wintering place for migratory birds. In recent years, most of the tidal flats in Hangzhou Bay have been invaded by *S. alterniflora*. Owing to the rapid reproduction of *S. alterniflora*, the sea water exchange capacity has been affected, and the ecological niches of *Phragmites australis* and *Scirpus mariqueter* have been invaded. This has resulted in the decline of macrobenthos and the loss of food sources for migratory birds, which has affected the ecological environment of the tidal flats (Li et al. 2019). To control the *S. alterniflora* in the coastal tidal flats of Hangzhou Bay, the local government implemented a demonstration project of *S. alterniflora* control on tidal flats on the south bank of Hangzhou Bay from 2020 to 2021. The removal of *S. alterniflora* by mechanical excavation has produced a natural experimental site to explore the control mechanism of *S. alterniflora*.

In this study, following 1 year of natural restoration in the MRA, a survey of macrobenthos was carried out in the middle tidal zone of the MRA on the south bank of Hangzhou Bay during the flood season at the end of June 2022. Comparison survey areas were established around the MRA in the same tidal area,

according to different vegetation types and vegetation coverage (Mou et al. 2015), including the NMA, the SAA and the SMA (Fig. 1). Five sampling stations were established in each area to study the difference between the restoration area and the other areas.

2.2 Sample treatment

Four parallel samples were set up at each sampling station. The samples were collected within a quantitative frame of 25 cm × 25 cm × 30 cm (length × width × depth). The samples were washed on site with a sieve with a pore size of 0.5 mm. The collected macrobenthos samples were fixed in 5% formaldehyde solution for morphological identification in the laboratory. The number and weight of each species were recorded during the identification process, and the data were converted into the average abundance (ind./m^2) and biomass (g/m^2) per unit area according to the sampling area. While collecting macrobenthos, sediment samples were collected near each station and placed in sample bags, which were immediately frozen for analysis. The water content of the sediments was determined. The particle size of the sediments in each area was determined by a laser diffraction granulometer (Mastersizer 3000, Malvern Panalytical, UK), and the composition and type of the sediments were analyzed. Part of each sample was analyzed for organic carbon (TOC) and total nitrogen (TN) content. The sample was treated with 2M HCl solution to remove inorganic carbon, and then washed with purified water and dried for determination using an elemental analyzer (Elementar Vario MICRO cube, Germany) (Jiang et al. 2022).

2.3 Statistical analysis

The community structure of macrobenthos in the ecological restoration area of the south bank of Hangzhou Bay was analyzed using the species dominance index (Y), Margalef's richness index (d), Shannon-Wiener diversity index (H') and Pielou's evenness index (J'). The calculation formulas are:

$$Y = P_i \times f_i$$

$$d = (\mathrm{S}\text{-}1) / \log_2 \mathrm{N}$$

$$H' = -\sum_{i=1}^{s} P_i \log_2 P_i$$

$$J' = H' / \mathrm{H}_{max}$$

where, S is the total number of species in the sample, N is the total number of individuals in the sample, f_i is the frequency of species i at each station in the survey area, P_i is the ratio $\left(\frac{ni}{N} \text{ or } \frac{\omega_i}{W}\right)$ of the number of species i (n_i) or biomass (ω_i) to the total number of individuals (N) or total biomass (W).

This study compared and analyzed the differences in species composition, quantity and distribution, and biodiversity of macrobenthic communities among the different habitats (Zhou et al. 2006). When

analyzing the community structure of macrobenthos in different habitats, according to the results of the homogeneity of variance test, if $P > 0.05$, then one-way ANOVA was selected; while if $P < 0.05$ then the Welch test was selected. Data processing was performed using SPSS26.0 (IBM Corp., Armonk, NY, USA). In the post-hoc multiple comparisons, the parameters were selected to compare the significant results of graph-based HSD, LSD and Tamhani test. The analysis method of sediment parameters was the same as that of macrobenthos community, and the data were also processed in SPSS26.0. PRIMER 6.0 (Clarke and Gorley 2006) was used to construct the Bray-Curtis similarity matrix for the macrobenthic communities in the survey area. Cluster analysis and multidimensional scaling (MDS) analysis were performed. SIMPER analysis was used to calculate the relative contribution of each taxonomic unit to the overall average dissimilarity observed between two or more groups, and ANOSIM (similarity analysis) was used to test the significance. ANOSIM is a robust nonparametric hypothesis testing framework for similarity differences between sample groups (Anderson 2001). Abundance data were transformed into square roots before analysis, and then analyzed after standardization. Canonical correspondence analysis (CCA) was used to analyze the surface sediment factors. Canoco 5 (Ter Braak and Smilauer 2012) was used for data processing, and the selected species were the dominant species.

3 Results

3.1 Sediment parameters

The sediment parameters in the four habitats are shown in Table 1. The surface sediment types in the MRA and the NMA were found to be silt, and the sediment types in the SSA and the SMA were clayey silt. The physical and chemical results of surface sediments in the survey area showed that the average particle size was NMA > MRA > SAA > SMA, while the contents of organic carbon and total nitrogen were in the order of SMA > SAA > MRA > NMA. The water content was SMA > MRA > SAA > NMA. The results of the one-way ANOVA and Welch test showed that there were differences in the sediment parameters in different habitat types.

