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Assessing the changes in marine microalgae diversity in the Nanji Islands Nature Reserve over the past decades using sediment eDNA

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The Nanji Islands, designated as one of China's national nature reserves, are renowned for their abundant microalgal resources. Changes in microalgae can serve as indicators of marine environmental shifts. Despite this significance, the absence of comprehensive historical records on microalgal diversity has limited the evaluation of marine environmental protection efforts. This study addresses this gap by analyzing surface sediments and sediment cores from the Nanji Islands, utilizing ^{210}Pb dating and employing environmental DNA (eDNA) technology to trace historical shifts in the molecular diversity of microalgae. Chloroplast gene fragments were amplified using rbcL primers, and ^{210}Pb dating determined the deepest sediment layer (78-80 cm) to date back to 1994. From the 70-72 cm layer to the 60-62 cm layer, the number of microalgal reads increased rapidly from 27,716 to 65,143, signifying enhanced abundance over the 10 years following the establishment of the nature reserve. This was followed by a deceleration in microalgal abundance growth over the subsequent 20 years, potentially reflecting variations in primary production of microalgae. Concurrently, the rise in operational taxonomic units (OTUs) and the Margalef index suggest a boost in microalgal diversity, which may be attributed to improvements in the marine environment. The microalgal community composition has shifted from a dominance of Dinoflagellata and Streptophyta to a predominance of Bacillariophyta, the change in phosphate would be one of the impact factors worth noting. This study provides foundational data on the historical changes in microalgae in the Nanji Islands and serves as a reference for exploring the relationship between environmental conditions and microalgal dynamics.

KEYWORDS

Nanji Islands Nature Reserve, eDNA, marine microalgae, molecular diversity, environmental indicators

1 Introduction

Global warming has precipitated a crisis of rapid global biodiversity loss, necessitating the urgent development of innovative and efficient methods for biodiversity assessment (Vitousek, 1994). As integral components of biodiversity, plants—and particularly microalgae—play a fundamental role in maintaining the primary productivity of the marine ecosystem, accounting for approximately 40% of marine plant life (Moreno-Garrido, 2008). Moreover, microalgae can lead to Harmful Algal Blooms (HABs), posing grave risks to marine life, human health, and the global economy, as well as contributing to air pollution and ecological damage (Hallegraeff, 1993; Landsberg, 2002). Such blooms are often induced by rapid environmental changes in marine conditions, including precipitation, temperature, light intensity, salinity, and nutrients (Errera et al., 2008; Hambright et al., 2014). Consequently, shifts in marine microalgae community can serve as sensitive indicators of environmental changes.

The foundation of any biodiversity assessment is taxonomic composition (Ruppert et al., 2019). Traditional methods for identifying microalgae rely on detailed morphological examination, typically requiring trained phycologists and microscopy. However, since many microalgae do not leave effective records in marine sediments, it is challenging to trace historical changes in microalgal populations through morphological studies alone. This limitation hinders the effective use of microalgae communities as indicators of marine environmental shifts. Environmental DNA (eDNA)-based molecular approaches offer a promising alternative for biodiversity assessments (Beng and Corlett, 2020). Extracting eDNA from soil samples (Taberlet et al., 2012) allows for the simultaneous collection of DNA from multiple individuals and taxa, bypassing lengthy and costly field sampling processes. This non-invasive and non-destructive method is particularly effective in detecting rare or difficult-to-collect taxa (Alsos et al., 2018; Carrasco-Puga et al., 2021; Hartvig et al., 2021). eDNA-based surveys provide rapid assessments and monitoring of biodiversity changes in specific regions, which is essential for understanding ongoing biodiversity loss and marine environmental shifts. Soil eDNA has proven successful in identifying current and past biodiversity (Yoccoz et al., 2012) and reconstructing past environmental changes (Rawlence et al., 2014), offering insights into "hidden diversity" (Jørgensen et al., 2012; Parducci et al., 2013). The historical variation revealed by eDNA have been widely reported (Alsos et al., 2016; Armbrecht et al., 2022; De Schepper et al., 2019; Garcés-Pastor et al., 2022). Nevertheless, the application of soil eDNA for exploring historical changes in marine microalgal diversity is still in its infancy, presenting significant knowledge gaps (Ariza et al., 2023).

The Nanji Islands, located at the southern end of the East China Sea, represent a convergence zone for the Zhejiang Coastal Current and the Taiwan Warm Current. The numerous islands and capes in this region induce intense mixing of upper and lower seawater, fostering upwellings that lead to excellent water quality, abundant food sources, and favorable hydrological and climatic conditions. Consequently, this area boasts high biodiversity with a complex faunal composition. Prior to the establishment of the nature reserve, the Nanji Islands experienced significant human activity, including the artificial cultivation and processing of algae, fish, and shellfish. These activities introduced pollution and environmental stress (Yang et al., 1994; Li et al., 2017). However, there is a notable lack of historical records detailing the environmental impacts on the Nanji Islands, with baseline data on marine microalgae being particularly scarce. Therefore, new methods are urgently needed to address this information gap. Since the establishment of the Nanji Islands Nature Reserve in 1990, there has been heightened public and governmental interest in the environmental changes occurring in this region.

