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Positive effects of two decades of passive ecological restoration in a historically polluted marine site

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The Mediterranean Sea is one of the most exploited regions of the world's oceans. Here industrial activities have determined either acute or long-term impacts on coastal marine ecosystems. In this study, we investigated macrofauna distribution and diversity, and food-web functioning in a coastal area of the Mediterranean Sea facing an industrial chemical plant abandoned in the '90s to assess benthic ecosystem health. This area has been identified as a Site of National Interest (SNI) since 2002 and has been closed to any human activity awaiting to be remediated according to national laws. Our results indicate that, two decades after the SNI declaration (a decade after the plant decommissioning), there is no longer any sign of the impact of historical contaminations on macrofauna and benthic food web functioning. Overall, all the thirty-six sites showed high/good ecological quality according to the score assigned by AMBI and M-AMBI indexes, reflecting the absence of chronic impacts. Our findings reveal, for the first time, the positive effects of passive restoration (i.e., unassisted, or spontaneous recovery following cessation of anthropogenic impacts) on historically impacted coastal ecosystems since their health conditions, in terms of both abiotic (environmental variables and contaminant concentration) and biotic (macrofauna diversity and community composition, and benthic food-web structure) factors, were indistinguishable from surrounding non-impacted areas. These findings also suggest that other effective area-based conservation measures (OECMs) could be useful not only for biodiversity conservation of vulnerable and priority habitats in larger ocean sectors but also to promote the passive recovery of historically contaminated ecosystems.

KEYWORDS

passive restoration, benthic biodiversity, food webs, industrial contamination, environmental quality, Mediterranean Sea

1 Introduction

Coastal environments include essential marine habitats (e.g., seagrass meadows, rocky reefs, estuaries, soft-sediment habitats) that support marine biodiversity and food webs and provide key ecosystem goods and services for human well-being (Coll et al., 2010; Barbier, 2017). At the same time, coastal areas have a long-lasting legacy of environmental impacts due to increasing urbanization, industrialization, and the consequent wastewater discharge of contaminants, and resource exploitation, which have led to the progressive loss of marine biodiversity and habitats (Cardinale et al., 2012; Pereira et al., 2012; Halpern et al., 2015; Hay Mele et al., 2020; Soto-Navarro et al., 2021). Anthropogenic impacts impairing coastal environments also act in synergy with other stressors such as extreme climate events (i.e., heat waves), thus determining a progressive alteration of the trophic food webs and ecosystem functioning (Micheli et al., 2013; He and Silliman, 2019; Danovaro et al., 2018).

Along the Mediterranean coasts, there are more than 200 active, dismissed, or decommissioned refineries, petrochemical, and chemical plants that, over time, have determined persistent and chronic impacts on the ecosystems due to the release of inorganic and organic contaminants, including fertilizers, hydrocarbons, PCBs, and heavy metals (Gambi et al., 2020). Several industrial-contaminated sites have a relevant impact on coastal ecosystems even after decades of industrial activity cessation (Loures, 2015; Bertocci et al., 2019; Ausili et al., 2020; Morroni et al., 2020; Gambi et al., 2022). In particular, high concentrations of polycyclic aromatic hydrocarbons (PAHs) and heavy metals have been widely reported to cause adverse effects on benthic communities at all levels, from prokaryotes to metazoans, and alter the biodiversity, food-web structure, and ecosystem functioning (Louati et al., 2013; Aylagas et al., 2017; Gambi et al., 2020; Hay Mele et al., 2020; Mayer-Pinto et al., 2020; Morroni et al., 2020; Tangherlini et al., 2020; Raymond et al., 2021).

The Italian legislation has assigned the status of "Sites of National Interest" (SNIs) to the most polluted coastal and terrestrial sites affected by industrial activity, thus requiring specific recovery plans to reduce the risk for human and ecosystem health (Ausili et al., 2020; Corinaldesi et al., 2022). The SNIs are indeed characterized by persistent contamination levels even several decades after the cessation of industrial activities (Romano et al., 2004; Gambi et al., 2020; Morroni et al., 2020; Naidu et al., 2021). The coastal area of Falconara Marittima (Northwest Adriatic Sea, Mediterranean Sea), which is also included among the European "problem areas" based on the contamination levels in water, sediments and biological components (Andersen et al., 2019), has been declared a SNI since 2002 and has been interdicted to any anthropogenic activities (e.g., shipping, fishery, and bathing). Such levels of contaminants have been reported to be harmful to macrobenthic communities (Josefson et al., 2008). A recent investigation conducted on the benthic ecosystems of this SNI, in front of a chemical industry abandoned more than three decades ago, revealed that heavy metals (As, Hg, and Al), C > 12 aliphatic hydrocarbons and total PAHs are currently low and without significant impact on meiofauna assemblages (Corinaldesi et al., 2022). However, the observed biological response could be due to the specific tolerance and fast resilience of the meiofaunal component (Whomersley et al., 2009; Schratzberger and Somerfield, 2020) while other benthic components, i.e., larger animals such as macro-megafauna, could better reflect long-lasting negative effects on biodiversity and ecosystem functioning (Bessa et al., 2014; Kim et al., 2020; Raymond et al., 2021; Gammal et al., 2023; Hilgendag et al., 2022).

Macrofaunal assemblages respond to environmental changes depending on their species-specific sensitivity/tolerance (Han et al., 2021), providing information on the different degrees of impact on biodiversity and ecosystem functioning (Gray et al., 1988; Hewitt et al., 2016; Ellis et al., 2017; Momota and Hosokawa, 2021). Nonetheless, macrofaunal assemblages in coastal benthic ecosystems play crucial functional roles by i) channelling the matter and energy flow to higher trophic levels, ii) influencing nutrient cycling and secondary production; and iii) contributing to the burial, diagenesis, and transfer of pollutants along food webs (Constable, 1999; Jędruch et al., 2019). For all these reasons, macrofauna has been proven to be one of the most effective biological indicators (Borja et al., 2000; Nepote et al., 2022; Magni et al., 2023).

Food-web functioning is crucial for the good health of marine ecosystems and provides important indications of the impacts of anthropogenic activities on benthic ecosystems (Castro-Jiménez et al., 2021; Alp and Cucherousset, 2022). Stable isotope analysis (hereafter defined as SIA) is a useful tool for investigating the trophic interactions within marine assemblages (Boecklen et al., 2011; Fanelli et al., 2015) and the isotopic analysis of the niche size (quantified as convex hull, standard ellipse area, or kernel utilization density; Jackson et al., 2011; Eckrich et al., 2020; Bada et al., 2022) has been also applied to assess food web response to different stressors, including chemical pollution (Hogsden and Harding, 2012; Alp and Cucherousset, 2022).

In the present study, we assessed the effects of passive restoration (i.e., unassisted, or spontaneous recovery following cessation of anthropogenic impacts; Perrow and Davy, 2002; Jones et al., 2018; Fraschetti et al., 2021) on benthic biodiversity and food-web functioning in the SNI of Falconara Marittima (Northwest Adriatic Sea, Mediterranean Sea), by comparing taxa richness, biomass and diversity of macrofauna and their food-web structure within and outside the SNI. Results of this study expand the knowledge on the current health status and resilience of benthic ecosystems after the cessation of industrial activities.