Table 1
Sediment analysis of four areas. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area

Sediment	MRA	NMA	SAA	SMA
Mean diameter (μm)	$(15.75 \pm 0.96)^{a}$	$(18.65 \pm 0.50)^{a}$	$(9.10 \pm 0.09)^{b}$	$(6.43 \pm 0.05)^{c}$
TOC (%)	$(0.49 \pm 0.05)^{a}$	$(0.32 \pm 0.02)^{b}$	$(0.58 \pm 0.01)^{a}$	$(0.75 \pm 0.01)^{c}$
TN (%)	$(0.07 \pm 0.01)^{ab}$	$(0.05 \pm 0.01)^{a}$	$(0.08 \pm 0.01)^{b}$	$(0.11 \pm 0.01)^{c}$
Water content (%)	$(41.08 \pm 1.78)^{a}$	$(33.07 \pm 0.21)^{b}$	$(36.30 \pm 0.65)^{bc}$	$(43.69 \pm 0.66)^{a}$
Silty sand content (%)	$(78.95 \pm 1.01)^{a}$	$(86.47 \pm 0.39)^{b}$	$(71.88 \pm 0.11)^{c}$	$(62.21 \pm 0.14)^{d}$
Clay content (%)	$(16.23 \pm 1.07)^{a}$	$(11.07 \pm 0.31)^{b}$	$(25.37 \pm 0.28)^{c}$	$(34.54 \pm 0.04)^{d}$
(Note : Different letters in the upper right corner of the data indicate significant differences between the data (P < 0.05), Values are mean ± SEM)				

3.2 Species composition and dominant species of macrobenthos

A total of 48 species of macrobenthos were collected in the survey area, including 12 species of annelids, 19 species of mollusks, 12 species of arthropods, 4 species of vertebrates and 1 species of nemertean. The macrobenthos groups in the survey area were mainly mollusks, annelids and arthropods (Fig. 2). The number of macrobenthic species in the MRA was the least, at 13 species. The number of macrobenthos species in the NMA was the highest at 28 species, while the number of macrobenthos species in the SSA and the SMA was 22 and 21 species, respectively.

The species composition of macrobenthos in the NMA was the closest to that in the MRA, and the proportion of annelids, mollusks and arthropods was similar. The most difference was in the SAA, where the mollusk species were the most abundant, and a variety of marsh snails and shore snails were collected. Mollusca and arthropods accounted for the highest proportion in the SMA. The results showed that the species distribution of zoobenthos in the MRA and the NMA was similar, and the species distribution of zoobenthos in the SAA and the SMA was similar.

The dominant species (Y value ≥ 0.02) of macrobenthos in the four habitats are shown in Table 2. Comparing the dominant groups of the four habitats, it was found that the MRA was dominated by arthropods, followed by annelids, and *H. vadiculus* was the most dominant species. The dominant groups in the NMA, the SAA and the SMA were mainly mollusks, followed by annelids. *Y. similis* and *A. brevicula* were the most dominant species in the NMA and the SAA, respectively. In the SMA, the dominance of one species of *Assiminea* sp. and *Parheteromastus* sp. were the largest.

Table 2
Dominant species and dominance of macrobenthos in different habitats. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area.

Dominant species	MRA	NMA	SAA	SMA
Neanthes japonica	0.16	-	-	-
Nephtys oligobranchia	0.07	0.07	0.07	0.02
Harpiniopsis vadiculus	0.32	0.03	-	-
Yoldia similis	0.12	0.30	-	-
Eteone delta	-	0.02	-	-
Glycinde gurjanvae	-	0.05	-	-
Photis sp.	-	0.08	-	-
Lineus sp.	-	0.03	-	-
Bullacta exarata	-	0.20	-	-
Parheteromastus sp.	-	-	0.16	0.32
Glauconome sp.	-	-	0.08	-
Assiminea brevicula	-	-	0.39	0.28
Assiminea sp.	-	-	0.23	0.32

3.3 Abundance and biomass of macrobenthos

The average abundance of macrobenthos in the four habitats was NMA > SMA > SAA > MRA, and the average biomass was NMA > SMA > MRA > SAA (Fig. 3). The average abundance of macrobenthos in the NMA was approximately twice that of the MRA. The biomass of the NMA was approximately three times that of the MRA, while the biomass of the SAA was less than half of that of the MRA. However, the SPSS results showed that there were no significant differences in the abundance and biomass of macrobenthic communities in different habitats ($P > 0.05$).

3.4 Biodiversity index analysis of macrobenthos in different habitats

The macrobenthic community diversity index H' of different habitats in the ecological restoration area was NMA > SAA > SMA > MRA, with index values of 1.994, 1.528, 1.401 and 1.242, respectively. The evenness index J' was NMA > MRA > SAA > SMA, with index values of 0.711, 0.664, 0.626 and 0.601, respectively. The richness index d was NMA > SAA > SMA > MRA, with index values of 2.843, 2.043, 1.795 and 1.179, respectively. The H' and d of macrobenthos in the MRA were at the lower middle level, and the species distribution was relatively uniform. The H' and d of macrobenthos in the NMA were above the

middle level, and the species distribution was the most uniform among the four habitats. The *H′* and *d* of macrobenthos in the SAA were at a medium level, and the distribution among species was relatively uniform. The *H′* and *d* of macrobenthos in the SMA were at the lower middle level, and the distribution uniformity was the lowest among the four habitats. The results of Welch test showed significant differences in the *H′*, *J′* and *d* ($P < 0.05$). The specific differences are shown in Fig. 4.

3.5 Analysis of macrobenthic community structure

The results of similarity cluster analysis are shown in Fig. 5. The macrobenthic communities in the four habitats with a similarity of more than 35% could be divided into two groups. The macrobenthic communities in the SAA and the SMA had a high similarity of more than 60%. The similarity of macrobenthic communities in the NMA and the MRA was more than 45%. The similarity of each station in the four habitats was also high, and the clustering effect is obvious, indicating that the ecological restoration pilot had significant differences in community structure. The results of MDS were basically consistent with the cluster analysis, which further verified the results of the cluster analysis. According to the SIMPER analysis (Fig. 6), the species with the largest contribution in the MRA were *H. vadiculus* (39.63%), *Y. similis* (28.34%) and *N. oligobranchia* (14.32%). The species with a contribution of more than 10% in the NMA were *Y. similis* (23.24%), *B. exarate* (12.9%), *H. vadiculus* (11.57%) and *Photis* sp. (10.49%). In the SAA, the species with a more than 10% contribution were *A. brevicula* (30%), *Assiminea* sp. (24.8%) and *Parheteromastus* sp. (15.54%). The species with a contribution rate of more than 10% in the SMA were *Parheteromastus* sp. (28.19%), *Assiminea* sp. (26.82%) and *A. brevicula* (25.22%). The ANOSIM analysis showed that R = 0.881, and the difference between different habitats was greater than the difference of stations within each habitat.