To assess changes in microalgal diversity in the Nanji Islands over recent decades, we collected surface sediment samples from various stations (Daleidao, Houjishan, Huokunao, and Mazuao) and sediment core samples from Huokunao. The molecular diversity of microalgae was analyzed using eDNA data. Combining with ^{210}Pb dating, this study presents, for the first time from an eDNA perspective, the historical changes in microalgal diversity in the Nanji Islands, providing foundational data for the region. These findings are significant for developing informed environmental protection measures for the Nanji Islands.

2 Materials and methods

2.1 Sample collection

From May 9 to May 13, 2022, a field investigation was conducted on the Nanji Islands (121°01'-121°06'E, 27°27'-27°30'N), focusing on four coves: Guoxingao, Mazouao, Dashaao, and Huokunao (Chinese transliterated names). Huokunao, characterized by predominantly muddy sediment, was selected as the target station for core sampling based on its sediment characteristics. In alignment with the local tidal schedule, the low tide period was chosen to collect gravity cores from Huokunao using PVC tubes with diameters of 90 mm and 110 mm, along with a rubber mallet. Two sediment cores, HKA01 (70 cm) and HKA02 (80 cm), were successfully retrieved. Additionally, surface sediment samples were collected from the intertidal zone at the Mazuao (MZA) and Huokunao (HKA) stations, as well as from the shallow continental shelf areas of Houjishan (HJS) and Daleidao (DLD), which were designated as reference stations (Figure 1). This strategic and comprehensive sampling approach was designed to yield a robust dataset for analyzing historical changes in marine microalgal diversity.

2.2 Sample treatment

2.2.1 Sample splitting

After field sampling, the collected sediment cores underwent careful processing and preparation for subsequent analysis. Using an angle grinder, the PVC pipes containing the cores were split open vertically. Sterilized clean iron wire was used to carefully split the HKA01 and HKA02 cores. Upon visual inspection, core HKA01 displayed chaotic sedimentary layers, whereas core HKA02 exhibited well-stratified layers. Therefore, HKA02 was chosen for further investigation (Figure 1E). The lithology of core HKA02 was

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divided into four distinct sections with no signs of bioturbation: 0 ~ 16 cm was brown sandy; 16 ~ 30 cm was dark gray clay; 30 ~ 54 cm was dark brown silty; 54 ~ 80 cm was gray sandy silty. Using a ruler, the core samples were systematically subsampled using a sterilized scraper, with a 2 cm stratification standard. This meticulous sampling process resulted in a total of 40 discrete samples from core HKA02. The unsampled HKA01 core was carefully wrapped in plastic and stored at -20°C (Haier; Haier Freezer; BC/BD-829HEZ; 2105*860*905 mm; 829 L; China) alongside the HKA02 samples for potential future analysis.

2.2.2 Age dating

To establish the chronology of the sediment core, samples from three distinct depth intervals (10-12 cm, 30-32 cm, and 78-80 cm) were selected for ^{210}Pb dating. The samples were dried in a drying oven to remove any moisture content and then ground in an agate mortar to a fine 100-mesh particle size, ensuring a homogeneous sample for subsequent analysis. The prepared samples were sent to the Public Technology Center of the Institute of Oceanology, Chinese Academy of Sciences, for ^{210}Pb dating measurements. The analysis was conducted using the 920-8 alpha spectrometer produced by EG&G Ortec (USA), which has an instrument resolution of FW = 19 Kev.

2.2.3 Anti-contamination processing

To ensure data integrity and minimize the risk of contamination throughout the experimental process, the researchers implemented a comprehensive set of anti-contamination measures: (1) Personal Protective Equipment (PPE): Researchers wore protective clothing, masks, gloves, and rubber boots to maximum body coverage during the experiment. Strict adherence to PPE protocols helped prevent the introduction of extraneous DNA or contaminants. (2) Sterilization of Materials and Instruments: All experimental materials and instruments were thoroughly sterilized at high temperature and pressure. This rigorous sterilization process eliminated the possibility of cross-contamination from previous samples or external sources. (3) Sampling Tool Disinfection: Sampling tools were disinfected and sealed before and after each sampling event. This meticulous handling of the sampling equipment prevented the introduction of any contaminants during the field collection of the sediment cores and surface sediments. (4) Dedicated Laboratory Spaces: DNA extraction experiments were carried out in a dedicated laboratory, while the PCR amplification procedures were conducted in a separate laboratory. This spatial separation minimized the risk of DNA contamination from the laboratory environment. (5) Blank and Negative Controls: Appropriate blank and negative control samples were included throughout the experiment to detect and rule out any potential DNA contamination from the laboratory or reagents used.

