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Full length article

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ABSTRACT

The Anthropocene is characterized by dramatic ecosystem changes driven by human activities. The impact of these activities can be assessed by different geochemical and paleontological proxies. However, each of these proxies provides only a fragmentary insight into the effects of anthropogenic impacts. It is highly challenging to reconstruct, with a holistic view, the state of the ecosystems from the preindustrial period to the present day, covering all biological components, from prokaryotes to multicellular eukaryotes. Here, we used sedimentary ancient DNA (sedaDNA) archives encompassing all trophic levels of biodiversity to reconstruct the two century-natural history in Bagnoli-Coroglio (Gulf of Pozzuoli, Tyrrhenian Sea), one of the most polluted marine-coastal sites in Europe. The site was characterized by seagrass meadows and high eukaryotic diversity until the beginning of the 20th century. Then, the ecosystem completely changed, with seagrasses and associated fauna as well as diverse groups of planktonic and benthic protists being replaced by low diversity biota dominated by dinophyceans and infaunal metazoan species. The sedaDNA analysis revealed a five-phase evolution of the area,

where changes appear as the result of a multi-level cascade effect of impacts associated with industrial activities, urbanization, water circulation and land-use changes. The *sedaDNA* allowed to infer reference conditions that must be considered when restoration actions are to be implemented.

1. Introduction

The increasing human impacts and the concurrent threat on the environmental resources at both global (i.e., climate change) and local (e.g., pollution, overexploitation) scales have led to defining the current Epoch as the Anthropocene (Lewis and Maslin, 2015). The deterioration of environmental conditions is widespread in marine environments, particularly in coastal ecosystems that represent the main global economic assets in terms of ecological services (Costanza et al., 1997; Keyes et al., 2021). The impact on coastal marine ecosystems has pervasive consequences by leading to species extinction (Barnosky et al., 2011; Dirzo et al., 2014), impairing the ecological functions (Obura et al., 2021), and losing ecosystem goods (Worm et al., 2006). In the light of it, the monitoring of marine biodiversity changes at different spatio-temporal scales is becoming a priority tool for planning conservation strategies at the international level (Dornelas et al., 2014; Danovaro et al., 2020).

The emerging omics technologies (e.g., metagenomics) have recently offered a new perspective for understanding the impact of anthropogenic activities on biodiversity (Carraro et al., 2020). The environmental DNA (eDNA) has revolutionized the detection of species and found applications in biodiversity assessment and biomonitoring (Kelly et al., 2014; Cordier et al., 2021). Specifically, the application of eDNA metabarcoding improves the capability to simultaneously capture various biological components encompassing multiple trophic levels (Djurhuus et al., 2020). Indeed, the analysis of ancient eDNA preserved in sediment (*sedaDNA*) enables the reconstruction of the present-day to past biological communities and paleo-environmental conditions (Pedersen et al., 2015; Brown and Blois, 2016; Capo et al., 2021). In lakes sediments, prokaryote communities (cyanobacteria) were targeted to evaluate temperature rise and eutrophication effects (Monchamp et al., 2017), while changes in the composition of micro-eukaryotic communities allowed to follow anthropogenic impacts in Alpine lakes (Keck et al., 2020). Until now, only few studies have been conducted on sediment cores collected in marine coastal environments. Siano et al., (2021) showed anthropogenic effects around the Bay of Brest in the last 5000 years based on microbial eukaryotes community, while Armbrecht et al., (2022) targeted phytoplankton to track harmful algal blooms over ∼ 9000 years off the East Australian coast. These studies allow for the comprehension of the effects of human activities on biodiversity over time and the definition of reference conditions (e.g., baseline or pre-industrial). However, both studies focused on a single marker that altogether prevents a holistic comprehension of community shifts or diversity changes and hampers the observation of their potential interactions.

In this work, we investigated changes in biodiversity and community composition spanning from prokaryotes to multicellular organisms, through a multi-markers metabarcoding approach. Our *sedaDNA* multimarker data consistently reveal a five-phase variation of the paleocommunity that perfectly matches with the anthropogenically-induced environmental changes as supported by the historical archives and geochronology in one of the most polluted European (Armiento et al., 2022; Sprovieri et al., 2020), likely worldwide, marine sites of Bagnoli-Coroglio (Italy; Supplementary Fig. 1). The bay and hinterland area of Bagnoli-Coroglio have a long-lasting legacy of environmental impacts emerging from a complex interplay of anthropogenic activities (i.e., urbanization, industry, polluted-sediment disposal, land-use, coastline, and water-circulation changes) (Bertocci et al., 2019). The first industrial activities started to develop in the first decades of 1900 and reached their production peak in the late 1960 s-early 1970 s (Cavaliere et al.,

2021). The site hosted the second-largest Italian steel factory (Ilva/Italsider), active till the early '90 s (Trifuoggi et al., 2017), as well as asbestos (Eternit) and concrete (Cementir) factories. Due to the concentrations and hazardousness of pollutants, and their impacts on the ecosystem and human health, the area was declared a contaminated Site of National Interest (SIN) in 2000 (Law 388/2000). Today, Bagnoli-Coroglio is a large brownfield area, with marine sediments and benthic assemblages still intensively impacted by metals and polycyclic aromatic hydrocarbons (PAHs) (Ausili et al., 2020; Gambi et al., 2020; Tangherlini et al., 2020).

2. Methods

2.1. Core collection and processing

The AB01 sediment core (110 cm in length) was collected at 55 m water depth in the Bay of Bagnoli (Gulf of Pozzuoli, Tyrrhenian Sea; 40° 48.150′ N, 14° 08.913′ E) (Supplementary Fig. 1) on December 5th, 2018, with an SW-104 corer Carma®, equipped with a liner of 10.4 cm in diameter. After collection, the core was transported to the laboratory, sliced through extruder equipment at 1 cm resolution discarding the outermost centimeter in contact with the liner, to avoid smearing effects downcore. Sediment layers were sliced using sterile spatulas, photographed, weighed and the color was scored following Munsell Soil Chart. For the first 41 cm and starting from the top layer (0–1 cm = layer #1), odd layers were used for grain size and chemical analyses, while even layers were used for biological analyses and organic matter (OM) characterization (Supplementary Table 1). Samples for eDNA metabarcoding were collected by cutting the central portion of each layer with a disposable, sterile petri dish (diameter 5 cm) and placed in 5 ml cryovials (two biological replicates for eukaryotes and two replicates for prokaryotes), frozen in liquid nitrogen and stored at −80 °C.

2.2. Grain-size analyses

Each core sediment sub-sample (odd layers) was washed with deionized water, then disaggregated using 1 % sodium metaphosphate, and analyzed by a laser particle size analyzer (Helos KF SympaTEC). Grain-size results from the top core to 40–41 cm sediment layer are reported as a volume percentage of sand and mud (as silt and clay fractions) against the correspondent age, accordingly to the sediment core dating described below.