3.6 Relationship between macrobenthos community and sediment environment

The results of the CCA (Fig. 7) show that the sediment factors in the MRA were quite different from those in the other three habitats, while the surface sediment environmental factors in the MRA and the NMA were relatively close. This may be due to the lack of vegetation cover in the restoration area, which was similar to the mudflat environment. The environmental factors of surface sediments in the SAA and the SMA were similar because of the invasion of *S. alterniflora* and its occupation of the niche of the native plant *Scirpus mariqueter*. There were significant differences in mean diameter, TOC and TN ($P < 0.01$), but no significant differences in water content, silty sand content and clay content. The contribution of mean diameter was the largest (53.0%), and this played a leading role in the distribution of macrobenthos. The results of CCA showed that the correlation between mollusks and water content, TOC, TN and clay content was high, and the correlation between polychaetes and mean diameter and silty sand content was high. *A. brevicula*, *Assiminea* sp., *Glauconome* sp. and *Parheteromastus* sp. were mainly distributed in the SAA and the SMA, and these were positively correlated with clay content, TN, TOC and water content. The species of *N. oligobranchia*, *G. gurjanvae*, *E. delta*, *Lineus* sp., *Y. similis*, *B. exarata* and *Photis* sp. were mainly distributed in the NMA, and these were positively correlated with sediment mean diameter and silty sand content. *N. japonica* and *H. vadiculus* were mainly distributed in

the MRA. *N. japonica* is less affected by sediment factors, and *H. vadiculus* is greatly affected by mean diameter and silty sand content.

4 Discussion

4.1 Macrobenthic community structure

Habitat type is the main factor that determines the difference in the macrobenthic community structure. From the results of this study, the macrobenthic communities in the restoration area and the non-restoration area of different habitats had differences in species composition, abundance, biomass and various diversity indexes. The removal of *S. alterniflora* by human intervention inevitably causes damage to the ecological environment and has a negative impact on the abundance, biomass and diversity of macrobenthos in the habitat. In this survey, the number of macrobenthic species in the MRA was the smallest, the abundance was the lowest, the species composition was relatively simple, and the species diversity index and richness index were the lowest.

4.1.1 Comparison between the MRA and the NMA

Owing to the implementation of the *S. alterniflora* control project, the *S. alterniflora* habitat in the MRA changed to the bare mudflat habitat after the treatment. The species number, abundance, biomass and biodiversity index of the MRA were approximately half those of the NMA. This was mainly because the ecosystem is fragile in the early stage of tidal flat restoration. The benthic animals are less resistant to an adverse environment and human disturbance and are vulnerable to diseases and pests (Wei et al. 2012). The ecosystem in the NMA was stable, there was a soil environment suitable for the growth of benthic animals, and the benthic diatoms provide a rich source of food for benthic animals. Eventually, the rich benthic resources on the mudflat will attract a large number of birds to forage, forming a good ecosystem. The findings demonstrated that the macrobenthos in the MRA were in a transitional state. It is necessary to give them natural recovery time to develop towards the stable state of the NMA, which can gradually restore and improve the living environment of benthic animals, increase their diversity and develop into a stable ecosystem.

4.1.2 Comparison between the MRA and the SAA

The MRA was formerly an *S. alterniflora* habitat before the implementation of the control project. In this survey, the number of macrobenthos species in the SAA was higher than that in the restoration area, and the biological abundance was close to the restoration area, but the biomass was lower. Compared with the survey results of Wu Ming et al. (2011) in Hangzhou Bay in 2009 (Table 3), except for the large difference in abundance, there was not significant difference in species number, biomass and diversity index. The main reason for the high abundance and diversity index of macrobenthos in this study may be that the sampling of *S. alterniflora* habitat survey in 2009 was 25cm × 25cm × 20cm, and the sampling in this survey was 25cm × 25cm × 30cm, with deeper depth and more biological samples collected. After the implementation of the ecological restoration project, the number of species in the MRA and the

diversity index *H′* were lower than that before the implementation of the project. Although the biodiversity index of the MRA was still relatively low, on the whole, after 1 year of natural restoration, the macrobenthos had recovered rapidly and gradually approached the state before the implementation of the project. The diversity index of macrobenthos in the *S. alterniflora* habitat was smaller than that in the natural mudflat habitat. This may be related to the invasion of *S. alterniflora*, which changed the original habitat characteristics and occupied the niche of native species (Wan et al. 2009). The formation of a single, closed and patchy plant population changed the sediment on the beach and fragmented the living space of the original community. This resulted in a corresponding change in the structure of the macrobenthic community and reduced the biodiversity of the habitat to a certain extent (Jiang et al. 2016). When sampling in the SAA, the mud was found to be harder. This was due to the strong root system and high growth density of *S. alterniflora*, which promoted silt deposition and made the central area of the tidal flats very black in color. Benthic animals cannot live in the bottom anaerobic layer, thus there are few species and a low quantity of polychaete benthic animals distributed in the bottom layer (Wei et al. 2012).

Table 3
Comparison of tidal flat restoration area and *Spartina alterniflora* area. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area

Investigating area	Species	Abundance (ind./m^2)	Bioss (g/m^2)	*H′*
SAA(2009)	20	20	5.08	0.93
MRA(2022)	13	662	17.08	1.24
SAA(2022)	22	786	4.56	1.53