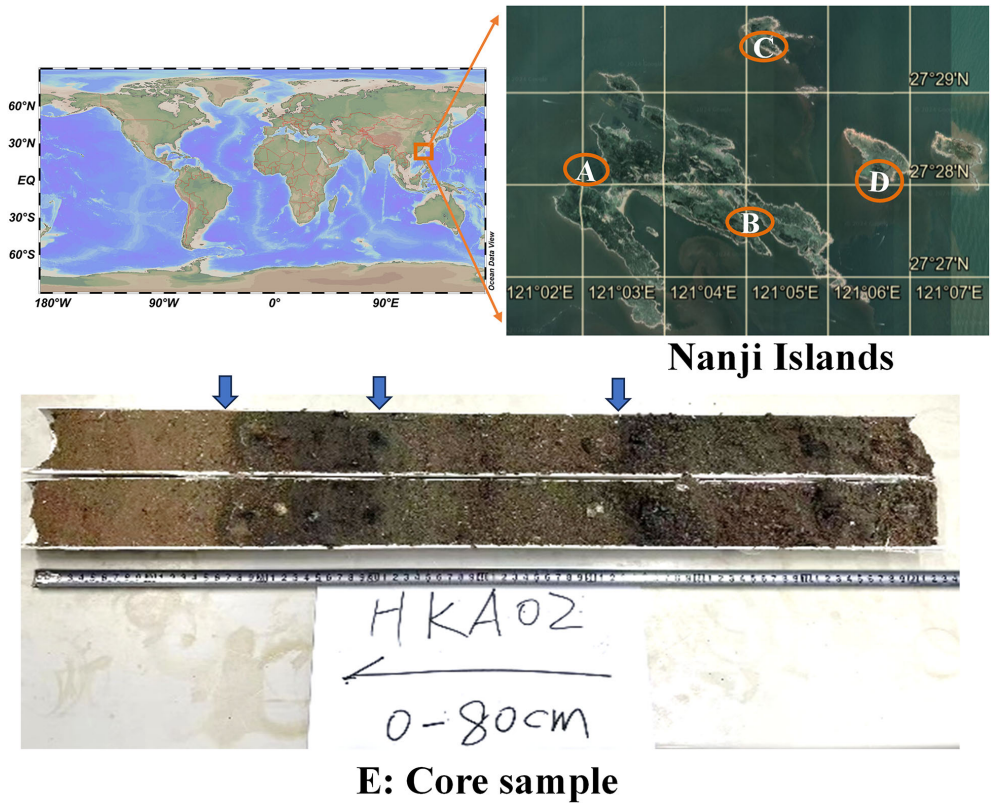

FIGURE 1
Surface sampling stations of Nanji Islands and the core sample from the Hunkunao station. The surface sampling stations included **(A)** Mazuao station (MZA); **(B)** Huokunao station (HKA); **(C)** Daleidao station (DLD); **(D)** Houjishan station (HJS).

2.2.4 eDNA processing

Environmental DNA (eDNA) was extracted from the sediment samples using the MoBio PowerSoil DNA Extraction Kit, which is designed to efficiently isolate high-quality DNA from complex soil and sediment matrices. To target marine microalgal diversity, specific primers were used to amplify the chloroplast rbcL gene via polymerase chain reaction (PCR) (Bradley et al., 2007). The primer information and detailed PCR amplification procedures are provided in Table 1. The 50 μL PCR reaction mixture consisted of the following components: 25 μL of high-fidelity enzyme (2×TransTaq HiFi PCR SuperMix), 1 μL each of forward and reverse primers (10 μM), 17 μL of DDH2O, and 6 μL of the extracted eDNA template. Throughout the entire eDNA processing workflow, appropriate blank and negative control samples were included to monitor and rule out any potential contamination.

The PCR amplicons with clear target bands were selected for subsequent sequencing, which was conducted at Novogene, a leading genomics service provider. The raw sequencing data obtained from the Illumina HiSeq platform contained some interfering information. To ensure more accurate and reliable analysis results, a series of quality control measures were implemented on the sequencing data: First, the sequencing data was demultiplexed into individual samples based on the unique barcode and primer sequences. The barcode and primer sequences were then trimmed from the raw reads. FLASH (V1.2.7) (Magoč and Salzberg, 2011) was used to assemble the trimmed sequences for each sample, generating the "Raw tags". According to the quality control procedures of QIIME (V1.9.1) (Caporaso et al., 2010) and Usearch (v11.0.667) (Edgar, 2013), the Raw tags were further filtered to remove chimeric sequences and low-quality reads, resulting in high-quality effective data. The unoise3 non-clustering denoising method was employed to generate operational taxonomic units (OTUs) from the high-quality sequences. Notably, there was no specific clustering threshold in OTU generation, as the UNOISE algorithm does not rely on a pre-defined clustering cutoff. Instead, it uses an error-correction approach to denoise the sequence data and identify accurate biological sequences (Edgar, 2016). The representative OTU sequences were compared against the NCBI database using BLAST (V2.7.1) to obtain the taxonomic information for each OTU, yielding an average identity value of 96.47%. Unassigned OTUs were labeled as "Unknown" and treated as "Other" in subsequent analyses (Supplementary Tables 1, 2).

2.3 Statistic analysis

The sequencing data were normalized based on the sample with the smallest amount of data. This normalization step ensured that diversity analyses were conducted on a comparable basis across all samples. To analyze alpha diversity indices, such as the Shannon-Wiener and Margalef index, the normalized OTU table data was used. The data was further standardized by resampling to the minimum number of OTUs observed across the samples. This additional step guaranteed that the diversity metrics were calculated on a consistent basis, ensuring that diversity metrics were calculated consistently for meaningful comparisons. The alpha diversity indices were calculated using the "alpha_diversity.py" command in QIIME software (Caporaso et al., 2010). Figures depicting sampling stations were generated using Ocean Data View software (version 5.6.2) and combined with other components using Microsoft PowerPoint (version 2022). Microalgal community plots were created in Origin software (version 2021). To determine the average sedimentation rate, the study employed the Constant Initial Concentration (CIC) model (Wan, 1997). The calculation formula is as follows:

$$v = \frac{h \star \gamma}{\ln[210_{Pb_{ex}(0)}/210_{Pb_{ex}(h)}]} \quad (1)$$

Where v is the sedimentation rate. $^{210}Pb_{ex}(0)$ is the excess ^{210}Pb activity concentration at the surface. $^{210}Pb_{ex}(h)$ is the excess ^{210}Pb activity concentration at depth h. γ is the decay constant of ^{210}Pb. h is the depth of the sediment.