2 Materials and methods

2.1 Study area and sampling design

The study area is located on the western coast of the central Adriatic Sea (Mediterranean Sea, Figure 1), in Falconara Marittima (Italy).

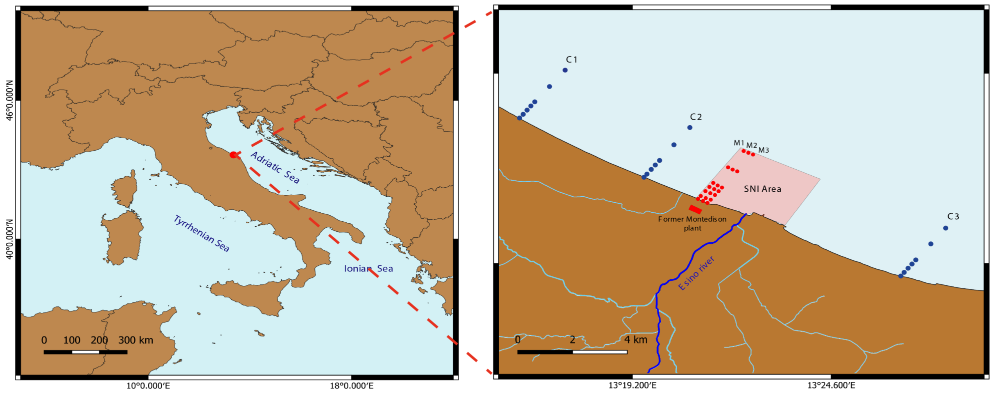

FIGURE 1
Study area and location of Control (C1 and C2= transects to the north of the SNI area, and C3= transect to the south of the SNI area) and impact (M1, M2, and M3) transects.

The marine area of the SNI of Falconara Marittima has an extension of 1200 ha and is characterized by the presence of the abandoned "Montedison SPA" and the still-active "API SPA" Oil Refinery. In addition, the SNI includes the mouth of the Esino River. Our investigation has been carried out in 2019 in front of the former Montedison industrial plant (Figure 1). This company started its production of superphosphate in 1919, then in 1944 the plant was acquired by the Montecatini Company and used as a warehouse by the British Royal Army Service Corps, and finally, from 1966 to 1990 (decommissioning year) the chemical pole has produced fertilizers using pyrite and phosphorous, which have been reported to contaminate soil and groundwater of the surrounding area, together with heavy metals, fluorides, hydrocarbons and PAHs (Regione Marche, 2009). The SNI of Falconara Marittima was declared in 2002 and the coastal area was confined and included in the high-risk area in 2003.

Three transects (defined as M1, M2, and M3) were investigated in the SNI area in front of the Montedison plant, and three transects (C1, C2, and C3) were selected outside the SNI, two transects to the north (C1 and C2) of the SNI area, and one to the south (C3). Each transect, perpendicular to the coastline, included 6 stations located at 3, 4, 5, 6, 10, and 12 m depth (see SOM Table S1). In each station, three independent replicates of sediment samples were collected onboard the R/V Actea with a Van Veen grab of 40 x 20 cm (surface of 0.08 m^2). Sediment samples were sieved on board with a 500 µm mesh-size sieve to retain the macrofauna organisms. Each sample was fixed in 70% ethanol solution and stored at 4°C.

At all sampling stations, water temperature and salinity were measured using a CTD probe (SBE19 equipped with additional sensors for O_2, fluorescence, turbidity, and pH911-plus sensor). Grain size and contaminants concentration ($C > 12$ and $C< 12$ aliphatic hydrocarbons, PAHs, PCBs, heavy metals, and metalloids) as well as phytopigments and biochemical components of the sedimentary organic matter contextually analyzed with macrofauna samples have been previously reported (Corinaldesi et al., 2022) and summarized in Supplemental Online Material.

To analyze the main current regime, a 1MHz Nortek ASC AWAC Acoustic Profiler, deployed at 1.8 km far from the coast (13.36E, 43.66N, close to the M3 transect in the SNI area) from February 13 to April 14, 2020, was used. The current meter was placed at 0.5 m from the bottom and the currents were monitored for 10 minutes at intervals of 30 minutes over 4 depth cells with a 2 m vertical spacing. To avoid the side lobe echo near the sea surface the topmost cell was removed. To avoid the side lobe interference, the closest cell to the sea surface was removed. The Integrate Marine Observing System toolbox (IMOS toolbox by Australian National Mooring Network and Australian Ocean Data Network) was used for data parsing including quality control to remove spikes and outliers. Data processing was performed using the software Storm (Nortek) and hourly currents were computed from the 30 min data by averaging three sequential half-hour observations in the time range ±30 min around each hour. The data are represented in the Supplementary Material (Figures S1, S2).

2.2 Macrofauna abundance, taxonomic diversity, and biomass

Samples of macrofauna were sorted under a stereomicroscope (x10 and x40), and organisms were identified at the lowest taxonomic level possible and counted. Specimens were then oven-dried at 60°C for 24 hours and weighted for dry biomass estimates. Both abundance (ind/m^2) and biomass (g DW/m^2) were obtained by standardizing abundance and biomass data to the grab surface. Polychetes and molluscs (many of them represented by juveniles) were mostly identified to the genus and family levels, while Crustacea and Echinodermata were identified to the species level. Therefore, since part of the samples was identified at the family or genus level (see the complete list of all taxa identified in Table S1), the level of family was used for the calculations of the various metrics. It is commonly acknowledged that family-level identification is sufficient to assess the impacts on marine soft bottom assemblages in routine monitoring programs (e.g., Somerfield and Clarke, 1995; Lampadariou et al., 2005; Chessman et al., 2009; Dauvin et al., 2016; Romano et al., 2016; Pitacco et al., 2019). Some authors even concluded that environmental quality assessments conducted at the family and genus levels are even more accurate than those at conducted the species level (Checon and Amaral, 2017). Families are also likely to be a good predictor of species-level variation related to natural environmental gradients (De Biasi et al., 2003; Dethier and Schoch, 2006). This is because there is a considerable degree of redundancy in species-level data (Clarke and Warwick, 1998; Olsgard et al., 1998) and thus, the analysis at higher taxonomic levels might act to reduce the confounding effects of species-level responses to natural variation and allow the effects of anthropogenic disturbance to be more clearly detected (Dauvin et al., 2003). Taxa richness was estimated by the DIVERSE routine in PRIMER7 (Clarke and Gorley, 2006). As species and higher taxa richness strongly correlate with each other (Volvenko et al., 2023), species richness can be assessed using genus, family, or order richness and here taxa richness was calculated using the level of family, as in other studies (Cooper and Barry, 2017; De Smet et al., 2017; Bevilacqua et al., 2018). The AZTI Marine Biotic Index (AMBI, Borja et al., 2000) and Multivariate AMBI indices (M-AMBI, Carvalho et al., 2006; Dauvin and Ruellet, 2007; Cai et al., 2014; Tweedley et al., 2015) were calculated to assess the environmental status of the different communities according to condition, transects, and depths. Both indices were estimated through the AMBI software, freely available at https://ambi.azti.es/download/. They were both robust to aggregation of macrofaunal data to the family level (Forde et al., 2013).