4.1.3 Comparison between the MRA and the SMA

Compared with the MRA, the number, abundance, biomass and biodiversity index of macrobenthos were higher in the SMA. As an endemic species in the Yangtze Estuary, *Scirpus mariqueter* plays an important role in the wetland ecosystem. It can provide a natural habitat for foraging, activity and rest for many wintering migratory birds. At the same time, the rhizomes and bulbs of *Scirpus mariqueter* are also important food for the national first-class protected Hooded Crane. However, the large-scale invasion of the exotic species *S. alterniflora* has caused significant competitive exclusion for *Scirpus mariqueter*, and its area of suitable habitat is shrinking (Chen and Sun 2011). This has resulted in a large reduction in the distribution of the species (Chen et al. 2004), which has severely impacted the *Scirpus mariqueter* community (Liu and Han. 2009). The shift in species has led to a change in the structure and function of the tidal wetland ecosystem (Chen and Sun 2011). The germination time of *S. alterniflora* is early, and the plant grows rapidly, so that it inhibits the normal growth of adjacent plants in its distribution area. *S. alterniflora* has obvious advantages over *Scirpus mariqueter* in height, coverage, aboveground biomass and underground biomass (Lu and Zhang 2012), which creates conditions for it to access more light and nutrients and quickly replace *Scirpus mariqueter* (He et al. 2011). Therefore, the existence of an ecotone between the distribution of the two plants is extremely unfavorable to the survival of the *Scirpus*

mariqueter population. In this survey, although the biodiversity of macrobenthos in the SMA was not as high as that in the NMA, the unique importance of *Scirpus mariqueter* for birds cannot be ignored. Therefore, after the removal of *S. alterniflora* and the stable development of the mudflat in Hangzhou Bay, the restoration of *Scirpus mariqueter* needs to be considered.

4.2 Correlation between macrobenthic community characteristics and sediment factors

The species composition and distribution of macrobenthic communities reflect the comprehensive response of each species to environmental factors and habitat quality (Zhang et al. 2023). The physical and chemical properties of sediments are the key factors that affect the distribution of benthic communities (Neira et al. 2007). Larger soil particle sizes are a favorable factor for the biodiversity of macrobenthos (Huang et al. 2021). From the perspective of sediment particle size, the particles in the MRA and the NMA were significantly larger than those of the SAA and the SMA. The MRA is a tidal flat that is newly silted after the removal of *S. alterniflora*. The sediment particle size is large. Owing to the presence of the developed root systems of their respective vegetation, the sediment particle size in the SAA and the SMA is small, and the proportion of clay is higher than the other two habitats. In all four habitats, the proportion of silt is high, which provides a good habitat for organisms such as annelids and mollusks. The carbon and nitrogen content of the original *S. alterniflora* in the restoration area of the tidal flat was higher than that in the NMA. Jiang S et al. (2022) found that the sediment grain size was finer after the invasion of *S. alterniflora*, which was attributed to the enhancement of the deposition process of *S. alterniflora*, contributing to the accumulation of organic matter in the sediment. They also found that the content of TOC and TN increased with the development time of *S. alterniflora* because it produces a large quantity of litter and roots. The results of sediment analysis in the current study found that vegetation-covered habitats had smaller sediment grain sizes and higher TOC and TN contents, which is consistent with the results of Jiang S et al. (2022). Organic carbon is the main food source for benthic animals, and the biomass and density of macrobenthos generally increase with an increase in organic debris (Honkoop et al. 2003). This is inconsistent with the results of this study, and it may be related to other sediment factors. The specific reasons need to be further studied. Surface sediments are the location for the growth and development of benthic animals, and the sedimentary composition characteristics and various physical and chemical factors have an important impact on benthic animals.

4.3 Opinions for the management of *Spartina alterniflora* on tidal flats

At present, many studies have been carried out on *S. alterniflora* in China, and the most effective treatment method has proved to be a combination of several methods, such as mowing + shading and mowing + flooding. According to the characteristics of each place, or using native plants for biological substitution, some good results have been achieved. However, owing to the difficulty of prevention and control of *S. alterniflora*, the overall coordination in the region is not sufficient, the protection and supervision capability is still relatively weak, and the trend of diffusion and spread is still severe. No

detailed research on the impact of *S. alterniflora* on organisms and the environment, or on the response of organisms in the recovery process, is available. In the process of *S. alterniflora* management, we should adhere to the combination of ecological concepts and engineering measures, scientifically manage *S. alterniflora*, adjust measures to local conditions, and restore the original local ecosystem to the conditions before the invasion of *S. alterniflora* as much as possible. At the same time, we should clarify the responsibility for *S. alterniflora* management and encourage and guide social forces to participate in the prevention and control of *S. alterniflora*. One important group is the local fishermen who have been affected by the invasion of *S. alterniflora*, which has led to a decrease in the number of tidal flat organisms and a decrease in fishing catches.

The macrobenthos can reflect the health status of the tidal flat ecosystem and it can be used as an indicator for the *S. alterniflora* control project to track and monitor the implementation effect of the ecological restoration project. According to the research results of Ma et al. (2017), after the implementation of the ecological restoration project, there was a certain negative impact on macrobenthos in the short term, but this change was only temporary. After the treatment of *S. alterniflora*, with the development of the restoration area, wetland beaches and local vegetation can be gradually restored, the living environment of macrobenthos can be improved, and the diversity of macrobenthos can be increased. The implementation of the *S. alterniflora* control project will inevitably cause damage to the original ecological environment. The restoration of vegetation and macrobenthos destroyed by the project will take many years. Therefore, the improvement of biodiversity lags behind the completion of the restoration project. Owing to the lag in the restoration of macrobenthic communities and the obvious seasonal changes in their distribution characteristics, future studies should select important seasons to monitor the restoration project for a longer period of time, and a long-term macrobenthic community database should be established (Zhang et al. 2023). Continuous monitoring of environmental factors in the restoration area is required to prevent secondary invasion of *S. alterniflora* and increase the possibility of local or complete eradication of *S. alterniflora*.

5 Conclusion

This study was found that after the implementation of the *S. alterniflora* treatment project, the macrobenthic community structure in the MRA was most similar to the community structure in the NMA. In the follow-up monitoring process of ecological restoration effect, the changes of macrobenthic community structure need to be continuously observed. The implementation of the *S. alterniflora* control project in Hangzhou Bay is an example for the full implementation of the *S. alterniflora* control measures in China. The study shows that the replicable empirical methods can measure changes in the macrobenthos following restoration. At the same time, ecological restoration after treatment, using satellite remote sensing, UAV and other high-tech methods, combined with field investigation, should be continuously promoted to improve the ability to monitor *S. alterniflora* dynamically.