3 Results

3.1 Data review

As shown in Table 2, the high-throughput eDNA sequencing generated a total of 1,443,961 raw sequences, with an average of 120,330 sequences per sample. The 0-2 cm layer sample from the Huokunao (HKA) station had the highest number of raw sequences, reaching 139,073. After sequence assembly, quality filtering to remove low-quality reads, and the elimination of chimeric sequences, a total of 1,303,331 high-quality, non-chimeric sequences remained. The average effective rate of the sequencing data was 90.25%, indicating a robust and reliable dataset for further analysis.

TABLE 1 Primer information and PCR amplification procedures for samples from the Nanji Islands.

Primer Name	Sequence (5′-3′)	PCR Amplification Procedures
rbcLZ1	5′ATGTCACCACAAACAGAGACTAAAGCAAGT3′	94°C Pre-denaturation for 5 minutes; 92°C Denaturation for 15 seconds, 60°C Annealing for 60 seconds, 72°C Extension for 60 seconds, 45 cycles; 72°C Final Extension for 10 minutes
rbcL19	5′CTTCTTCAGGTGGAACTCCAG3′	

TABLE 2 Statistical summary of the basic eDNA sequencing data information for the different layers (0-72 cm) of the sediment core from Huokunao (HKA) station, as well as the surface sediments from Daleidao (DLD), Houjishan (HJS), Huokunao (HKA), and Mazuao (MZA) stations.

Type	Sample	RawPE	Qualified	Nochime	Effective%
Core sample (0-72cm)	0-2 cm	139073	135526	131760	0.9474
	10-12 cm	136614	127509	121009	0.8858
	20-22 cm	135054	125763	122101	0.9041
	30-32 cm	135000	126877	120398	0.8918
	40-42 cm	134275	121361	117816	0.8774
	50-52 cm	132951	123348	119494	0.8988
	60-62 cm	136736	128384	127088	0.9294
	70-72 cm	66191	62643	60977	0.9212
Surface sample (0-2cm)	DLD	133315	124976	116051	0.8705
	HJS	86287	80127	74200	0.8599
	HKA	113928	108986	106244	0.9326
	MZA	94537	89172	86193	0.9117

3.2 Chronological information

Based on ^{210}Pb dating, age data for different sediment layers, including 0-2 cm, 10-12 cm, 30-32 cm, and 78-80 cm layers, were obtained (Table 3). Using formula (1) for calculation, sedimentation rates at different layers were determined. The sedimentation rate between 30 and 80 cm layer was the highest, reaching 2.83 cm/year. The sedimentation rate for the 0-10 cm layer was the lowest at 1.19 cm/year. Additionally, the sedimentation rate for the 10-30 cm layer was 2.55 cm/year. The estimated ages of the sediment layers were as follows: the deepest layer dated to 1994, the 10-12 cm layer to 2014, and the 30-32 cm layer to 2010. Assuming constant sedimentation rates across different intervals, the ages of other layers of the core sample were calculated based on known depths: the 20-22 cm layer was estimated to be from 2012, the 50-52 cm layer from 2004, the 60-62 cm layer from 2000, and the 70-72 cm layer from 1997.

3.3 Microalgae community parameters

After comparing with the NCBI nt database, a total of 623,197 reads were annotated at the species level. The average number of reads for the different layers of the Huokunao sediment core was 45,882, which was lower than that of the surface sediment samples from DLD, HJS, HKA, and MZA (Table 4).

3.3.1 Surface sediment samples

Among the surface sediment samples collected from DLD, HJS, HKA, and MZA stations, the DLD station exhibited the highest read number, with 56,664 reads. Conversely, the HJS station recorded the lowest read number. However, the OTU count, Margalef index, and Shannon-Wiener index were highest at the HJS station, with values of 2,180, 175.63, and 7.12, respectively (Table 4). In contrast, these indices were lowest at the MZA station.

3.3.2 Sediment core samples (0 -72 cm)

For the Huokunao sediment core samples, microalgal read counts exhibited an increasing trend from deeper layers to surface layers. Both the OTU number and Margalef index of the microalgal community also showed a consistent upward trend, peaking in the surface layer samples—OTUs peaked at 1,813 in the 0-2 cm layer, while the Margalef index reached a peak of 133.54 in the 10-12 cm layer. The minimum values for both indices were observed in the 60-62 cm layer (Figure 2). The Shannon-Wiener index, though fluctuating overall, demonstrated an increasing trend as sediment depth decreased, with its minimum value of 1.80 recorded in the 40-42 cm layer.

3.4 Microalgae community composition

3.4.1 Surface sediment samples

The microalgal community compositions varied among the surface sediment samples from different stations. Bacillariophyta was the most abundant microalgae phylum at the DLD, HJS, and HKA stations, accounting for 67.62%, 55.39%, and 46.32% respectively, followed by Dinoflagellata and Chlorophyta. However, at the MZA station, Dinoflagellata emerged as the predominant group, accounting for 91.07%, followed by Streptophyta (Table 5). Comparisons with the NCBI nt database annotated a total of 552 species. Among these, *Alexandrium pacificum*, *Thalassiosira profunda*, *Odontella* sp. TN-2014, Bacillariophycidae sp. TN-2014, and *Navicula cryptocephala* each accounted for more than 5% of the reads and are considered dominant species (Table 6). Specifically, *A. pacificum* was the most abundant microalgae at the DLD, HJS, and MZA stations, while *N. cryptocephala* was the most abundant species in the surface sediment sample from the HKA station.