2.3 Stable isotope analysis

The most abundant taxa identified in the investigated area were used for stable isotope analyses. Selected taxa were oven-dried for 24 hours at 60°C. Dried samples were converted into a fine powder with a mortar and pestle. For each taxon, two or three replicates (when possible) were weighed (ca 0.3 - 1.3 mg) and placed into tin capsules. Since inorganic carbon could lead to an increase of $\delta^{13}C$, because it is isotopically heavier than most carbon of organic origin and could reflect the isotopic signature of environmental carbon (Schlacher and Connolly, 2014), samples containing carbonates (i.e., spicules or exoskeleton) were acidified with HCl 1M, by adding it drop by drop to the sample until bubble cessation. Samples for the analysis of N were not acidified, as several studies demonstrated that the acidification procedure can alter the N signal (Kolasinski et al., 2008). After that, samples were oven-dried again for 24 hours at 60°C. Samples were analyzed through an elemental analyser (Thermo Flash EA 1112) for the determination of total carbon and nitrogen, coupled through a continuous flux to an isotope-ratio mass spectrometer (IRMS, Thermo Delta Plus XP) for $\delta^{13}C$ and $\delta^{15}N$ at the Laboratory of Stable Isotopes Ecology of the University of Palermo (Italy). A stable isotope ratio was expressed, in relation to international standards (atmospheric N_2 and Vienna PeeDee Belemnite for $\delta^{15}N$ and $\delta^{13}C$, respectively), as:

$$\delta^{13}C \quad or \quad \delta^{15}N:[(R_{sample}/R_{standard})^{-1})]*10^3$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Analytical precision based on standard deviations of internal standards (International Atomic Energy Agency IAEA-CH-6; IAEA-NO-3; IAEA-N-2) ranged from 0.10 to 0.19‰ for $\delta^{13}C$ and 0.02 to 0.08‰ for $\delta^{15}N$.

2.4 Data analysis

2.4.1 Changes in abundance, biomass, and assemblage composition

To test for differences among all variables investigated between SNI and Control transects and among water depths, uni- and multivariate distance-based permutational analyses of variance were applied (PERMANOVA; Anderson et al., 2008). All the statistical analyses were carried out considering 3 factors as sources of variance: Condition (fixed, 2 levels: SNI vs. Control); Transect (random and nested in Condition, 3 levels for each Condition: northern Control C1, northern Control C2, southern Control C3, SNI M1, SNI M2, and SNI M3) and Depth (fixed and crossed, 6 levels: 3, 4, 5, 6, 10, 12 m). PERMANOVA was run on the Euclidean distance matrix for univariate data (abundance, biomass and taxa richness) and on the Bray-Curtis resemblance matrix of 4th root-transformed abundance data for multivariate data (taxa composition), with 9999 permutations and with "permutation of residuals under a reduced model" as permutation method, significant p-values were set at $p<0.05$. A CAP analysis (Canonical Analysis of Principal Coordinates, Anderson and Willis, 2003) was run on the factor(s) found to be significant according to PERMANOVA results to visualize the pattern found. A SIMPER analysis was then performed to identify the species/taxa that most contributed to the similarity/dissimilarity in each condition and transect, and that was responsible for the dissimilarities. SIMPER test was run using Bray-Curtis similarity on the 4th root-transformed abundance square matrix with a cut-off percentage for low contribution at 60%.

2.4.2 Drivers of changes in abundance and community composition

Multivariate multiple regression analysis (Distance-based linear models, DistLM) was performed to determine whether macrofaunal taxonomic composition (used as the response variable) was potentially influenced by environmental characteristics (grain size, depth, temperature, and salinity), presence of different contaminants (PAHs, C > 12 and C< 12 aliphatic hydrocarbons, PCB, heavy metals, and metalloids such as As, Cu, Hg, Ni, Cr) and/or trophic resources (phytopigments, biopolymeric C concentrations -BPC) (all abiotic data were tested for differences by univariate PERMANOVA using the same design described above). Before running the subsequent analyses, the mean-effects range medium-quotient (m-ERM-q quotient) was calculated for both heavy metals and PAHs based on the available ERM values for these contaminants (Kowalska et al., 2018, see also Corinaldesi et al., 2022). Then variables were tested for collinearity, excluding values >0.6 according to the number of samples (Dormann et al., 2013). The DistLM was run using the "step-wise" selection procedure and the AIC (Akaike Information Criterion) model selection criterion (Anderson et al., 2008). In the model, only the concentration of contaminants present in the first 10 cm depth of the sediments was used since most of the macrofaunal organisms usually inhabit the shallowest layer of sediment with abundance decreasing with depth (Janas et al., 2019).

2.4.3 Changes in stable isotope composition and trophic structure

For the Stable Isotope Analysis (SIA), since the biomass obtained was not sufficient to allow the analysis of each taxon/species per transect and depth (the minimum required biomass for SIA is 1 mg of dry weight per replicate), for each taxon/species specimens, from transects within each condition, were merged and depths were unified as follows: depth 1 comprises samples from 3 m, depth 2 from 4-6 m and depth 3 from 10-12 m. Additionally, each taxon was assigned to a trophic guild (TG), based on literature evidence for that specific species/taxon or a similar taxon (i.e. co-generic/same family, etc.), as follows: Ca (carnivore), OS (opportunistic scavenger), DF (deposit feeder) and SF (suspension feeder). Then, uni- and multivariate PERMANOVA tests were conducted on a modified experimental design from the one used for community analysis, with three crossed factors, Condition (fixed, 2 levels: SNI and Control), Depth (fixed, 3 levels: 1, 2 and 3) and TG (fixed, 4 levels: Ca, OS, DF, and SF).

To reveal key aspects of trophic structure and to detect any effect of contamination across the food web, niche-based metrics (traditional convex Hull - TA, standard ellipse area - SEA, containing ca. 40% of the data and representing the core isotopic niche, and standard ellipse areas corrected for small sample size – SEAc, Layman, et al., 2007; Jackson et al., 2011) were also estimated through the R routine SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al., 2011).

Finally, to provide an overview of the trophic structure of the macrofauna assemblage of the area, stable isotope data were merged at the taxon level and a cluster analysis and a nMDS were run to visualize the separation of species/taxon according to the TG. The trophic level (TL) of each taxon was calculated according to Post (2002) as TL=[($\delta^{15}N_i$- $\delta^{15}N_{base}$)/TEF]+λ, where $\delta^{15}N_i$ is the mean $\delta^{15}N$ value of the i-species, $\delta^{15}N_{base}$ is the $\delta^{15}N$ of the species used as a baseline, here *Tellina* spp., a primary consumer, TEF is the trophic enrichment factor here set at 2.56 (Vanderklift and Ponsard, 2003) and Λ is the trophic level of the species used as a baseline, that is 2, being a primary consumer. Uni- and multivariate PERMANOVA, pairwise tests, CAP, DistLM, Draftsman plot, and SIMPER tests were carried out using the routines included in the software PRIMER 7+ (Clarke and Gorley, 2006).