Declarations

Acknowledgements

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Figures

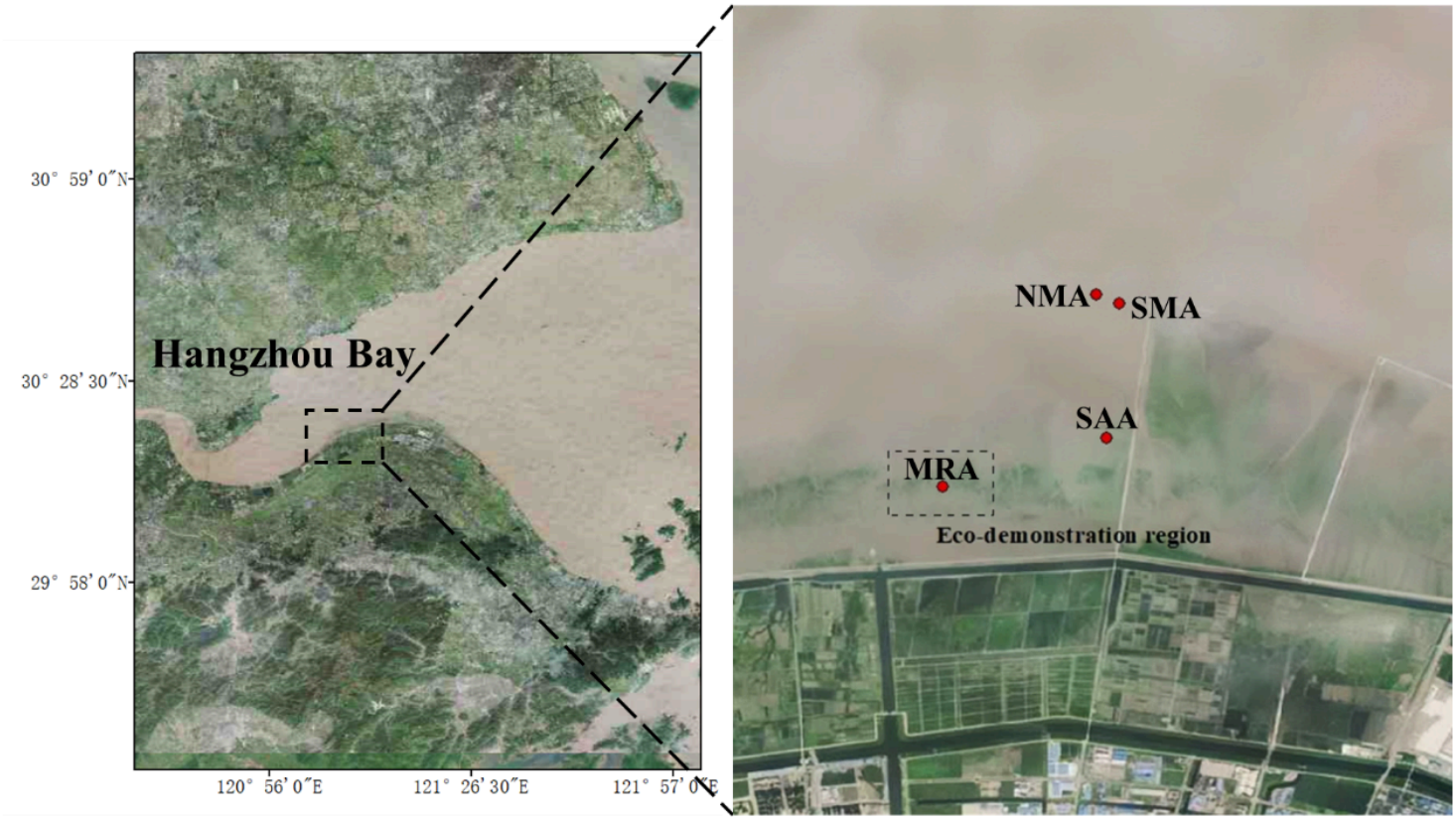


Figure 1

Sampling area of macrobenthos on tidal flats in Hangzhou Bay, China. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area

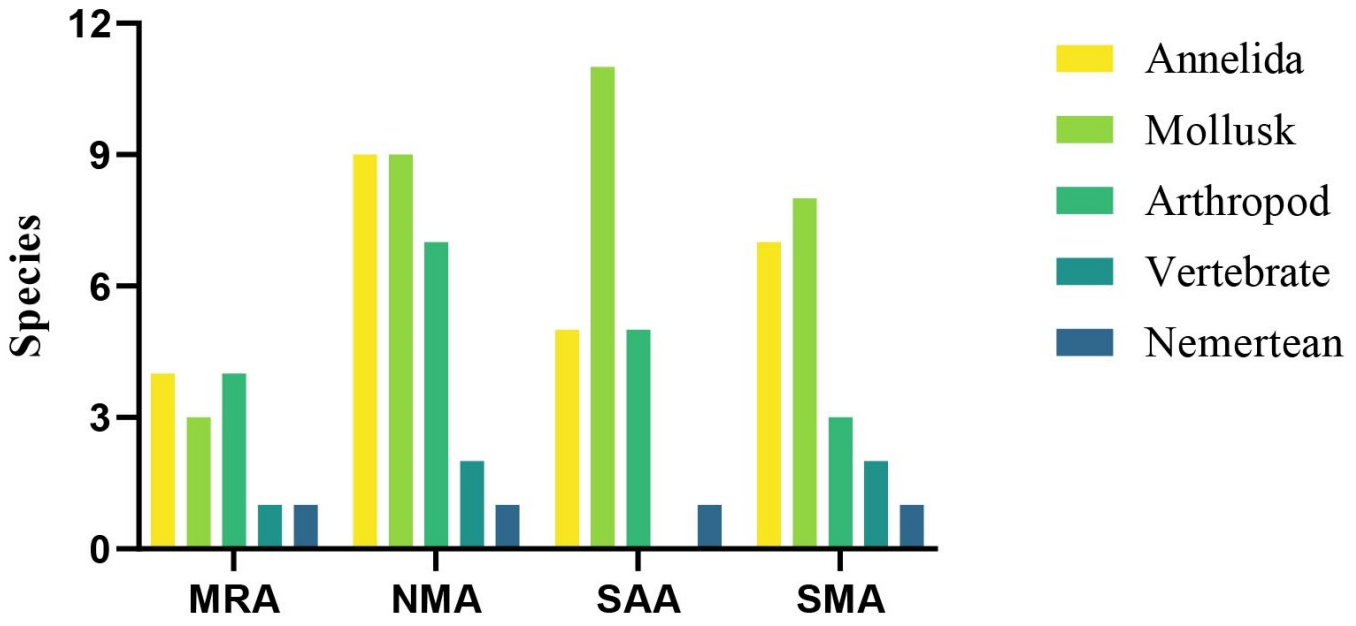


Figure 2

Species composition of macrobenthos in different habitats. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area