TABLE 3 Information on ^{210}Pb dating of intertidal sediment cores from Huokunao station.

Depth (cm)	^{210}Pb (Bq/kg)	^{226}Ra (Bq/kg)	Excess ^{210}Pb (Bq/kg)	Sedimentation rate (cm/a)	Year
0-2	23.71	60.18	36.47	/	2022
10-12	19.98	48.05	28.07	1.19	2014
30-32	20.72	46.01	25.29	2.55	2010
78-80	20.31	35.8	15.49	2.83	1994

3.4.2 Sediment core samples (0 -72 cm)

As detailed in Table 5, Bacillariophyta was the most annotated microalgal phylum, with 372,621 reads accounting for 56.50% of the total reads. This phylum peaked in the 10-12 cm layer of the Huokunao sediment core. The next two dominant groups were Dinoflagellata and Streptophyta, which accounted for 22.60% and 13.85%, respectively. Chlorophyta accounted for 6.80%, while Haptista and other minor microalgal phyla made up less than 1%. As sediment depth decreased, the read number of Bacillariophyta increased, reaching a maximum at the 10-12 cm layer, while the read number of other microalgal phyla decreased (Figure 3, left). However, the number of OTUs for both Bacillariophyta and other microalgal groups increased with decreasing sediment depth (Figure 3, right).

A. pacificum was the species with the highest number of reads, accounting for 10.57% of the total reads. In the Huokunao sediment core samples, *A. pacificum* reached its maximum read count in the 50-52 cm layer, significantly higher than in other layers. Reads of *T. profunda*, accounting for 9.17%, gradually increased with decreasing sediment depth, showing an upward trend. *Odontella* sp. TN-2014 and *N. cryptocephala* accounted for 7.68% and 5.14% of the reads respectively, reaching their maximum values in the surface 0-2 cm layer or the 10-12 cm layer.

4 Discussion

On September 30, 1990, the State Council of China officially approved the establishment of five marine nature reserves, including the Nanji Islands. Renowned for their rich biodiversity, particularly in mollusks and algae, the Nanji Islands have earned the nickname "Mollusk and Algae Kingdom." This exceptional biodiversity has attracted considerable attention from the scientific community. Researchers have conducted surveys on coralline algae (Tang et al., 2014), benthic diatoms (Li et al., 2017), and phytoplankton (Li et al., 2010) in the Nanji Islands, revealing the biodiversity characteristics from various perspectives and across different biological groups.

4.1 Changes in microalgal community of core sample (0-72 cm)

The average Shannon-Wiener index obtained in this study exceeds 5, indicating a high level of microalgal diversity. Since the establishment of the nature reserve, sporadic reports on microalgae investigations in the Nanji Islands have been published (Wang and Zhu, 1998; Li et al., 2017), making it challenging to study historical environment changes in the region through microalgae community.

TABLE 4 The changes in read number, OTU number, Margalef index, and Shannon-Wiener index for the different layers (0-72 cm) of the Huokunao (HKA) sediment core, as well as the surface sediments from Daleidao (DLD), Houjishan (HJS), Huokunao (HKA), and Mazuao (MZA) stations.

Type	Sample	reads	OTU	Margalef index	Shannon-Wiener index
Core sample (0-72cm)	0-2 cm	68704	1813	130.59	6.6
	10-12 cm	56897	1783	133.54	5.46
	20-22 cm	53232	1380	106.85	6.01
	30-32 cm	55206	1232	93.31	7.08
	40-42 cm	57404	947	69.07	1.8
	50-52 cm	55365	923	68.58	3.61
	60-62 cm	65143	495	33.75	4.48
	70-72 cm	27716	535	51.9	4.5
Surface sample (0-2cm)	DLD	56664	1934	141.88	5.5
	HJS	34435	2200	198.98	7.44
	HKA	55668	2180	175.63	7.12
	MZA	36763	739	64.76	3.67

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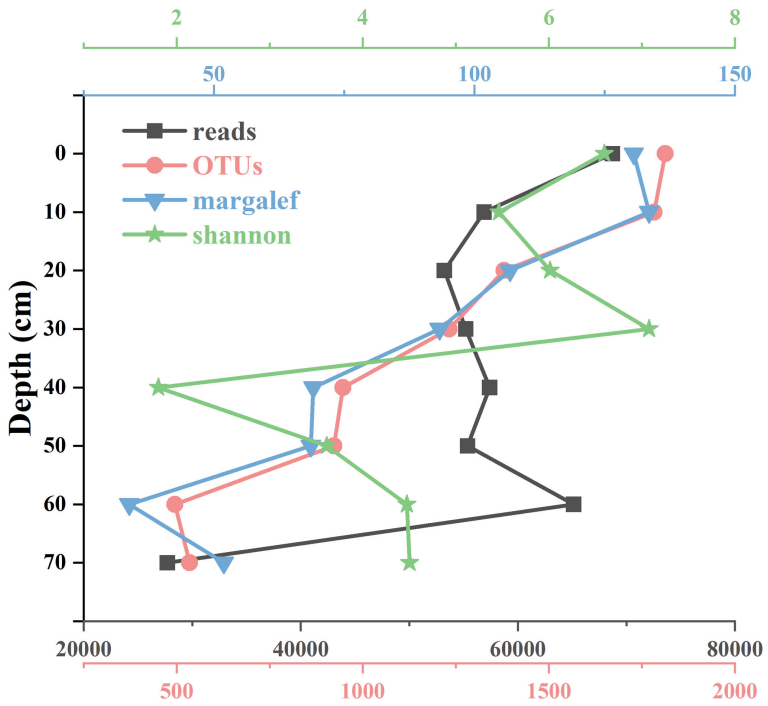

FIGURE 2
Microalgal community parameters at different depth layers of the Huokunao sediment core (0-72 cm), including the number of reads, number of OTUs, Margalef index, and Shannon-Wiener index.

