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Anthropogenically induced environmental changes in the northeastern Adriatic Sea in the last 400 years (Panzano Bay, Gulf of Trieste)

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Abstract

Shallow and sheltered marine embayments in urbanized areas are prone to the accumulation of pollutants, but little is known about the historical baselines of such marine ecosystems. Here we study foraminiferal assemblages, geochemical proxies and sedimentological data from 1.6m long sediment cores to uncover ~400 years of anthropogenic pressure from mining, port and industrial zones in the Gulf of Trieste, Italy.

From 1600 to 1900 AD, element concentrations and foraminiferal assemblages point to negligible effects of agricultural activities. The only significant anthropogenic activity during this period is mercury mining in the hinterlands of the gulf, releasing high amounts of mercury into the bay and significantly exceeding today's Italian sediment quality guidelines (SQG) and the standards on the effects of trace elements to benthic organisms (ERL and ERM). Nonetheless, the fluctuations in the concentrations of mercury do not correlate with changes in the composition and diversity of foraminiferal assemblages due to its nonbioavailability. Intensified agricultural and maricultural activities in the first half of the 20th century caused slight nutrient enrichment and a minor increase in foraminiferal diversity. Intensified port and industrial activities in the second half of 20th century increased the normalised trace element concentrations and persistent organic pollutants (PAH, PCB) in the topmost part of the core, with solely Ni exceeding Italian SQG, ERL and ERM. This increase caused only minor changes in the foraminiferal community because foraminifera in Panzano Bay have a long history of adaptation to naturally elevated trace element concentrations.

Our study underlines the importance of using an integrated, multidisciplinary approach in reconstructing the history of environmental and anthropogenic changes in marine systems. Given the prolonged human impacts in coastal areas like the Gulf of Trieste, such long term baseline data are crucial for interpreting the present state of marine ecosystems.

Keywords

Marine pollution, Trace elements, Nutrients, Persistent organic pollutants, Benthic foraminifera

1. Introduction

The northern Adriatic Sea is densely urbanised and polluted (Lotze et al., 2006; Cozzi and Giani, 2011), and the areas around the Po River, the Venice Lagoon and in the Gulf of Trieste bear the highest pressure (Solis-Weiss et al., 2007; Raccanelli et al., 2009). Panzano Bay, located in the northeastern part of the Gulf of Trieste, is a shallow and sheltered embayment prone to the accumulation of pollutants, with recent anthropogenic pressure coming from agricultural, maricultural, mining and industrial activities (Horvat et al., 1999).

The impact here started nearly 500 years ago with the onset of mercury mining in the hinterland of the bay (Singh and Turner 2009, Covelli et al., 2012), enhanced in the late 20th century with intensifying agriculture and mariculture (Aleffi et al., 2006; Rampazzo et al., 2013; Finch et al., 2014), and continued to the present times with increasing port and industrial activities (thermoelectric plant) of the City of Monfalcone (Notar et al., 2001; Pozo et al., 2009).

Such intensive anthropogenic pressures have prompted a growing scientific effort to estimate the

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effects of pollution on ecosystem composition here. Most attempts have addressed modifications of the marine habitats that occurred in the 20th century, using only geochemical (Horvat et al., 1999, Faganeli et al., 2003; Acquavita et al., 2012) or biological proxies (Solis-Weiss et al., 2007). There is however, a growing tendency towards integrated assessments of its present state (Cibic et al., 2007; Melis and Covelli, 2013, Franzo et al., 2015), but until today there are no multidisciplinary studies assessing the long term history of the environmental changes in the northeastern Adriatic and thus capturing its preindustrial, undisturbed state.

Such a historical record requires an integrated geochemical and paleoecological approach. Benthic foraminifera, among the most abundant microorganisms in shallow and marginal marine environments, are often used in paleoecological studies. This is because they are highly sensitive to short term environmental changes (Schönfeld, 2012), they have a high preservation potential and thus provide an excellent temporal record of ecosystem states over the past hundreds to thousands of years (Yasuhara et al., 2012).