3 Results

3.1 Environmental variables and sedimentary chemical contaminants

Results of the environmental variables, chemical contaminants, and biochemical components of the sediment organic matter in the investigated sediments have been reported by Corinaldesi et al. (2022) and are briefly presented in Table S2. To summarize, the sedimentary grain size was generally characterized by a high percentage of sand (from 39 to 99%) within a 10-m depth. An increase of the silt-clay fraction (15–45%) was observed between 10 and 12-m depth, being such differences significant (Table S3). However, the gravel fraction was generally very low in all the stations investigated (<5%). The temperature of the bottom water of the Control transects ranged from 19.62 to 21.27°C, whereas in the SNI transects from 19.93 to 20.97°C. Salinity in the Control and SNI transects ranged from 37.59 to 37.92 and from 37.38 to 37.75, respectively, with slightly, though significant, higher values at 10-12 m (Table S3). Steady currents showed a mean speed of 13 cms-1 flowing southeasts, notwithstanding the proximity to the coast (see Figures S1-2).

Heavy metal and metalloid concentrations varied among the different transects investigated without a clear pattern. However, the highest concentrations of Hg, Cu, Cd, and As were observed in the sediment of the station at 12 m depth in the M3 transect, being such differences almost significant only for Cu (Table S3). The highest concentrations of aliphatic hydrocarbons C > 12 were observed in some stations of the SNI transects, especially at 12 m depth in the M1 transect (4,000 mg kg^{-1}) as well as the total PAH concentrations at 3 m (51.98 µg kg^{-1}). The concentrations of C< 12 and C > 12 hydrocarbons in surface sediments (0-10 cm) were similar or higher than in the sub-superficial layers (10–20 cm). PCB concentrations showed values much lower than thresholds established by the national (D. M. 152/2006, 173/2016) and international laws (e.g., Canadian Council of Ministers of the Environment, 2002). However, all the observed differences related to metals and metalloids, PAHs and hydrocarbons were not significant (Table S3).

The biochemical components of the sedimentary organic matter significantly changed with water-column depth both in the SNI and Control transects. In addition, the concentrations of phytopigments were significantly higher in the C3 Control transect (4.76 ± 0.83 µg g^{-1}) than in the other transects, where values were very similar. Protein concentrations were on average lower in M1 and M2 transects (0.44 ± 0.09 – 0.52 ± 0.6 mg g^{-1}) than in the other transects, while carbohydrate and lipid concentrations were significantly higher in the C3 transects. Overall, the highest average values of BPC concentrations were observed in the Control transects, especially in C3 (0.82 ± 0.06 mgC g^{-1}) compared to those observed within the SNI area. In both SNI and Control transects BPC concentrations were on average 0.6 mgC g^{-1}, while the contribution of autotrophic C to BPC was ca. 23.5%.

3.2 Macrofauna abundance and biomass

Overall, a total of 69 taxa, 53 families (22 polychaetes, 13 molluscans, 16 crustaceans and 2 echinoderms), and 18 species were identified (see Table S1). Juvenile bivalves were the most abundant group at all transects, especially at 3-4 m depths (Figure S3). Spionid polychaetes and venerid bivalves were dominant in the whole area (Figure S3). Macrofaunal abundances along the SNI transects ranged, on average, from 1649.4 ± 337.4 to 2016.7 ± 296.2 ind. m^{-2}, in M2 and M1, respectively, and along the Control transects from 1799.4 ± 338.5 to 2278.6 ± 465.1 ind. m^{-2}, C1 and in C3, respectively (Figure 2A). In general, macrofaunal biomass was higher at 4-6-m depth than at 3 m and 10-12 m depth (Figure 2B), and in the Control transects varied from 37.7 ± 7.2 to 58.4± 2.6 g m^{-2} (in C2 and C1, respectively) and in the SNI transects from 44.7 ± 7.9 to 56.9 ± 7.3 g m−2 (in M3 and M2 transects, respectively; Figure 2B). Taxa richness (indicated as SR) was similar in the Control (SR=17.42 ± 1) and SNI (SR=16.68 ± 0.92) transects (Figure 2C), being on average higher at 5-m depth in the Control than in the SNI transects (57.7 ± 1.5 in the Control vs. 50.3 ± 2.3 in the SNI transects) (Figure 2C).

3.3 Composition of macrofaunal assemblages

Macrofaunal assemblages were typically dominated by polychaetes, crustaceans, and molluscs (Figure 2D). PERMANOVA did not show any significant difference for the factor "Condition" (i.e., between assemblages at SNI and Control transects; Table S5A), while significant differences were observed among all Control transects, and M1 vs. M3 transects of the SNI. In addition, differences were found across bathymetric gradients (3 m vs. 4-m depths and 6 m vs. 10-m depths; Tables S5A–D). Although the PERMANOVA output indicated the significant interaction "condition x depth" (Table S5A), the related pairwise test did not provide significant differences between Control and SNI transects at any depths (Table S5C).

The CAP plot carried out on the factor "Depth" highlighted a clear horse-shoe distribution of the samples as a function of the depth (Figure 3, left), while CAP run on the factor "Transect" separated partially the SNI from the Control transects (Figure 3, right). A clear segregation was evident for some samples of the C1 and C3 transects along the x-axis.

SIMPER analyses showed similar average dissimilarities among the composition of macrofaunal assemblages at the different depths of Control (on average, 38.40%, range 32.86 to 47.32%) or SNI (on average 36.73, range 30.53 to 43.83%) transects (Table S6), separately, with the highest dissimilarity observed at 12-m depth in both cases. A higher average dissimilarity was found comparing the Control and SNI transects in macrofaunal assemblage compositions, ranging from 52.2 to 64.0% (at 12 and 5-m depth, respectively).

The AMBI and M-AMBI indices revealed a generally good environmental status for the whole area, with all the stations