2.3. Radiometric analyses and geochronology

Gamma spectrometry analyses were performed on 20 g geometry of slightly ground sediment samples to determine ^{210}Pb and ^{226}Ra activities. Calibration, quality checks, and measurement procedures have been described in Delbono et al. (2016). The excess ^{210}Pb ($^{210}Pb_{ex}$) activity was calculated as the difference between the total ^{210}Pb and the fraction in equilibrium with the parent radionuclide ^{226}Ra. Mass depth (g cm^{-2}) was used to account for the compaction of the sediment layers. The Constant Rate of Supply (CRS) model (Appleby and Oldfield, 1978) was applied to determine the age of each layer of the sediment core, assuming a CRS of unsupported ^{210}Pb. The ^{210}Pb dating was validated and extended over several centuries using information taken from ^{226}Ra activities. In fact, the ejected sediments by Vesuvius volcanic activities are marked by a high ^{226}Ra concentration (Voltaggio et al., 2004), and its recent historical activity (1631–1944) is well-known and continuous (Scandone et al., 2008) giving a series of well-recognized time-markers:

in the AB01 sediment core, ^{226}Ra peaks due to Vesuvius eruptions in 1944, 1906 and 1822 were identified (Armiento et al., 2022).

2.4. Chemical analyses

Sediment samples were transferred to the laboratory in polyethylene high-density (PEHD) containers at a temperature of 4 °C, where the phases of pre-treatment and analysis for metals and PAHs were performed. A detailed description of analytical procedures is reported in Armiento et al. (2020). Briefly, after extraction by microwave-assisted acid digestion of the samples, metals (Cd, Cu, Cr, Hg, Pb, and Zn) analytical determinations were carried out, according to the EPA 6020b method, by inductively coupled plasma-mass spectrometry (ICP-MS, Agilent 7800) except for Hg that was analyzed by atomic absorption spectrometer (AMA-254) according to EPA 7473 method. PAHs were extracted by an Accelerated Solvent Extractor (ASE 200 Dionex) and the analytical determination was carried out according to EPA 8270D method with the Agilent 7890A-5975C GC–MS system.

2.5. Organic matter

Sediment samples were dried in an oven (60 °C) and subsequently ground and homogenized. Sub-samples were then weighed (~10–20 mg) in silver capsules and treated with increasing HCl concentration solutions (v/v 8 %, 18 %, and 25 %) to remove the inorganic carbon fraction (carbonates) completely. Total organic carbon (TOC) and total nitrogen (TN) were analyzed using a Thermo Electron Flash Elemental Analyzer (EA 1112).

2.6. Prokaryotic metabarcoding

Total DNA was extracted in two replicates from 1 g of selected sediment layer (i.e., 1–2, 5–6, 9–10, 13–14, 17–18, 25–26, 27–28, 53–54) by using the DNeasy PowerSoil Kit (QIAGEN©, Düsseldorf, Germany) following manufacturer's instructions. The amount of DNA isolated was estimated by the absorbance at 260 nm and the purity by 260/280 and 260/230 nm ratios, by a NanoDrop spectrophotometer (ND-1000 UV–vis Spectrophotometer; NanoDrop Technologies, Wilmington, DE, USA). The amplification of 16S rRNA V4-V5 regions (~410 bp) was performed using 515F-Y forward and 926R reverse primers (Parada et al., 2016). Libraries were prepared from each sample by pooling three PCR replicates and sequencing was performed with all libraries on a single Illumina MiSeq flow cell by LGC Genomics GmbH (Berlin, Germany).

The raw reads were filtered low-quality base calls (<30 Phred score) using the QIIME2 (Bolyen et al., 2019). Primer sequences were discarded using Cutadapt (Martin, 2011) using default parameters. Sequence reads were error-corrected, merged and amplicon sequence variants (ASVs) were identified using DADA2 with –trunc-len-f 272 and –p-trunc-len-r 192 (Callahan et al., 2016). ASVs were taxonomically classified using VSEARCH (Rognes et al., 2016) against the SILVA database (release 138) created trimming to the amplified region. Taxonomic information was used to remove eukaryotic, chloroplast and mitochondrial-related sequences before subsequent analyses. To reduce analytical biases due to different sequencing depths among samples, the ASV table was randomly subsampled to the same number of sequences (13600). This normalized table was subsequently converted to a phyloseq class object in R for further analysis (McMurdie and Holmes, 2013). The same dataset was utilized to construct a table within the microeco package (Liu et al., 2021), which was then used as an input for a LefSe analysis (Segata et al., 2011) which allows assessing the differences in assemblage composition across the historical periods identified. The raw data is available from the Sequence Read Archive public database under the accession number: PRJNA822883.

2.7. Eukaryotes, foraminiferal and metazoan metabarcoding

For each sediment layer, 5 g of sediment was extracted using the DNeasy® PowerMax® Soil kit (QIAGEN©, Düsseldorf, Germany) following the manufacturer's instructions. All eDNA extractions and manipulations were performed to avoid cross-contamination between samples and contamination by modern eDNA. Three genetic markers, commonly used in eukaryotic biodiversity surveys (Frontalini et al., 2020; Lanzén et al., 2021; Pawlowski et al., 2021), were used to obtain a wide range of marine organisms. Eukaryotes, foraminifera, and metazoans were targeted by amplifying the V9 region of 18S rRNA (~150 bp) with 1389F − 1510R primers (Amaral-Zettler et al., 2009), 37F region of 18S rRNA (~190 bp) with s14F1- s15 primers (Pawlowski and Lecroq, 2010), and mitochondrial cytochrome oxidase 1 gene (~390 bp) with mlCOIintF and dgHCO2198 primers (Leray et al., 2013), respectively. Eight nucleotides were attached to the 5′ extremity of primers to allow the multiplexing of samples (Esling et al., 2015). Primer sequences and PCR programs are detailed in Supplementary Table 2. Per sediment sample, three replicates and one control (blank) were performed and checked on 1.5 % agarose gels. The triplicates were merged and the amount of DNA was quantified for the pool and its PCR control using high-resolution capillary electrophoresis with the QIAxcel system (QIAGEN©, Düsseldorf, Germany). The PCR products were equimolarly pooled into one 1.5 ml tube per marker and were purified using the High Pure PCR Product Purification kit (Roche Molecular Systems©, Basel, Switzerland). The final DNA concentration of pools was quantified using QubitTM HS dsDNA (Invitrogen, Thermo Fisher Scientific, Massachusetts, U.S.) kit. The libraries were prepared using the TruSeq® DNA PCR-Free Library Preparation Kit (Illumina©, California, U.S.) and then quantified by qPCR using the Kapa library quantification kit (Kapa Biosystems, Massachusetts, U.S.) for Illumina platform. Paired-end sequencing was performed on a MiSeq instrument (Illumina©, California, U.S.). We used the v2 kit with 300 cycles for V9 and foraminiferal amplicons and the v3 with 500 cycles for COI amplicons. The bioinformatic analysis was performed using SLIM software (Dufresne et al., 2019). The raw sequencing data were demultiplexed with the module DTD. We performed DADA2 (Callahan et al., 2016) with the pseudo-pool option to filter the low-quality sequences, trim the primers, merge the paired-end reads and remove the chimeras. This DADA2 module produced ASV sequences and count tables. We applied a LULU (Frøslev et al., 2017) curation on all markers datasets to filter PCR and sequencing errors or intra-individual variability as recommended in (Brandt et al., 2021). Taxonomic assignments for the representative ASV-LULU V9 sequences were performed using standalone blast in the blast + suite (Camacho et al., 2009) against PR2 database version 4.14.0 (Guillou et al., 2013) integrated with some private references sequences from diatoms strains. Assignments with a similarity of < 90 % and query coverage > 90 bp were removed and assignments at the species level were manually checked. Taxonomic assignments for ASV-LULU foraminiferal sequences were done using VSEARCH (Rognes et al., 2016) at 95 % similarity against our local database. The ASV-LULU COI sequences were taxonomically assigned using blast + against the NCBI database with 95 % similarity and a query coverage of > 230 bp. The raw data from foraminifera, COI and V9 sequencing are available on the Sequence Read Archive public database under the accession number: PRJNA824838.