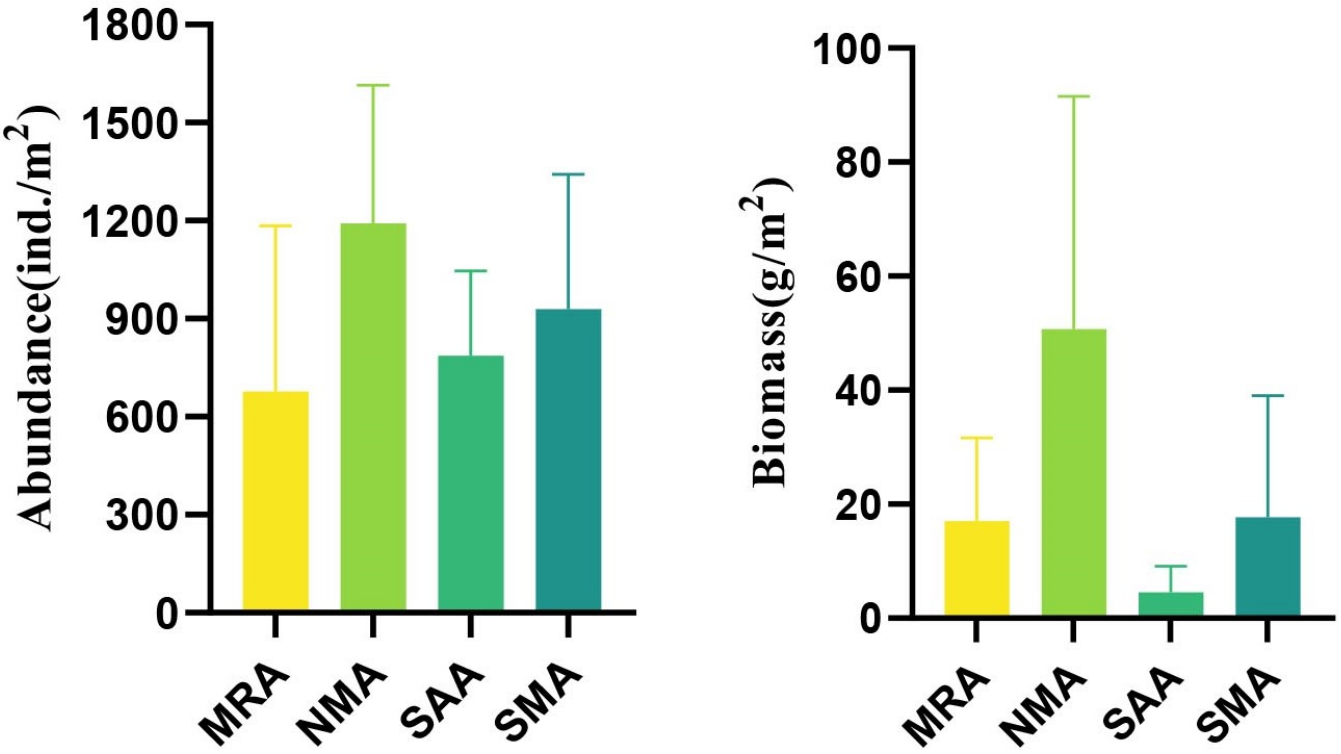


Figure 3

Abundance and biomass of macrobenthos in different habitats. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area