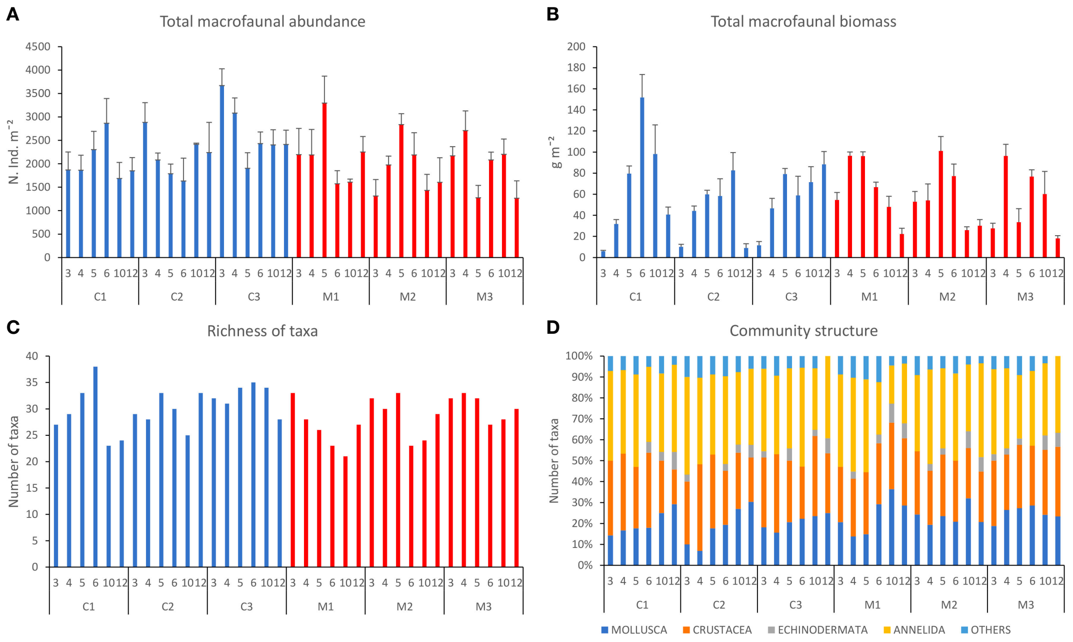

FIGURE 2
Bathymetric distribution (from 3 m to 10 m depths) of macrofaunal abundance, biomass, richness of taxa, and community structure in the sediments of Control (C1, C2, and C3) and SNI (M1, M2, and M3) transects (mean ± ES for abundance and biomass). **(A)** Macrofauna abundances in the different transects and depths, **(B)** biomasses in the different transects and depths; **(C)** richness of taxa in the different stations and transects, and **(D)** community structure in the different transects and depths. The group "Others" contains Echinodermata, Phoronida, and Nemertea. The PERMANOVA analyses showed no significant effect of the factor "Condition" on macrofaunal abundance, biomass, and richness of taxa (Table S4A). A significant effect of the interaction "Transect (Condition) × Depth" was observed on macrofaunal abundance and biomass, indicating differences among transects and depths within each area (Control and SNI). Biomass significantly varied between 3 m and 4 m depths (Table S4B), while analyzing changes between pairs of adjacent depths (i.e., 3 vs. 4 m, 4 vs. 5 m and so on) within the Control or SNI condition, significant differences were highlighted (Table S4C). Comparing biomass values among transects within the Control or SNI conditions, significant differences were observed within pairs of Control transects (C1 vs. C2 and C2 vs. C3; Table S3), and between pairs of SNI transects. Taxa richness significantly varied with the factor depth (p< 0.001). In particular, the pairwise tests revealed significant differences among the two extremes of the depth range explored (i.e., 3 m vs. 12 m), whereas no significant differences were present when comparing adjacent depths (Table S4B).

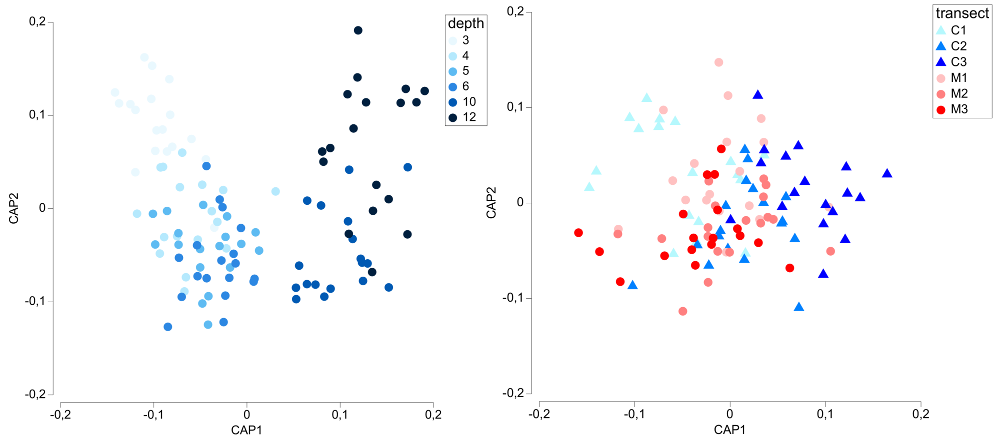

FIGURE 3
CAP plot representing the separation of samples collected in the SNI and Control transects, as a function of depth (left) and transect (right).

classified as undisturbed or slightly disturbed, according to the AMBI index (Figure S4), or defined in a high-good ecological status, according to the M-AMBI index (Figure S5).

3.4 Environmental drivers

The DISTLM model was carried out by excluding depth and temperature from the dataset due to their significant correlation with other variables. DISTLM results for the whole dataset (e.g., SNI and Control transects together) indicated that the percentage of sand was the main explanatory variable accounting for 26% of the total variance (AIC=102.66): the model run only considering Control transects provided similar results, with salinity and % sand as the main drivers contributing together for 74% of the total variance. In the SNI transects the main driver was salinity, which explains alone 48% of the total variance (AIC=46.97) (Table S7).

3.5 Stable isotopes analysis

The most abundant taxa, according to the previous results and occurring at both SNI and Control areas, selected for the SIA were four taxa of bivalves (*Chamelea gallina*, *Tellina* spp., *Donax* spp., and *Spisula subtruncata*), two taxa of gastropods (*Tritia neritea* and *Tritia* spp.), two crustacean taxa (the amphipod *Ampelisca* sp. and the tanaid *Apseudopsis latreillii*), one species and three families of polychaetes (*Owenia fusiformis* and Nephthyidae, Flabelligeridae, Spionidae). Additionally, other two non-indigenous bivalves were found and analyzed for SIA, *Ruditapes philippinarumn* and *Anadara transversa*.

Overall, the highest and the lowest δ^{13}C values were recorded for the two species of bivalves, i.e., *Tellina* spp. (14.02‰ ± 0.45) and *Chamelea gallina* (-19.32‰ ± 0.18), respectively, both from the SNI area. The highest δ^{15}N values were detected in nephtyid polychaetes (10.74‰ ± 0.36) from the SNI and the lowest in *Tellina* spp. (4.29‰ ± 0.12) from the Control area, respectively (Table S8). The PERMANOVA analysis performed on both δ^{13}C and δ^{15}N, evidenced significant differences for factor “depth” and “trophic group (TG)” (Table S9A), with communities located at intermediate depths (4-6 m) showing greater δ^{15}N values (Table S9B and Figure 4A) than those inhabiting deeper bottoms (10-12 m), but no differences occurred for the factor “Condition”. The pairwise comparisons for trophic groups highlighted a significant separation between carnivores and suspension feeders, deposit feeders and suspension feeders, and suspension feeders and opportunistic scavengers for all the combinations tested (i.e., δ^{13}C-δ^{15}N and δ^{15}N and δ^{13}C separately) (Table S9B). As far as δ^{15}N values are concerned, significant differences were observed also for the factor “Condition”, specifically at the trophic group level between Control and SNI for suspension feeders and opportunistic scavengers (Table S9, Figure 4B), being the δ^{15}N values of suspension feeders lower and that of opportunistic scavengers higher in the SNI than in the Control transects. Conversely, δ^{13}C values of the different TG were similar in the SNI and Control areas (Figure 4C).

Standard ellipses showed that the two macrofauna communities (at the Control and SNI area) have similar δ^{13}C and δ^{15}N variability (Table 1), with almost complete overlap of standard ellipses (SEAc) (Figure 5).