2.8. Benthic foraminifera morphology

Benthic foraminifera were analyzed from 20 samples in the sediment fraction > 125 μm, where possible at least 300 specimens were picked. The taxonomical identification largely followed Cimerman and Langer (1991), Sgarella and Zei (1993) and Milker and Schmiedl (2012).

2.9. Ecological indices

2.9.1. Metazoan gAMBI and Foram-AMBI indices

For marine macrofauna, we applied gAMBI index, an adaptation based on the genetic metazoan data of the AZTI's Marine Biotic Index (AMBI; Borja et al., 2000). The gAMBI values were calculated using AMBI Index Software v6.0 (Borja and Muxika, 2005) where a list of macrofaunal taxa with their ecological values is included (https://ambi. azti.es; Species List v. Dec2020).

To evaluate the Paleo-EcoQS based on benthic foraminifera, the Foram-AMBI (Jorissen et al., 2018), an adaptation of the AMBI macrofauna (Borja et al., 2000) was calculated.

2.9.2. MicrogAMBI and prokaryotes indices

Based on the prokaryotic taxa encountered in the different sediment layers, the microgAMBI was calculated following Aylagas et al. (2017). Putative indicator taxa of the ecological quality were identified based on their relationships with the pollution index as the probability of toxicity m-ERM-q (Kowalska et al., 2018; Long et al., 2000) by linear model regression analysis (lm) performed in R (R Core Team, 2013).

2.10. Statistics

For each group, the Shannon Weiner (H') alpha diversity was calculated. Taxa relative abundances were used to group layers (i.e., years) by using a constrained hierarchical clustering analysis (HCA) along the core depth. A similarity tree was produced using the Euclidian distance. Coniss (Grimm, 1987) was used as the clustering method. The analysis was performed using the package vegan (Oksanen et al., 2007) and rioja (Juggins, 2020) in RStudio. A Spearman's Rho correlation was used to identify significant relationships between environmental variables (i.e., PLI, PAHs, TOC, C/N, TN, Mud) and the diversity of the main taxonomical groups. A biological and environmental (BIO-ENV) analyses (Clarke and Ainsworth, 1993) were performed to detect the best subset of environmental variables with maximum (rank) correlation with morphological and molecular groups. This procedure finds the correlation (Spearman's Rho) between community dissimilarities (based on Bray-Curtis matrix) and environmental distances (Euclidian matrix), and for each size of subsets provides the best result. Canonical Correspondence Analyses (CCAs) were performed to observe the relationships between the environmental parameters and the assemblage turnover of main taxonomical groups. Data analyses were performed using the R software (3.6.3) and following packages: "vegan", (Oksanen et al., 2007), "PerformanceAnalytics" (Peterson et al., 2014) and "lattice",
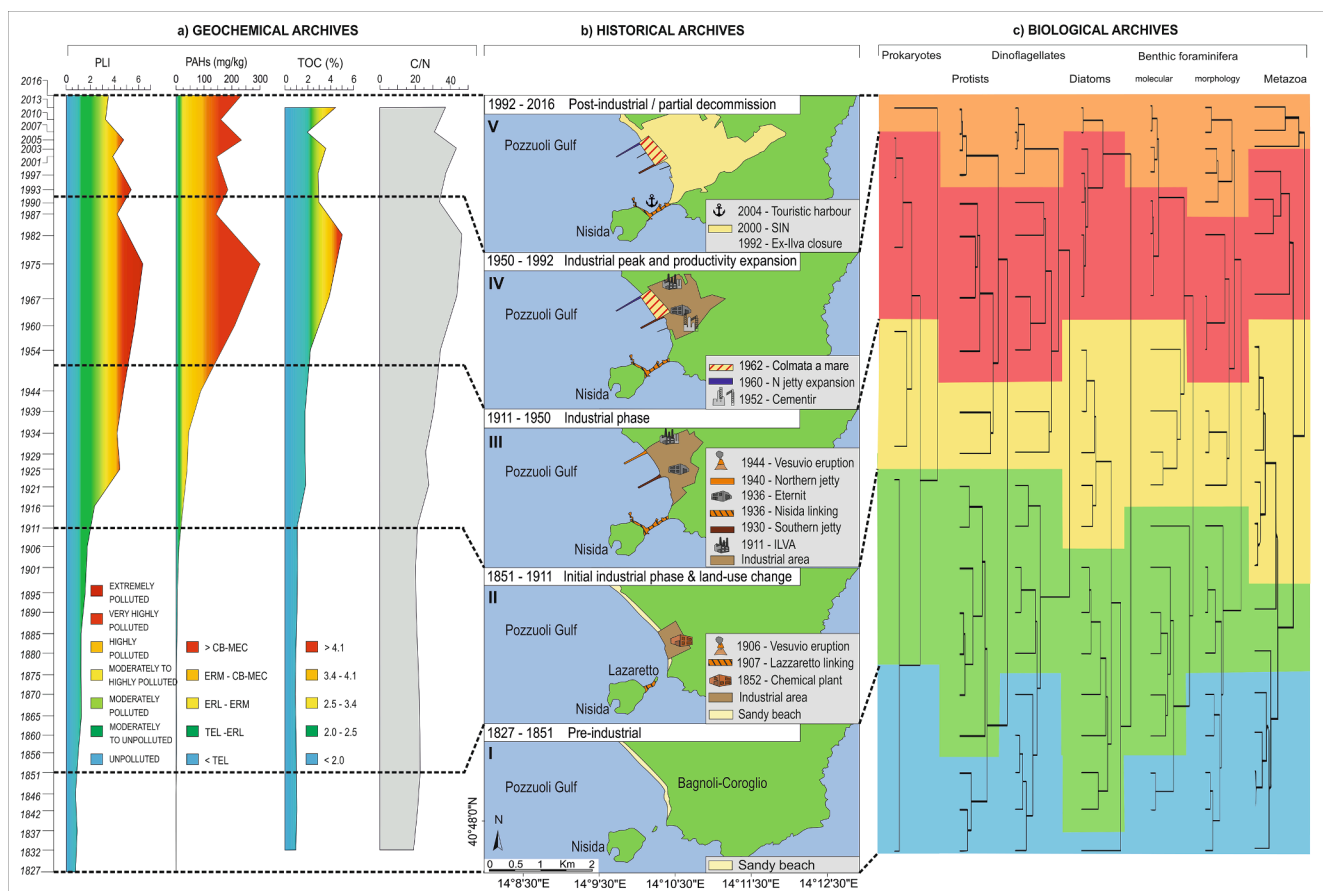
Fig. 1. Geochemical, historical, and biological archives. Plot of selected geochemical proxies (PLI: Pollution Load Index; PAHs: Polycyclic Aromatic Hydrocarbons, TOC: Total Organic Carbon; C/N: carbon–nitrogen ratio). Classification of PLI after (Zhang et al., 2011) with modification, sediment quality guidelines for PAHs (TEL: threshold effect level; ERL: effects range low; ERM: effect range median; CB-MEC: Consensus Based - midrange effect concentration) after (Burton and Burton, 2002) and TOC after (Bouchet et al., 2018). Historical archives with significant human-induced changes and activities. Hierarchical Cluster Analyses (HCAs) based on prokaryotes, protists, dinoflagellates, diatoms, benthic foraminifera (molecular and morphological) and metazoa. The colour-shaded areas over HCAs denote the stepwise temporal phases along the AB01 core in the Bagnoli-Coroglio SIN (Gulf of Pozzuoli, Tyrrhenian Sea), namely I: Pre-industrial (1827–1851; blue), II: Initial industrial phase & land-use change (1851–1911; green), III: Industrial phase (1911–1950; yellow), IV: Industrial peak and productivity expansion (1950–1992; red) and V: Post-industrial/partial decommission (1992–2016; orange). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Sarkar, 2008).