The microalgal communities in Guoxingao, Dashaao, and Shangmaan of the Nanji Islands are primarily dominated by diatoms, accounting for more than 90% of the community (Wang and Zhu, 1998). Li et al. (2017) provided detailed accounts of the benthic diatom community in the intertidal zone of the Nanji Islands in 2013-2014 and compared these data with pre-reserve establishment surveys. They observed an increase in diatom diversity, with the number of families rising by 7, and significant increases in the number of genera and species—from 29 genera and 55 species to 49 genera and 120 species—indicating a rise in the diversity of diatom communities.

Previous studies primarily relied on morphological data, which limited the comparative analysis of changes in the molecular diversity of microalgae. In this study, we utilized ^{210}Pb dating to obtain age data for sediment layers at depths of 0-2 cm, 10-12 cm, 30-32 cm, and 78-80 cm (Table 1). By integrating sediment depth

TABLE 5 The read number of microalgae phyla in different layers (0-72 cm) of the Huokunao (HKA) sediment core, as well as the surface sediments from Daleidao (DLD), Houjishan (HJS), Huokunao (HKA), and Mazuao (MZA) stations.

Type	Sample	Bacillariophyta	Dinoflagellata	Streptophyta	Chlorophyta	Haptista	Other
Core sample (0-72cm)	0-2 cm	125845	3762	1029	1460	150	22
	10-12 cm	130285	3152	457	378	4	13
	20-22 cm	23803	23116	1077	1007	29	16
	30-32 cm	22428	6476	7170	6433	0	499
	40-42 cm	2282	1768	4434	855	10	10
	50-52 cm	1997	45730	1121	364	0	29
	60-62 cm	2709	1838	55610	10842	0	3
	70-72 cm	518	558	8660	6157	0	11
Surface sample (0-2cm)	DLD	25824	9926	461	1531	4	444
	HJS	12084	7184	317	1864	2	364
	HKA	23534	4890	8744	13545	19	76
	MZA	1312	40626	2254	409	0	9

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TABLE 6 Reads information of dominant microalgal species in different layers (0-72 cm) of the Huokunao (HKA) sediment core, as well as the surface sediments from Daleidao (DLD), Houjishan (HJS), Huokunao (HKA), and Mazuao (MZA) stations.

Type	Sample	*Alexandrium pacificum*	*Thalassiosira profunda*	*Odontella* sp. TN-2014	Bacillariophycidae sp. TN-2014	*Navicula cryptocephala*
Core sample (0-72cm)	0-2 cm	151	2852	13624	764	3657
	10-12 cm	348	17414	4426	2751	6746
	20-22 cm	1823	337	48	5771	155
	30-32 cm	413	195	101	5226	159
	40-42 cm	340	101	31	176	72
	50-52 cm	20420	83	24	210	72
	60-62 cm	165	43	21	44	26
	70-72 cm	85	24	18	38	19
Surface sample (0-2cm)	DLD	1201	949	260	491	687
	HJS	435	425	120	183	250
	HKA	224	247	331	320	872
	MZA	580	44	15	66	29

information, we estimated sedimentation ages, finding that the deepest sediment layer corresponded to 1994. The chronological information from the core samples was continuous, suggesting no significant disturbance occurred. From the 70-72 cm layer to the 60-62 cm layer, the number of microalgal reads increased rapidly from 27,716 to 65,143 (Figure 2). Although the number of eDNA reads cannot exactly correspond to the absolute abundance of organisms due to various factors (Barnes et al., 2014; Gardes and Bruns, 1993; Milivojević et al., 2021), the trend in read counts correlates with changes in abundance, indicating a potential increase during this period. Although definitive quantitative relationships between microalgae abundance and primary productivity are not well-established due to factors such as temperature (Grant, 1986), light intensity (Gómez et al., 2009), nutrition type (Zhang et al., 1998), many studies have reported a linkage between microalgae biomass (abundance) and primary productivity (Barranguet et al., 1998; Gilbert, 1991; Vuppaladadiyam et al., 2018; Chatterjee, 2014). Research indicates that peaks in microalgae primary productivity often coincide with peaks in biomass (Gilbert, 1991), and a positive correlation exists between biomass and primary production rates (Vuppaladadiyam et al., 2018). Therefore, the observed rapid increase in microalgae reads during this period indicates a rise in microalgae abundance, potentially linked to enhanced primary productivity over the subsequent 10 years following the establishment of the nature reserve. Furthermore, we observed a relatively slow growth in the number of reads during the 0-60 cm layer, suggesting a possible deceleration in microalgal productivity growth over the subsequent 20 years. However, it is important to consider that DNA degradation may introduce bias in the eDNA data analysis from core samples of varying ages, as the degree and proportion of eDNA degradation increase over time (Handt et al., 1994; Lindahl, 1993). In this study, DNA degradation was observed but did not significantly affect the results. There was no notable difference in the total raw sequences among the layers, except for the 70-72 cm layer (Table 2). The deeper layer (60-62 cm) exhibited the second highest read count of microalgae (Table 4). Changes in microalgal phyla and species did not appear to be influenced by DNA degradation (Figures 3, 4), likely due to the relatively short