Both nMDS and cluster analyses, carried out on δ^{13}C and δ^{15}N values merged by taxon, irrespective of depth and condition showed a food web organized into three trophic levels, with carnivore polychaetes (Nephthyidae) and two taxa of scavenger gastropods (*Tritia neritea* and *Tritia* spp.) occupying the highest trophic level (TL=4) and all suspension and deposit feeders distributed between TL 2 and 3, and clustered together (Figure 6A). A further separation within TL 2 occurred between DF which generally showed higher δ^{13}C and δ^{15}N values and SF (Figure 6B).

4 Discussion

Recent insights obtained at the SNI of Falconara Marittima (Corinaldesi et al., 2022) showed that meiofaunal abundance, biomass and community structure changed among stations regardless of the distance from the abandoned chemical industrial

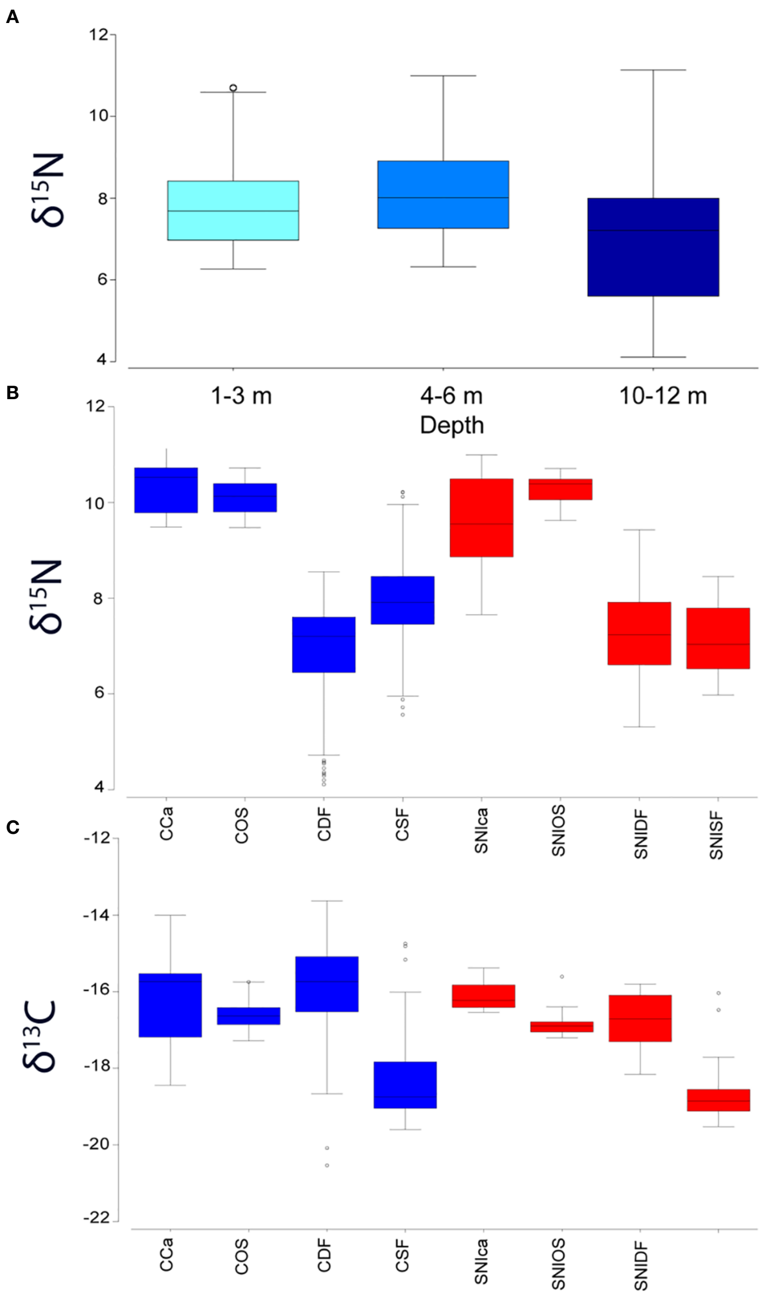

FIGURE 4
Box plots of the **(A)** mean λ^{15}N values of the macrofauna most representative species in the whole area according to depth, **(B)** mean λ^{15}N, and **(C)** λ^{13}C values of the different trophic groups in Control **(C)** and SNI area. Depth: 1 = 1-3 m; 2 = 4-6 m, 3 = 10-12 m; TG: Ca, carnivore; OS, opportunistic scavenger; DF, deposit feeder; SF, suspension feeder.

TABLE 1 Standard ellipse area (SEA), standard ellipse area corrected for sample size (SEAc), and Total Convex Hull for Control and SNI macrofauna collected in the study area.

Condition	SEA	SEAc	TA
Control	7.74	7.79	31.95
SNI	6.60	6.66	24.17

plant, which was dismissed three decades before the investigation, while all human activities ceased with the SNI establishment in 2002. The concentrations of heavy-metals and organic contaminants in the sediments of the investigated area were, indeed, generally lower than those expected to induce harmful biological effects (Long et al., 1995). However, studies on macrobenthic communities reported that the full recovery of coastal marine ecosystems from inorganic and organic pollution

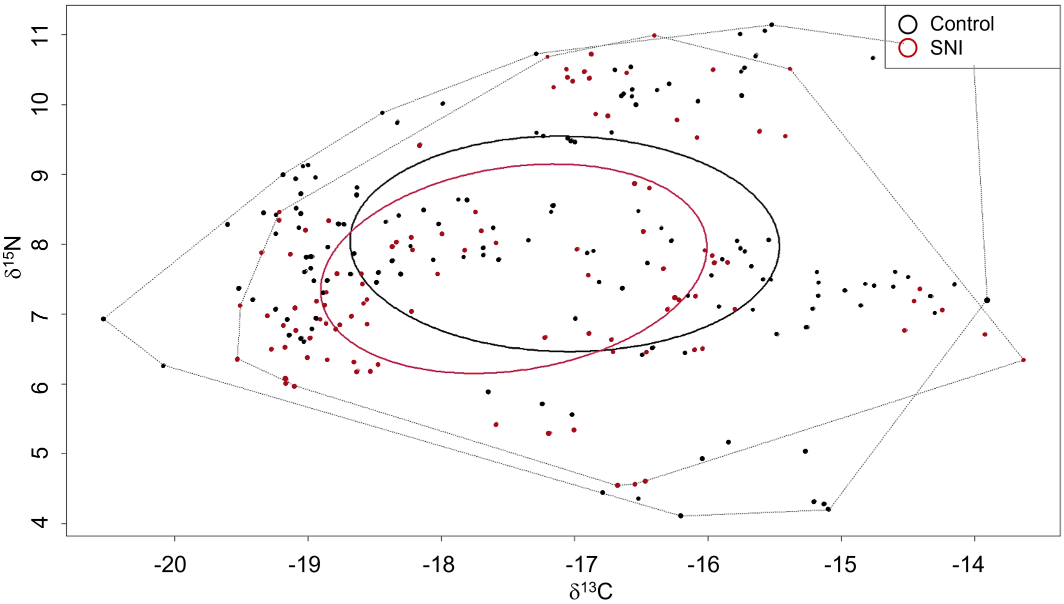

FIGURE 5
$\delta^{13}C$ - $\delta^{15}N$ scatterplot with standard ellipses corrected for small sample size population (SEAC) of the two macrofauna communities (p interval=0.4) in the study area.

may be slow, and can take more than a decade (Josefson et al., 2008; Schwing et al., 2020) or even more for attainment of the composition and diversity of original biotic components (especially considering short-lived and high-turnover biological component; Borja et al., 2010). The present study in the SNI of Falconara Marittima expands information on the effect of historical industrial contaminations on macrobenthic communities and their food-web structure. Since the investigated area of the SNI was interdicted to any anthropogenic activities since 2002, this study provides insights into the responses of benthic ecosystems to passive restoration.