3. Results

3.1. The geochemical signature of the pollution history at the Bagnoli-Coroglio SIN

The analysis of the AB01 sediment core provides a high-resolution (4.6 yr cm^{-1}), continuous, complete, and well-constrained (i.e., ^{210}Pb and ^{226}Ra) record for the reconstruction of paleo-environmental changes in the Bagnoli-Coroglio SIN in the last ~ 190 years (Fig. 1 and Supplementary Table 1) (Armiento et al., 2022).

The geochemical profiles highlight a progressive increase in the concentrations of inorganic (i.e., trace elements as underlined by the Pollution Load Index (PLI) and organic (i.e., Polycyclic Aromatic Hydrocarbons: PAHs) pollutants as well as in the quantity (i.e., TOC) and quality (i.e., C/N) of the OM at the very beginning of the 20th century (i. e., 1901) and a marked peak in the period 1954–1992 (Fig. 1, Supplementary Tables 3–4). The PLI record reveals moderately polluted conditions since 1921 that turns from highly to extremely highly polluted in 1944 up to 1990. A similar pattern is observed in the total concentration of PAHs whose origin is mostly pyrogenic (i.e., Anthracene to Anthracene plus Phenanthrene > 0.1, Supplementary Fig. 2).

The TOC trend shows a progressive increase since 1916, with a peak between 1954 and 1992. Based on the C/N values that are persistently > 19, a terrigenous origin of the OM has been inferred (Meyers, 1994). However, the significant increase of C/N in the 1960–1987 interval suggests a higher input of carbon of terrestrial origin, possibly resulting from the "*Colmata a Mare*" that corresponds to the disposal of contaminated soil between the two piers in 1962–1967 to enlarge the industrial area and/or from contamination by hydrocarbons (i.e., PAHs with a highly unbalanced C/N ratio).

3.2. Community composition shifts from past to present

The HCAs based on community changes in multimarker *sedaDNA* datasets and foraminiferal morphospecies consistently identified five main groups corresponding to different time intervals related to the development and exploitation of the area based on historical archives (Fig. 1). These stepwise temporal phases were defined as pre-industrial (1827–1851), initial industrial phase and land-use change (1851–1911), developing industrial phase (1911–1950), maximal industrial expansion (1950–1992), and post-industrial/partial decommission (1992–2016) (Fig. 1). These findings were supported by the CCAs analyses. They showed that layers deposited before 1911 (i.e., the pre-industrial and the beginning of industrialisation phases) were mostly associated to mud and lowest contaminations (i.e., negatively correlated to PLI, PAHs and TOC). In contrast, layers deposited during the industrial peak and the post-industrial phases were related to the highest values of PLI, PAHs, TN, TOC, and C/N. The detailed analysis of community changes for different groups along the vertical profile of the sediment is presented below.

The prokaryotic metabarcoding analysis showed that the ASVs belonging to *Bathyarchaeia* accounted on average for 13.5 % of the prokaryotic assemblage, followed by *Anaerolineaceae* (on average, 7.9 %), and *Aminicenantales* (on average 4.8 %). However, the relative abundance of the prokaryotic taxa changed in relation to the analyzed sediment layers corresponding to different historical periods (Supplementary Fig. 3). In particular, LefSe analysis showed that several taxa (considering all taxonomic levels from phylum to species) were associated with one or more temporal phases (Supplementary Fig. 4a); at the Family level, we found that groups such as Aminicenantales, Woesebacteria, Thermoflexaceae were associated with early stages of industrialization (with Aminicenantales representing ca. 6 % of the assemblages at pre-industrial and initial industrial phases), while others, like Thermoanaerobaculaceae (ca. 4 %), Flavobacteriaceae (2 %),

Woeseiaceae (2 %), Rhodobacteriaceae, Cyclobacteriaceae, Deslfobulbaceae, were more represented in the post-industrial phase (Supplementary Fig. 4b).

The eukaryotic metabarcoding data revealed clear temporal faunal turnover when considering the major groups of multi- and unicellular organisms (Fig. 2). The eukaryotic assemblage was dominated by seagrasses (*Posidonia oceanica*) in the oldest layers (1832–1851). A clear DNA signature of *P. oceanica* persisted until the beginning of the industrial phase (up to 1911). A similar temporal trend was also detected for terrestrial plants, whose signature almost disappeared after 1911 (Fig. 2). The plant DNA signatures were progressively replaced by those of microbial eukaryotes (protists) from 1860 to 2003 and metazoans from 2007 to 2013. The CCA analysis showed that before 1911 (i.e., the pre-industrial and the beginning of industrialisation phases), the layers clustered and were related with mud, whereas layers from the industrial peak and the post-industrial phases formed a group that was mainly related to PLI, PAHs, TN, TOC, and C/N (Supplementary Fig. 5a). The BIO-ENV analysis identified PLI, PAHs and mud as the environmental factors affecting the most the eukaryotes communities and PLI and mud for seagrasses and terrestrial plants communities (Supplementary Table 5).