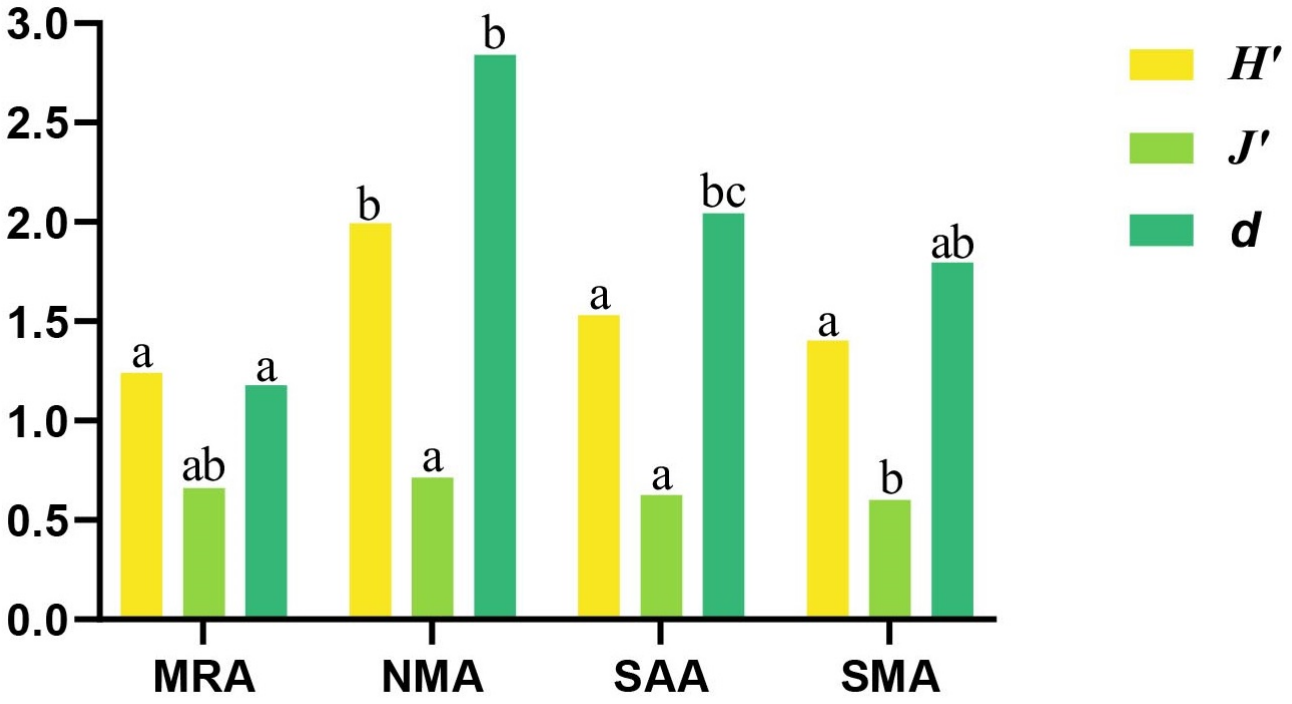


Figure 4

Macrobenthic diversity index in different habitats. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area

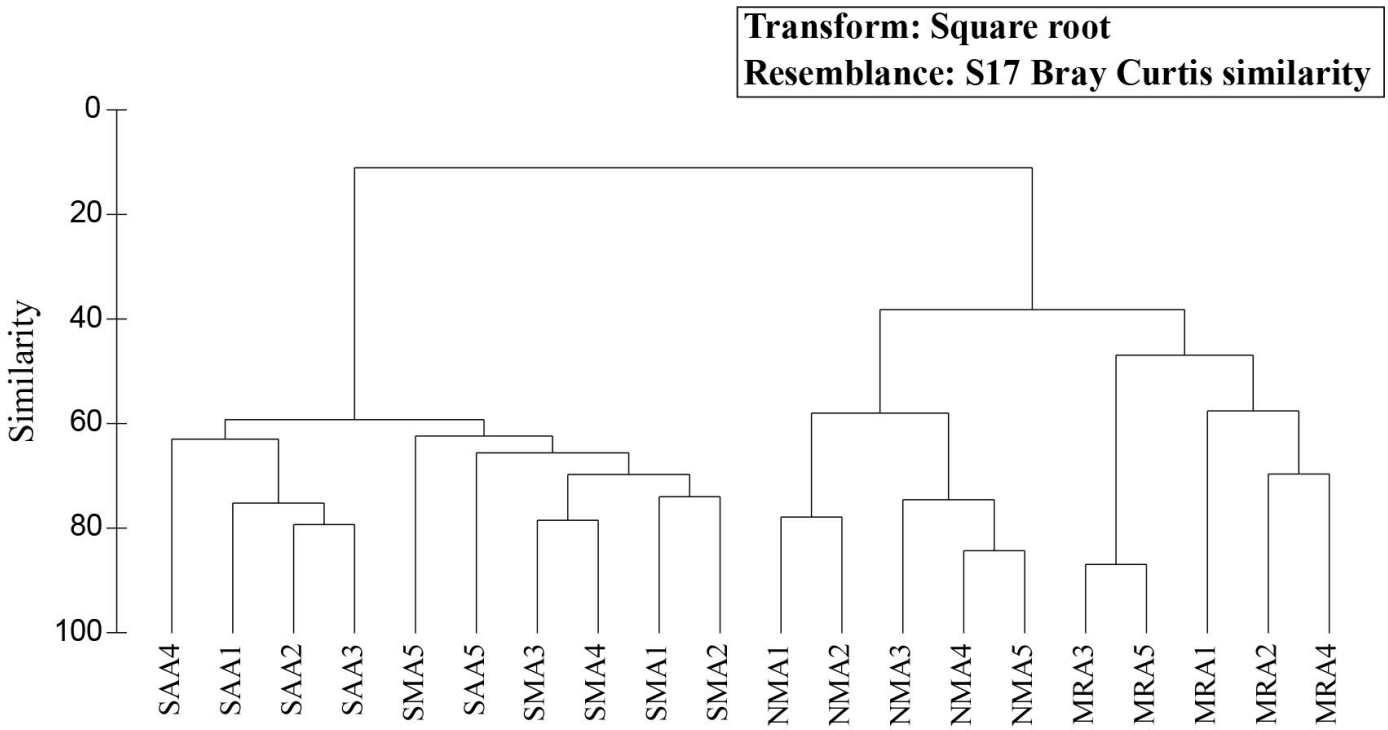


Figure 5

Cluster of macrobenthic community in ecological restoration area. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area