We found that macrofaunal abundance and biomass within the SNI were similar to the values found in the Control areas. Also, the cumulative richness of taxa was similar in the SNI and the Control areas. Further confirmation of the lack of negative impacts from the industrial activities on macrofaunal assemblages was obtained by the analysis of their taxonomic composition, which showed similar bathymetric gradients in the area facing the decommissioned industrial plant and the Control sediments. Therefore, our results indicate that the entire area, including both the SNI and the Control sites, was characterized by a homogeneous composition of the macrofaunal assemblage, mostly represented by opportunistic and tolerant taxa which are typically dominant in the coastal area of the North and Central Adriatic Sea (Simonini et al., 2009; Spagnolo et al., 2019). In particular, we found that all the investigated area was dominated by venerid bivalves (mostly *Chamelea gallina*), characteristic of the well-sorted fine sands, and widely reported in the Adriatic Sea from ca. 4-5 m down to 10-12 m depth (Romanelli et al., 2009). Among polychaetes the most abundant taxa were represented by spionids and the species *Owenia fusiformis*, which are indicators of impacted environments often present near river mouths (Putro, 2007; Pinto et al., 2009; Sivadas et al., 2010) and commonly found in the North and Central Adriatic Sea (Semprucci et al., 2010; Frontalini et al., 2011; Nasi et al., 2020). Since these two taxa were equally distributed within the Control and SNI transects, we can argue that the whole area was characterized by a similar benthic macrofauna diversity.

The most common crustacean family found was Apseudidae, represented exclusively by the species *Apseudopsis latreillii* (Milne-Edwards) (Table S1), a sensitive species to sewage discharge (de-la-Ossa-Carretero et al., 2010). These results suggest the lack of impacts due to anthropogenic contamination at present. Accordingly, the AMBI and M-AMBI indices indicated a homogeneous environmental status within and outside the former industrialized area with all the investigated stations showing a good/high ecological status. These results are consistent with those previously reported in the same area based on m-ERM-q (Effects-Range-Median; Long et al., 1995) values for heavy metals and PAHs, which indicated low environmental risk levels, with the only exception of a station in the SNI site very close to a stream outfall, classified as at high risk (Corinaldesi et al., 2022). As further support of the lack of differences between the SNI and the Control area's communities, the analysis of drivers revealed that the macrofauna composition in the whole area was mostly influenced by sedimentary characteristics (the % of sand in the sediments) and thermohaline conditions (salinity and temperature, which were highly-correlated), while neither hydrocarbons nor heavy metals were found to be the main controlling factors of macrofauna communities at the Control or SNI transects. Previous studies revealed that changes in salinity and temperature may influence benthic communities (Semprucci et al., 2010). Consistently, in the present study, the changes in the thermohaline conditions observed in both the Control and SNI areas (variation between the maximum and minimum salinity and temperature values, 0.34 PSU and 1.58°C and 0.37 PSU and 1.04°C;

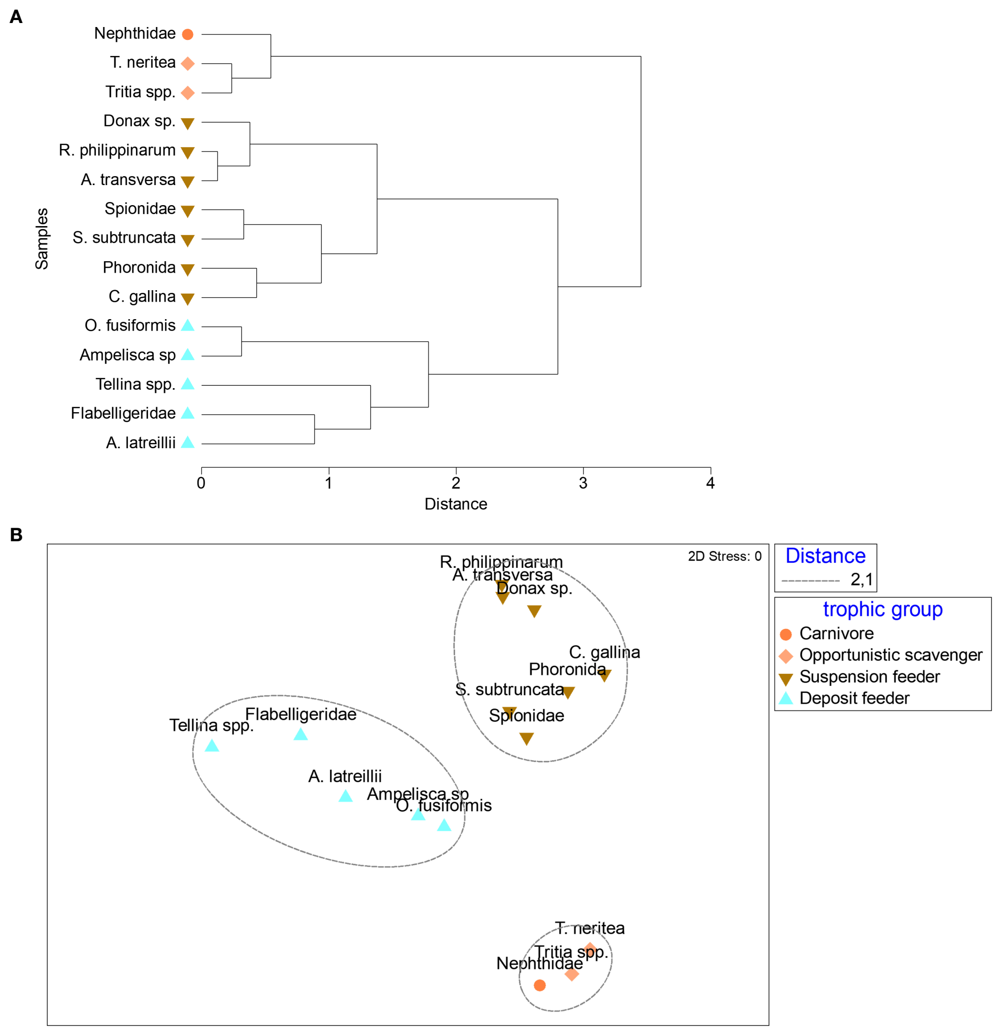

FIGURE 6
Output of cluster **(A)** and nMDS **(B)** analyses carried out on the average values of δ^{15}N and δ^{13}C of taxa collected in the whole area for the factor trophic group (TG). Ca, carnivore; OS, opportunistic scavenger; DF, deposit feeder; SF, suspension feeder.

respectively), which were probably attributed to the presence of fresh water inputs (Esino river and the Rubiano torrent), could explain the significant role of these factors in shaping the composition of macrofauna assemblages.