The dramatic shift in the paleo-community composition observed at the beginning of the industrial period (i.e., after 1911) was also detected within protists (Fig. 2). The CCA analysis revealed a separation of layers before, during and after industrialisation (Supplementary Fig. 5b). Like other eukaryotes, protists before the industrial period were more influenced by mud, whereas after this period strongly by PLI, PAHs, TN, TOC, and C/N. The protist diversity showed a strong negative correlation with these environmental parameters (Supplementary Fig. 6). Indeed, among them, PLI and PAHs were revealed to be the most important parameters in explaining these community changes (Supplementary Table 5). Before 1911, the protist assemblage was highly diversified, represented by free-living (Dinophyceae) and parasitic (Syndiniales) dinoflagellates, Apicomplexa, ciliates, diatoms, and Rhizaria (Radiolaria, Foraminifera, and Cercozoa). A remarkable decrease in the abundance of cercozoans and radiolarians (dominated by planktonic spumellarians and nassellarians), as well as Apicomplexa, was observed after 1911 (Fig. 2). From 1921, the protist community was dominated by Dinophyceae, mainly including genera known to produce resting stages (e.g., *Protoperidinium*, *Scrippsiella* and other taxa belonging to the family Thoracosphaeraceae, *Gymnodinium*) (Supplementary Fig. 7). Among other groups, diatoms represented a rather constant percentage of the protist assemblage. They were markedly dominated by planktonic taxa that produce resting stages, among which the genus *Chaetoceros* was retrieved all along the core, while *Biddulphia*, *Skeletonema* and *Thalassiosira* became abundant after the main industrialization (i.e., after 1954) (Supplementary Fig. 8). From 1967, an increasing abundance of ciliates was also detected. The CCAs based on dinoflagellates and diatoms provided a clear separation of layers in pre- and industrial periods (Supplementary Fig. 5c-d). During the industrialization phase and after, dinoflagellates and diatoms showed an opposite relationship with environmental variables and diversity. The diversity of dinoflagellates was negatively correlated with PLI, TN and mud, whereas the diversity of diatoms was positively correlated with PLI, PAH, TOC, TN, C/N and negatively with mud (Supplementary Fig. 6). The BIO-ENV analysis revealed that PLI and PAH were amongst the most important parameters shaping the dinoflagellate and diatom assemblages. Mud was also a very influential factor for diatoms (Supplementary Table 5).

A community composition shift after 1911 (beginning of industrial phase) was also observed in benthic foraminifera analyzed with both molecular and morphological approaches (Fig. 2). Before 1911, the foraminiferal assemblages inferred from *sedaDNA* data were dominated by monothalamids and representatives of taxonomically non-identified environmental clades. In 1911, a small number of reads corresponding mainly to one ASV was identified. Since 1967, buliminids and other calcareous globothalaminids were continuously present. In the
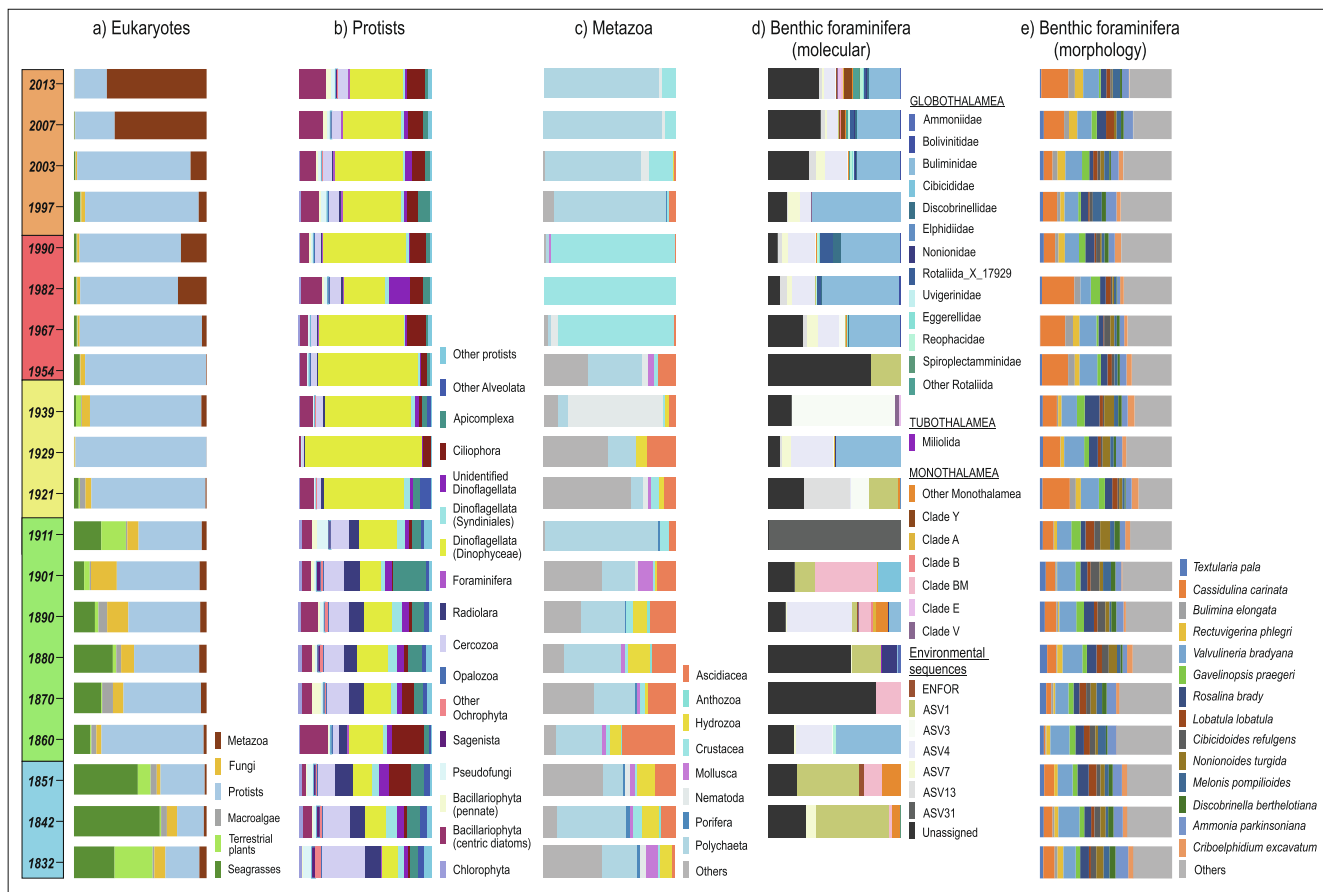
Fig. 2. Paleo-community composition shift. Eukaryote, protists, metazoa, and benthic foraminifera (molecular and morphological) composition changes along the AB01 core in the Bagnoli-Coroglio SIN (Gulf of Pozzuoli, Tyrrhenian Sea) inferred from multimarker *sedaDNA* data from 1827 to 2013. The different colours over the ages refer to the main phases (I: blue; II: green; III: yellow; IV: red and V: orange) as identified in Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

morphological dataset, the most abundant species were *Valvulineria bradyana, Cassidulina carinata, Rosalina bradyi, Ammonia parkinsoniana, Gavelinopsis praegeri,* and *Lobatula lobatula*. The abundance of *C. carinata* showed a marked increase between 1911 and 1921 (from 8 to 20 %) that was associated with a lower abundance of *A. parkinsoniana* (1880–1997), *Textularia pala* (1911–1990), *L. lobatula* (1921–2001), *Cibicides refulgens* (1901–2013) and *Nonionella turgida* (1954–2013). *Bulimina elongata* exhibited a significant increase after 1954 (Fig. 2, Supplementary Table 5). There is a difference between pre and industrial layers in CCA analysis in molecular and morphology foraminiferal assemblages (Supplementary Fig. S5e-f). On overall, the environmental factors that influenced the most foraminifera were PLI (molecular data) and TOC (morphology data) (Supplementary Table 5). The diversity of benthic foraminifera was negatively correlated to PLI, PAHs, TN, TOC, and C/N for the morphological dataset, whereas an opposite trend was found in the molecular one (Supplementary Fig. 6).