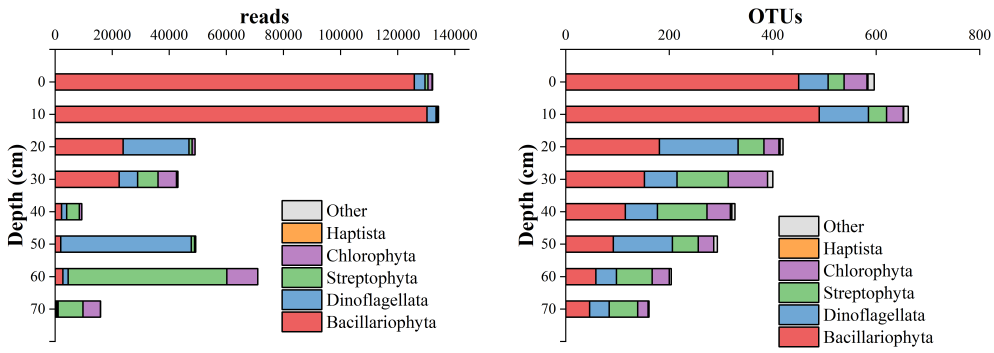

FIGURE 3
The changes in reads (left) and OTUs (right) of different microalgal phyla along the depth of the Huokunao sediment core.

and recent age of the core samples. Analyzing changes in different microalgae groups revealed that the increase in read numbers primarily stemmed from the Bacillariophyta group. Meanwhile, the ratios of Dinoflagellata and Streptophyta decreased, indicating a shift in the microalgae community from being dominated by Dinoflagellata and Streptophyta to predominantly Bacillariophyta following the establishment of the nature reserves (Figure 3). Zhou et al. (2017) studied the seasonal succession of Dinoflagellata and Bacillariophyta, finding that phosphate is the most critical factor for the decline of diatom blooms and the succession from diatom to dinoflagellate blooms, with increased temperatures favoring dinoflagellate growth. Although annual succession differs from seasonal patterns, the impact of phosphate remains significant. After the establishment of the nature reserve, reduced human activity likely contributed to a decline in terrigenous phosphates, which may explain the increase in Bacillariophyta. The Margalef index initially decreased but subsequently showed a stable upward trend, while the OTU numbers of groups such as Bacillariophyta, Dinoflagellata, Streptophyta, and Chlorophyta all increased (Figure 3). This indicates an improvement in the biodiversity of microalgae in the Nanji Islands, suggesting that the marine environment has benefited from the establishment of the protected area. This finding aligns with Li et al. (2017), who reported an increase in diatom taxa following the establishment of the protected area. Although no direct correlation between microalgae diversity and environmental quality changes has been reported, biodiversity loss is often linked to global climate change (Blois et al., 2010; Buisson et al., 2013; Thuiller et al., 2005). Thus, the establishment of nature reserves and the subsequent improvement of the local environment provide a reasonable explanation for the enhancement of local biodiversity.

4.2 Validation of red tides

Microalgae species annotations were obtained through comparisons with the NCBI nt database. Among the identified species, *Alexandrium pacificum* exhibited the highest read proportion, reaching 10.57%. This species belongs to the genus *Alexandrium*, many of whose members produce potent algal toxins, including paralytic shellfish toxins (PST) and spirolides. The massive proliferation of *Alexandrium* species pose serious threats to human health, ecosystems, and the mariculture industry (Anderson et al., 2012). Eutrophication and harmful algal blooms are significant marine environmental issues in China's coastal areas, especially near the Yangtze River Estuary (Zhou et al., 2008; Wang and Wu, 2009). *A. pacificum* is widely distributed in the Bohai Sea, Yellow Sea, and East China Sea (Dai et al., 2020; Gao et al., 2015). Significant blooms of this species have been reported in the South China Sea, coastal areas near the Yangtze River Estuary in the East China Sea, and the northern part of the Yellow Sea (Song et al., 2009; Wang et al., 2011). In this study, the read number of *A. pacificum* was exceptionally high in the 50-52 cm layer of the Huokunao sediment core, far exceeding its read numbers in other layers (Figure 4). Assuming a constant sedimentation rate, the sedimentation age of the 50-52 cm layer was estimated to be around the 2000s. Previous research has documented *Alexandrium* red tide events in China's coastal areas, noting seven *Alexandrium*-related red tide events in the Zhejiang region between 2002 and 2003, with a cumulative area exceeding 1000 km², including three events near Nanji Islands and surrounding waters in Wenzhou, Zhejiang (Liang et al., 2019). The outbreaks of *Alexandrium*-related red tides in the Nanji Islands and adjacent sea areas likely contributed to the accumulation of *Alexandrium* DNA in the sediment core, as reflected in the high read abundance at this sediment layer. This