From the analysis of the benthic food web, we observed the typical structure of benthic macrofauna inhabiting the Mediterranean soft bottoms (Romano et al., 2016), which is organized into three main trophic levels, from suspension feeders (located at trophic level-TL2) to carnivores and opportunistic scavengers (TL 4). The similar isotopic values (both δ^{15}N and δ^{13}C together and separately) found in the common macrofaunal taxa in the SNI and Control transects further support the finding of the absence of a specific impact on the macrofaunal assemblages due to the chemical plant of the Falconara SNI. The last industrial activity of the chemical plant was the production of fertilizers, which are known to provoke an increase in δ^{15}N values in marine sediments and in turn in the benthic fauna living therein (Alonso-Hernández et al., 2017 and references cited), while the fossil fuel emission necessary for the industrial plant's functioning has been reported to determine a λ^{13}C decrease (Rumolo et al., 2011 and reference cited). However, we did not find ^{13}C-depletion and ^{15}N-enrichment in the analyzed taxa of the SNI and Control areas. The significant differences observed in the δ^{13}C and δ^{15}N values between specimens inhabiting intermediate depths (4-6 m) and deeper bottoms (10-12 m) can be attributed to the gradual decrease of terrigenous inputs offshore, as observed in other coastal areas of the Mediterranean influenced by river runoff (Darnaude et al., 2004). When only δ15N values were considered, some significant differences for suspension feeders and, to a lesser extent, for scavengers between SNI and Control transects were observed. Since the majority of the investigated samples from the Control sites were collected south of the mouth of the Esino River (C3 transect), changes in δ^{15}N values could be attributed to local environmental variability of the nitrogen baseline related to river inputs (Galloway et al., 2004). River outflows usually increase the nitrogen load in the coastal environment (Boyer et al., 2006), which could have caused in turn an enrichment in the δ^{15}N levels in the species living close to the river mouth (Fanelli et al., 2013).

However, since the food web structure of the macrofauna assemblages at the two investigated sites (Control vs. SNI) was similar, all the differences concerning the benthic food web of macrofauna assemblages observed in the whole area are mainly due to natural variability. A similar trophic structure without any clear signs of the effects of contamination was also revealed by stable isotope-based community metrics, with Standard Ellipse Areas (as a proxy of isotopic niche width) showing similar values in SNI and Control benthic communities, and almost complete overlapping, suggesting the lack of anthropogenic impacts on the food web attributes (Alp and Cucherousset, 2022).

Overall, our findings indicate that although few stations in the SNI showed a certain degree of organic contamination, no stations can be defined as having a low-quality ecological status. We argue that the dispersion of contaminants due to the West Adriatic current, which flows along the Italian coast southward (Wang and Pinardi, 2002; Bignami et al., 2007), which can be intensified by Bora wind events (Paklar et al., 2001; Cushman-Roisin et al., 2013), can have contributed to the passive restoration of the area. The current measurement collected during the sampling period confirmed the expected pattern (Figures S1, S2) of a flowing southward current. These high-energy hydrodynamic conditions allow the biocoenosis of Fine Well-Sorted Sands or FWSS (SFBC, Sables Fins Bien Calibres in French, Pérès and Picard, 1964) to take place in the coastal area of the whole Region (Semprucci et al., 2010). The FWSS biocoenosis is recognized to be resistant and resilient under an array of human pressures (Dauvin et al., 2017) and the highly- hydrodynamic conditions likely prevent from accumulating contaminants, also when they are associated with organic matter.

Other factors besides the hydrodynamic regime could have concurred to the natural recovery such as microbial degradation (Röling and Van Verseveld, 2002; Acosta-González and Marqués, 2016; Dell'Anno et al., 2021) and bioturbation of opportunistic or tolerant burrowing macrofauna (including for example Spionidae, Owenidae and Paraonidae among polychaetes, and Photidae among crustaceans; Dauvin et al., 2017) that can adapt to human-impacted conditions (Fabi et al., 2009; Simonini et al., 2009). Bioturbation may represent a nature-based remediation strategy as it can improve sediment oxygenation, thus promoting resilience to anthropogenic impacts (Lam-Gordillo et al., 2022).

As further evidence of the good health status of the SNI area, we also discovered habitats with high ecological value, such as sabellarid bioconstructions (Franzitta et al., 2022 and Figure S6). These habitats are known to provide important ecosystem services such as sediment stabilization and mitigation of coastal erosion (Desroy et al., 2011; Lisco et al., 2017; Jones et al., 2020) and supporting coastal biodiversity (Bertocci et al., 2017; Gravina et al., 2018; Ingrosso et al., 2018; Bonifazi et al., 2019) and ecosystem functioning (Jones et al., 2020; Muller et al., 2021). *Sabellaria spinulosa* reefs are also included in the Habitat "Reefs" (code 1170) and listed under Annex I of the Habitats Directive 92/42/EEC.

Overall, this study expands knowledge on the health of the coastal benthic ecosystems affected by historical contaminations such as the SNI of Falconara Marittima, where the lack of a significant impact on macrofauna assemblages and food-web functioning, corroborates information obtained from the previous study on meiofauna (Corinaldesi et al., 2022). This two-pronged approach was used for the first time in a historically polluted marine site, confirming that the joint assessment of meio- and macrofauna are a robust tool to assess the environmental quality of marine ecosystems, as previously reported for others coastal environments (Fanelli et al., 2022; Magni et al., 2022) subjected to natural or anthropogenic impacts.

5 Conclusions

The present investigation reveals that after decades from the decommissioning of the industrial chemical plant located in the Falconara Marittima SNI, the coastal ecosystem recovery was achieved. It is likely that healthy conditions recovered even earlier but, due to the lack of previous studies in the area, this is difficult to ascertain. Our findings support the evidence that historically impacted marine coastal ecosystems and identified as "problem areas" under the law, can passively recover if characterized by highly performing self-purification conditions (i.e., hydrodynamic features) and that OECMs, by limiting marine resource exploitation and human pressures on ecosystems, can be useful not only to conserve marine biodiversity but also to favor the resilience of impacted ecosystems. At the same time, it is urgently necessary to better understand the passive ecological restoration times of historically polluted coastal areas in order to adopt management plans tailored to their specific characteristics, and eventually consider the implementation of an active restoration if these times are too long.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

CC, AD'A, and RD conceived the study. CC, ML, LM, and AD'A defined sampling strategy. ML, MM, and AC participated in the sampling activity. MM and ML performed laboratory analyses. EF, SB, and CG supervised laboratory activities. EF, CC, EN, CG, SB, SV, AD'A, PF, FM, and LM contributed to data elaboration and interpretation. EF, SV, and EN contributed to data visualization. EF, EN, and CC drafted the first version of the manuscript. All authors contributed to the article and approved the submitted version.

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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.2023.1199654/full#supplementary-material

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