Similar changes were also observed in the metazoan community, although their timing was slightly different. Older layers corresponding to pre-industrial and initial phases clustered together and were positively correlated with mud, whereas layers from post- and industrial peak tended to be negatively related with PLI, PAHs TN, TOC, and C/N (Supplementary Fig. 5g), also supported by the correlation analysis (Supplementary Fig. 6). The BIO-ENV analysis identified PAHs, mud and TOC as the most important parameters influencing the metazoan communities changes (Supplementary Table 5). Encrusting metazoans (ascidians, sponges) were constantly observed until 1911. After 1911, the encrusting and colonial ascidians were replaced by epibenthic and solitarian ascidiaceans that also disappeared after 1954 (Fig. 2). The

sequences of mainly planktonic Hydrozoa decreased throughout the core and almost disappeared in 1939. The most recent layers (i.e., 1997–2013) were mainly dominated by burial polychaetes (Annelida), whereas Crustacea were dominant in the phase of heavy industrialization (1967–1990).

3.3. Changes in diversity and ecological indices

The faunal turnovers were accompanied by changes in alpha-diversity (H') and ecological indices (Fig. 3 and Supplementary Table 6). From 1832 to 1921, relatively higher diversity values were identified for protists and dinoflagellates, and morphospecies of foraminifera. A similar trend between 1954 and 1967 was observed for metazoan diversity, while the opposite was instead found for prokaryotes, diatoms, and foraminifera. *Posidonia oceanica* meadows were consistently identified from 1832 to 1911 when it substantially disappeared along with terrestrial plants. This dramatic event was associated with a clear shift in foraminiferal assemblage dominated by epiphytic species and replaced by infaunal ones after 1911 (Fig. 2 and Supplementary Table 6). Encrusting metazoans followed a similar trend showing higher percentages in the pre-industrial and partly in the early industrial phases, except in 1939.

The microgAMBI values increased progressively from the oldest sediment layer to the recent ones with the highest values (3.5) corresponding to the period from 1967 to 1990 and then slightly decreased. These values allowed us to classify ecological quality status (EcoQS) conditions from good/moderate in 1901 to mostly poor in 1967–1990 (Fig. 3 and Supplementary Table 6). A similar trend was found for the
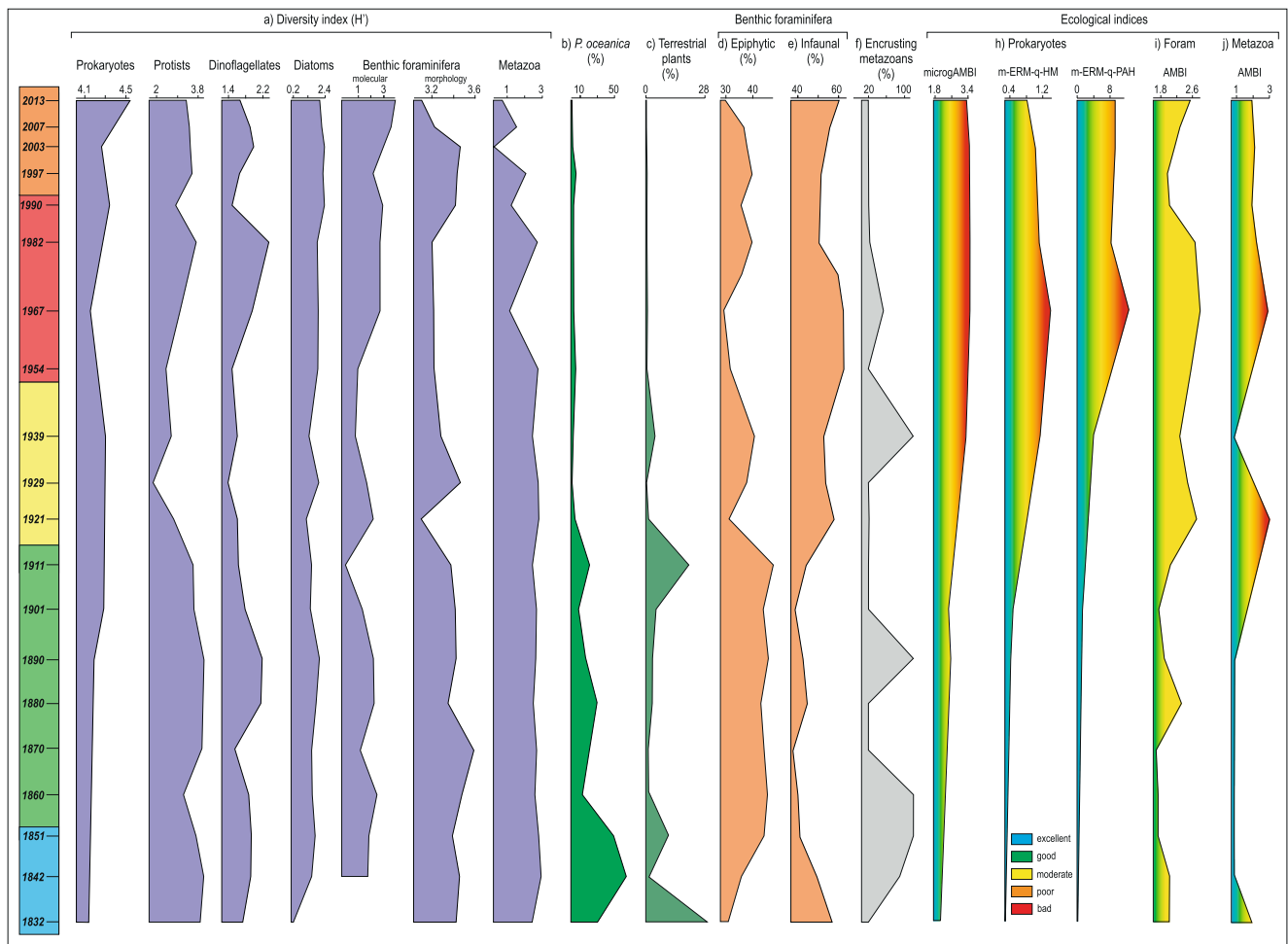
Fig. 3. Changes in diversity and ecological indices. **Shannon** diversity (H') plots of prokaryotes, protists, dinoflagellates, diatoms, benthic foraminifera (molecular and morphological) and metazoa coupled with relative abundance of *Posidonia oceanica*, land plants, epifaunal and epiphytic foraminifera and encrusting metazoa percentages. Ecological indices for prokaryotes (microgAMBI, m-ERM-q-HM, and m-ERM-q-PAHs), foraminifera (Foram AMBI) and metazoa (gAMBI) are also plotted. The different colour over the ages refer to the main phases (I: blue; II: green; III: yellow; IV: red and V: orange) as identified in Fig. 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

probability of toxicity (i.e., m-ERM-q) with a marked peak in 1967. The abiotic factors explaining the changes in prokaryotic diversity were mud, C/N, TOC and TN (Supplementary Fig. 6). Foram-AMBI showed a clear increasing trend from 1911 to 1922 to 1982–1990 and a shift from good-moderate to moderate EcoQS. A similar trend was also found for gAMBI values based on macroinvertebrate molecular data with two relevant peaks pointing to bad EcoQS in 1921 and 1967. Overall, the gAMBI mostly classified the EcoQS as excellent up to 1901 and moderate-to-bad afterwards (Fig. 3 and Supplementary Table 6).