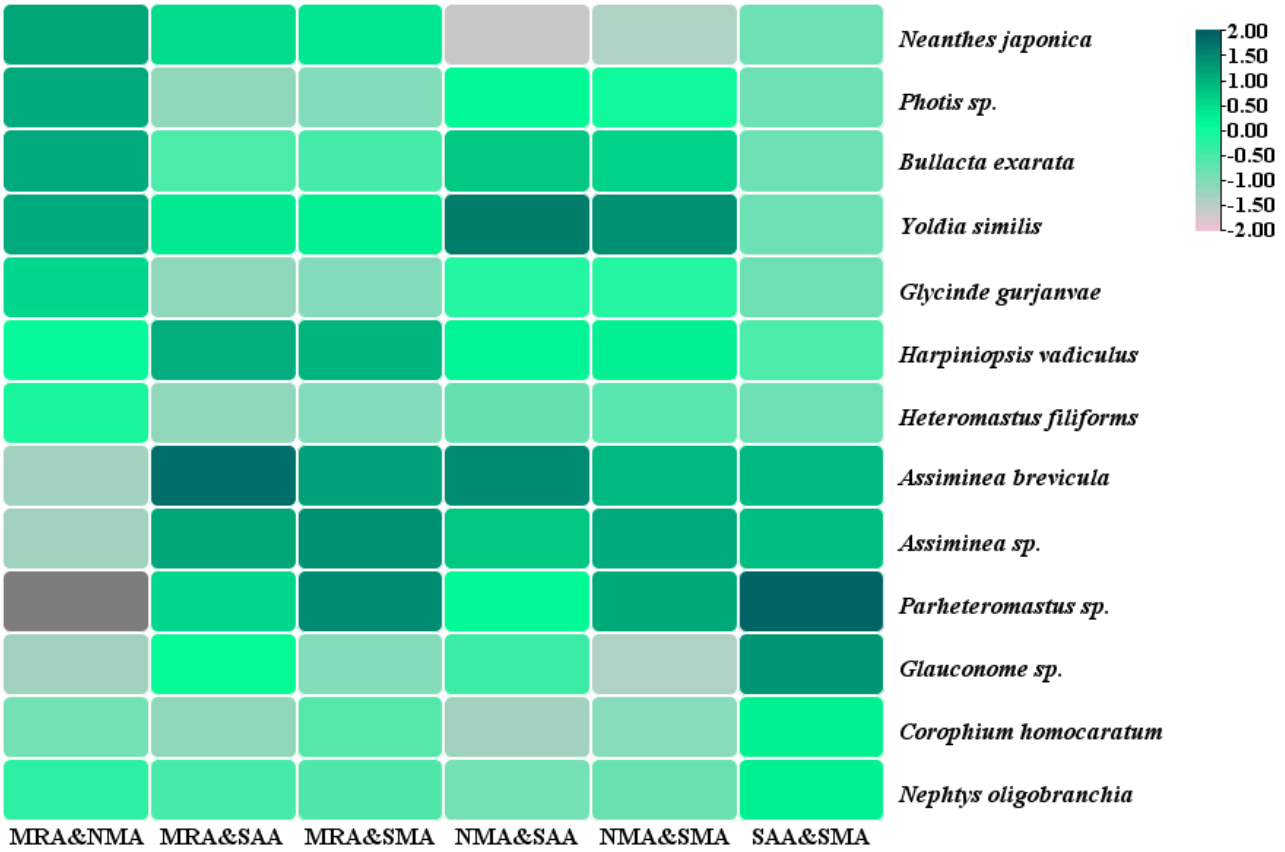


Figure 6

SIMPER between different habitats. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area

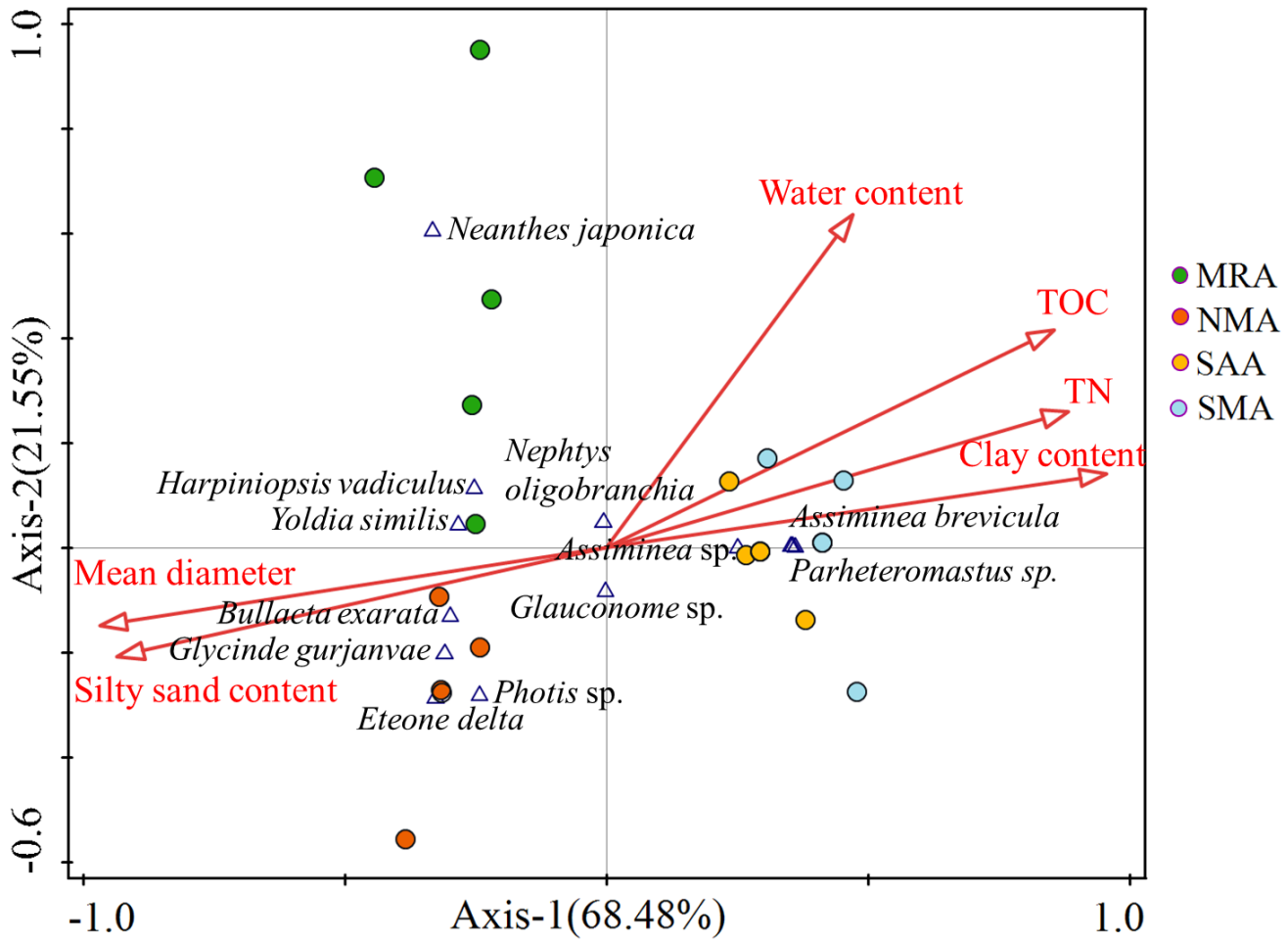


Figure 7

CCA analysis. MRA: mudflat recovery area; NMA: natural mudflat area; SAA: *Spartina alterniflora* area; SMA: *Scirpus mariqueter* area

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