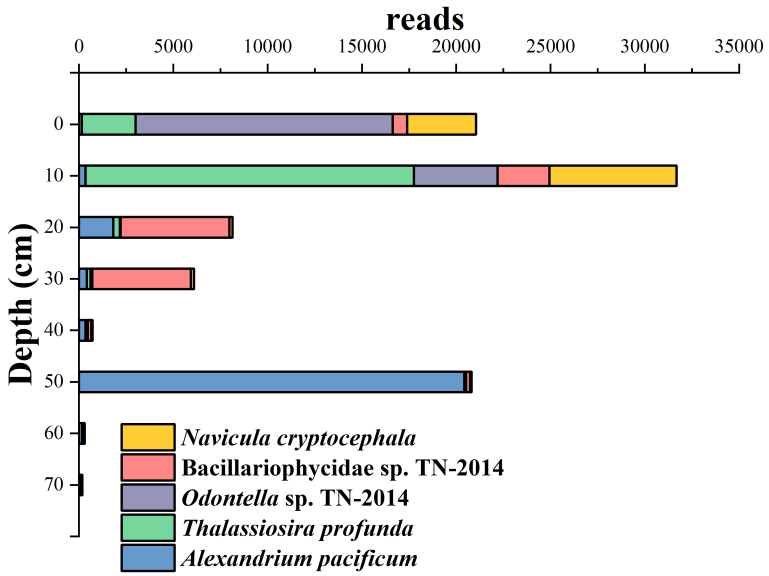

FIGURE 4
The stacked bar chart of the dominant microalgal species reads along the depth of the Huokunao sediment core.

confirms the validation role of microalgal eDNA in studying marine red tide events and other environmental changes.

4.3 Problems and reflections

The Huokunao intertidal area in Nanji islands was previously the site of the Nanji town government, resulting in relatively dense human activity in the past. Terrestrial pollutants, such as domestic sewage, frequently entered this marine environment. In this study, freshwater species like *Navicula cryptocephala* were detected. Similarly, *N. phylleptosoma*, *N. arenaria*, *N. cancellate*, and *N. perminuta* were identified as dominant species in this area in other studies (Li et al., 2017), indicating significant influence from terrigenous material input. Additionally, prior to the establishment of the protected area in 1990, a fish meal factory was built near Huokunao, with effluents from the factory affecting the area (Yang et al., 1994). Consequently, the Huokunao area has been significantly impacted by human activities, leading to a moderate degree of environmental pollution (Chen et al., 2016; Li et al., 2017). Frequent human activities may have led to a complex sedimentary environment, potentially disturbing the sedimentary sequence and complicating the acquisition of orderly sediment samples—this represents one potential sources of error in our dating study. Furthermore, the ^{210}Pb dating method used in this study assumes that measured excess ^{210}Pb activity increases with depth in sediment samples (Wan, 1997; Xia and Xue, 2004). However, historical human activities on Huokunao may have introduced pollution-related issues, leading to an excessive input of allochthonous ^{210}Pb, which could bias the dating results. Additionally, repeated tidal fluctuations in the Huokunao intertidal area may have caused disturbances in the sedimentary environment, affecting data accuracy. Regarding the eDNA research in this study, the use of high-throughput sequencing can be influenced by primer bias, leading to significant differences in the sequencing data (Pawlowski and Lecroq, 2010). Moreover, the target fragments obtained through high-throughput sequencing are generally relatively short, limiting the genetic information they contain (Ujiié and Ishitani, 2016). Currently, assessing the absolute information of species using metabarcoding or eDNA data remains challenging. Factors such as rDNA copy number (Milivojević et al., 2021), DNA degradation (Barnes et al., 2014), and experimental bias (Gardes and Bruns, 1993) may introduce further biases. The species annotation in this study was based on the NCBI database, which, while aiming to provide high-quality data, may vary due to the diversity of data sources. Some data may have undergone rigorous verification and quality control, while others may be less reliable. Additionally, the breadth and complexity of the bioinformatics field may result in missing or incomplete data for certain microalgae groups or species, limiting the application of eDNA technology in evaluating and researching microalgal molecular diversity. A specialized database for microalgae and plants is needed in the future.

5 Conclusion

This study collected sediment samples from stations such as Daleidao, Houjishan, Huokunao, and Mazuao in Nanji Island, employing eDNA technology combined with ^{210}Pb dating to reveal the historical changes in microalgal diversity in the Nanji islands region:

(1) The number of microalgal reads increased rapidly from the 70-72 cm layer to the 60-62 cm layer, indicating a significant rise in abundance during the 10 years following the establishment of the Nanji Islands Nature Reserve. This was followed by a slowdown in growth over the subsequent 20 years. (2) Simultaneously, the number of OTUs and the Margalef index increased, suggesting an improvement in microalgal diversity. The establishment of the protected area may have contributed to the enhanced environment and increased species diversity in the Nanji islands region. (3) The entire microalgal community underwent succession, transitioning from dominance by Dinoflagellata and Streptophyta to dominance by Bacillariophyta. This shift could be attributed to environmental changes, particularly related to phosphates.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: NCBI, PRJNA1136642.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

HL: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. AX: Writing – original draft, Methodology, Investigation, Data curation. ZY: Writing – original draft, Methodology, Investigation. YL: Writing – review & editing, Supervision, Project administration, Funding acquisition. JC: Writing – original draft, Methodology, Investigation. ZZ: Writing – original draft, Investigation. KX: Writing – original draft, Project administration. TL: Writing – review & editing, Funding acquisition. SX: Writing – original draft, Project administration.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1466434/full#supplementary-material

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