4. Discussion

The fluctuations of geochemical proxies, the *sedaDNA* records, the alpha-diversity (H') and ecological indices observed in the AB01 sediment core over two centuries reflect the ecosystem changes in the history of the area resulting from anthropogenic activities. The HCAs multimarker *sedaDNA* records consistently reveal a five-phase evolution of the area that perfectly matches with the anthropogenically induced environmental changes as supported by historical archives and other sources (Figs. 1 and 2). The environmental changes have not only resulted from contamination of the site but also from the geomorphological changes resulting from the expansion of the production site (i.e., reclaiming the area between the two piers "*Colmata a Mare*") and those that modified water circulation and sediment deposition (e.g.,

Lazzaretto and Nisida linking, piers' construction) (Fig. 1). Taken together, these anthropogenic activities led to a concurrent modification of the biological communities from prokaryotes and protists to higher plants and metazoans, impacting their diversity and ecological indices. The five phases of the ecological transformation of the area are discussed below.

Anthropogenic activities as demonstrated in Siano et al., (2021) and in this study led to irreversible shifts in biological communities. In our study all trophic levels were impacted from prokaryotes and unicellular eukaryotes to higher plants and metazoans. As a result, their diversity and ecological indices were altered.

4.1. Phase I (1827–1851)

Until the Industrial Revolution, the Bagnoli-Coroglio plain was a marshy back-barrier as supported by geomorphological evidence and the presence of palustrine sediments (Ascione et al., 2021) (Fig. 1-b). Moreover, biotic and abiotic values inferred an unpolluted environment. Agriculture was the main activity and the whole area was characterized by a few residential constructions and some touristic resorts along the beach, where thermal springs were just discovered. The lowest values of both organic and inorganic contaminants are recorded in this phase together with the highest diversity values of different biological communities (i.e., protists, dinoflagellates, foraminifera, metazoa). All

ecological indices (e.g., AMBIs and m-ERM-q) inferred excellent and good conditions. This finding indicates pristine environmental conditions, the ecosystem baseline with insignificant anthropogenic impacts. The most evident signature in our records is the very high relative abundance in this phase of higher plants and more importantly of the seagrass *P. oceanica* (Fig. 2).

4.2. Phase II (1851–1911)

From the mid-1800 to the beginning of the 20th century, a long sequence of anthropogenic interventions has deeply modified the natural landscape of the area. This period shows not significantly changes in biological composition and remains an environment almost unpolluted. The initial projects for settling the industrial district at Bagnoli plain dated back to 1852, when the glasswork Melchiorre - Bournique and the chemicals plant Lefevre went into operation (Cirillo et al., 2022) (Fig. 1-b). In 1907, the tuff reef of Lazaretto was linked to the Island of Nisida (Fig. 1-b). In 1905, the Ilva/Italsider company set up the construction of one of the largest Italian steel plants that started its production in 1911.

The environmental parameters and paleo-community records do not show any substantial change during Phase II still reflecting good environmental conditions with minor variations imputable to early urbanization and land-use changes. These data match well the information deriving from the historical archives and the mapping of benthic community where *P. oceanica* meadows were indeed recorded between 10 and 30 m depth up to the first decade of the pre-industrial period (1910) in front of the Bagnoli-Coroglio industrial area (Porzio et al., 2020). However, the signature of *P. oceanica* decreased in this period paralleled by a reduction of terrestrial plants, whose signature almost disappeared after 1911 (Figs. 2 and 3). This was probably due to a change in land use of the area where orchards were progressively replaced by buildings for workers (ca. 2000 people in 1910 and 4000 in 1918 at Ilva/Italsider) in the expanding industrial area (Selvaggio, 2015). This phase, as compared to the subsequent ones, was characterized by higher abundances of parasitic taxa, mainly Syndiniales (parasites of dinoflagellates, radiolarians, ciliates) and Apicomplexa (parasites of metazoans) (Fig. 2), which may be related to the presence of specific hosts and further indicates a marked change of the community composition in the area.

4.3. Phase III (1911–1950)

This period marks the beginning of the industrial phase. Pollutants concentration start to increase and notable changes in biological composition were observed. The environmental ecological status turned from unpolluted to polluted. In 1927, a factory for the production of blast furnace cement was built, and three years later, a pier was constructed for loading finished products and receiving coal and iron ores from vessels (Fig. 1-b). In 1936, the Island of Nisida was completely linked to the mainland by a 700-m-long strip of land that modified water circulation dynamics and the sedimentological pattern in the Gulf of Pozzuoli (Fig. 1-b). In 1936, a plant to produce asbestos (Eternit) started its activity and in 1940 a northern jetty was built. The enlargement of the factory continued till 1943, when the Second World War interrupted plant operations up to 1946. In this period, a marked contamination started with an overall increase of mostly inorganic chemicals (i.e., PLI) pointing to moderately-to-highly polluted conditions. PAHs started to increase with concentrations higher than the Effect Range-Low (ER-L) and, at the end of the Phase III, even higher than Effect Range-Median (ER-M). The extreme concentrations of PAHs (up to 87,000 μg g^{-1} d. w.) and their predominantly pyrogenic origin were also reported along with short cores in the beaches surrounding the former industrial plant (Passaro et al., 2020).

These changes are accompanied by radical paleo-community shifts in *sedaDNA* archives, namely the disappearance of *P. oceanica*, and important turnovers in the communities of protists and metazoans. *Posidonia oceanica* is an ecosystem engineer species endemic to the Mediterranean Sea that forms dense meadows in areas characterized by clean and clear waters (Marbà et al., 2014). The disappearance of this habitat-forming species was accompanied by a drastic decrease of epiphytic (i.e., plant-dwelling) foraminiferal taxa as well as of encrusting animals such as ascidians (*Botryllius* sp.) and sponges (Fig. 2). It can be reasonably hypothesized that the disappearance of *P. oceanica* was related to the construction of the connection between Lazaretto and the mainland in 1906 that altered the hydrodynamics of the area markedly and led to the deposition of finer sediments (silt and clay) leading to higher water turbidity and ultimately to reduced photosynthetic activity. However, grain-size data of AB01 sediment core show only minor variations in the sediment fine fractions (silt and clay) after 1906 (Supplementary Fig. 9). According to Porzio et al. (2020), the massive hydrocarbons (i.e., PAHs) released in this area could contribute to *P. oceanica* disappearance. The changes in water circulation could also explain a remarkable decrease of planktonic radiolarians, while the modification of the seabed could have led to the replacement of encrusting and colonial ascidian by epibenthic and solitarian ascidiaceans after 1911. In Phase III, considerable changes are observed in the protist community, with a decrease in diversity (Fig. 3) and a shift in its composition. Lower relative abundances of parasitic dinoflagellates (Syndiniales), Apicomplexa, Radiolaria, and Cercozoa were compensated by a marked dominance of Dinophyceae, mainly including taxa known to produce resting cysts (Figs. 2 and 3).

Many of these changes reflect a worsening of the environmental conditions triggered by the beginning of industrial activities. Overall, a reduction in diversity of eukaryotes, protists and foraminifera is associated with the beginning of the industrial activities in 1911 and becomes particularly evident during the maximum expansion of the industrial phase (i.e., 1920–1980) (Fig. 3). The increase in Foram-AMBI and microgAMBI, proxies for estimating the EcoQS, is associated with the increase of OM (i.e., TOC and C/N) and reliably follows its patterns. Indeed, this shift in the quantity (i.e., TOC) and possibly the quality (i.e., C/N) of OM strongly matches with the decrease of epiphytic foraminiferal species that are affected by the enhanced OM availability (i.e., higher TOC) with a more refractory origin (i.e., higher C/N). On the other hand, the concurrent increase of infaunal (i.e., organisms living within the sediment) taxa perfectly reflects the enhanced OM availability at the seafloor.

4.4. Phase IV (1950–1992)

This period defines the peak of the industrial phase and is mainly reflected by the highest level of contamination with highly-to-extremely highly polluted conditions based on PLI (Fig. 1-a). In addition, biological disturbances occur and are reflected in diversity (H') and ecological indices changes. In 1952, the Cementir started its production of concrete and the '60 s marked the production peak of steel reaching up to 2x10^6 tons yr^{-1} and involved ca. 5400 workers at Ilva/Italsider. Between 1962 and 1964, a large amount of contaminated soil from the industrial area was used to enlarge the industrial area by filling the gap between the two piers and creating the so-called "*Colmata a mare*". In the '70 s with 7698 workers at Ilva/Italsider, the steel production started to decrease, in 1985 the Eternit ceased its asbestos production and the steel factory permanently closed in 1992 (Fig. 1-b). The highest level of contamination (i.e., PLI and PAHs) was recorded between 1960 and 1987.

The ecological indices associated with prokaryotes, namely m-ERM-q-HM and -PAHs, clearly evidence an increasing trend and a marked peak in 1967 (Fig. 3). The Phase IV is also characterized by the worst EcoQS equally indicated by AMBI of prokaryotes, foraminifera, and metazoans, with a marked peak in 1967, in response to the enhanced OM availability. Despite the increase in C/N ratio suggesting a terrestrial origin of OM, the absence of higher plants record based on *sedaDNA* suggests an overall decrease over time of terrestrial inputs that implies a strong contribution of PAHs to the increase of C/N values (Meyers, 1994; Rumolo et al., 2011). In Phase IV, the protist community is still

characterized by the marked dominance of cyst forming dinoflagellates as in Phase III (Fig. 2). The remarkable abundance of these free-living heterotrophic and mixotrophic dinoflagellates can reflect a change in the trophic structure of the community. Alternatively, it could indicate a specific response of these unicellular organisms to the high concentration of pollutants in the sediments and the overlying water column. The transition between free-living motile stages to non-motile resting cysts occurs in the deeper layers of the water column, close to the sediments (Brosnahan et al., 2017) and it has been shown that high concentrations of heavy metals and PAHs are related to an increase of cyst production (Dale, 2001; Horner et al., 2011; Triki et al., 2017). The Phase IV was characterized by a marked reduction of metazoan diversity starting from 1954 (Fig. 3). The delayed response of metazoans compared to planktonic protists might be associated with the higher sensitivity behaviour of the latter to water chemical and physical variations that allow them to respond to changes rapidly. It might be speculated that the time lag response of metazoans is also linked to their higher position in the marine trophic web.

4.5. Phase V (1992–2016)

After the dismantling of the site started between 1994 and 2000 the Bagnoli-Coroglio area was included in the SIN list by the Italian Government, highlighting the threat to human health and the need to apply a remediation program (Ausili et al., 2020; Morroni et al., 2020; Romano et al., 2004). In the early 2000 s, the first remediation projects were planned but the complete remediation has not been completed (Fig. 1-b). This post-industrial phase shows an overall reduction of contamination, though PLI and PAHs still remain high, and a concurrent increase of diversity indices values. Metazoans dominated in the most recent layers (2013 and 2007) and were mainly represented by polychaetes that are currently abundant in the sandy bottom of the Bay of Bagnoli-Coroglio (Fasciglione et al., 2016; Morroni et al., 2020).

5. Conclusion and future perspectives

The present study provides the first holistic insight into the ecosystem changes that occurred in one of the most polluted marine coastal areas during the last two centuries. In the absence of long-term ecological studies of coastal communities, paleogenomics offers a unique opportunity to reconstruct past changes in biodiversity by linking them to environmental change and increased anthropogenic pressure. The investigation of *sedaDNA* allows us to retrospectively identify a five-stage stepwise evolution in the prokaryotic and eukaryotic communities, demonstrating how the industrial pollution and land-use changes have deeply modified their natural equilibrium. The site was characterized by seagrass meadows and high eukaryotic diversity until the beginning of the 20th century. Then, the ecosystem completely changed, with seagrasses and associated fauna as well as diverse groups of planktonic and benthic protists being replaced by low diversity biota dominated by dinophyceans and infaunal metazoan species. The *sedaDNA* approach enables to disentangle the multi-level cascade effects of industrialization, bringing to light the pre-industrial reference conditions and showing that after 20 years of decommissioning, the good ecological conditions have not fully recovered yet.

From the future perspective, it is important to highlight that paleogenomics is not only a tool for recording past ecosystems but might also help in assessing the recovery of degraded coastal marine ecosystems. Our metabarcoding approach is universally applicable. It is based on standardized and widely available protocols. It could be applied to any other polluted sites, as long as the reference database for local fauna and flora, especially the bioindicator taxa, is available. The open question is whether it is possible to recover the reference conditions revealed by paleogenomics. In the case of Bagnoli Bay, the ecosystem changes might be irreversible. The return to pristine conditions would require reintroduction of *Posidonia* meadows, which might be difficult if not impossible. However, in other less polluted sites, it might be still possible to conduct remediation actions that would lead to the recovery of preindustrial conditions. In such cases, the investigation of sedimentary DNA archives is highly recommended as this is the only way to obtain a holistic overview of the past biodiversity and ecosystem changes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Genomic data are available on the Sequence Read Archive public database under the accession number: PRJNA824838 and PRJNA822883

Author contributions

I.B.A., ML.RM and J.P. conducted the metabarcoding analysis of eukaryotes, foraminifera and metazoa. M.C. and F.F. conducted the morphological analysis of foraminifera. S.V., M.T., A.A. and C.C. conducted the metabarcoding analysis of prokaryotes. R.P., M.G.M. and M. M. performed the analysis on planktonic protists and interpreted the results. A.S., M.M.R. and I.D. performed the radiometric and grain-size analyses. F.M., S.C., M.R.M, J.R. and L.P. performed the geochemical analysis. F.Fra and F.F. conducted the statistical analysis. L.M. secured funding and planned the samples collection. F.F., M.M. and J.P. conceived and designed this work. F.F. wrote the original manuscript. All authors discussed and interpreted the results and contributed to the manuscript.

Data accessibility

The raw data for foraminifera, COI and V9 sequencing are available on the Sequence Read Archive public database under the accession number: PRJNA824838 and those of prokaryotes are available under the accession number: PRJNA822883.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107738.

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