The present multidisciplinary study is designed to provide a high resolution historical record of environmental changes in Panzano Bay, to obtain information on the state of the ecosystem prior to the onset of the most intensive impact, and to evaluate the effects of anthropogenic activities in the bay. We obtained geochemical data and foraminiferal assemblages from a 1.6m long sediment core containing a centennial scale record of environmental and anthropogenic changes. The core covers approximately the last 400 years, as indicated by radiocarbon calibrated AAR dating of the molluscs shells (Tomasovych et al., submitted).

Taking into account the history of potential anthropogenic stressors in Panzano Bay, we assess the following hypotheses: (1) agricultural and maricultural activities produce upcore increases in the concentrations of organic matter, nutrients and trace elements, (2) mining activities and thermoelectric plants generate a progressive enrichment of mercury and persistent organic pollutants, (3) increased pollutants alter the taxonomic composition of foraminiferal assemblages

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and cause a decline of species abundance and diversity.

To test these hypotheses, we evaluate the pollution in the bay using geochemical proxies (major, minor and trace elements, nutrients, persistent organic pollutants) and quantify the composition and diversity of foraminiferal assemblages. Finally, we reconstruct the chronology of environmental changes in Panzano Bay over the last 400 years and underline the applicability of our results to disturbed shallow coastal ecosystems elsewhere.

2. Study area

The Gulf of Trieste is a shallow marine basin in the northernmost part of the Adriatic Sea, occupying an area of about 500 km^2, with an average water depth of 17m and a maximum of about 25m (Fig. 1). Seasonal variations of water temperature range between 8 to 24ºC at the surface and 8 to 20ºC in the bottom layer. The salinity of the water in the gulf is typically marine, ranging between 33 and 38.5‰ (Ogorelec et al., 1991).

The water enters the gulf in the southeast and continues to the northwest, following the general anticlockwise circulation pattern of the Adriatic Sea. However, the water circulation in the gulf is mostly controlled by tides (range ~0.5m), winds (strong northeastern Bora) and seasonal variations of freshwater inflow. The Isonzo/Soča and Timavo rivers are the most significant sources of freshwater to the Gulf, with average inflows of about 100-130m^3/s each (Ogorelec et al., 1991).

The Gulf of Trieste generally shows mesotrophic to oligotrophic conditions, with episodic eutrophication events, accompanied by summer thermal stratification of the water column (Ogorelec et al., 1991; Horvat et al., 1999; Turk et al., 2007).

The main sediment supply comes from the Isonzo River in the north and from the weathering of the Paleogene flysch deposits outcropping along the southern coast of the gulf. The sediment accumulation rates are approximately 1 mm/yr in the central part of the gulf and increase to about 2.5mm/yr towards the mouth of the Isonzo River located in Panzano Bay (Ogorelec et al., 1991, our unpublished data). Surface sediments in this area are mostly silt clays and clay silts (Zuschin and

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Piller, 1994) occupied by a high biomass epifauna (Zuschin et al., 1999).

The Gulf of Trieste is affected by many sources of organic and inorganic pollutants, coming from agricultural and industrial activities in the hinterland as well as from tourist and maricultural activities along its coasts (Notar et al., 2001; Covelli et al., 2006). Panzano Bay is one of the highly impacted areas, with organic pollution coming from mussel farms located along the eastern part of the Gulf of Trieste (Melaku Canu and Solidoro, 2014) and industrial and port areas of the city of Monfalcone, including a thermoelectric plant and several coal, petroleum and other cargo handling piers (Fig. 1). The Monfalcone thermoelectric plant consists of four thermoelectric generator sets powered by coal and fuel oil and became operative in 1965 ("The Monfalcone Thermoelectric Plant"). Finally, there is substantial Hg pollution originating from the Idrija mercury mine in the hinterlands and delivered to the bay through the Isonzo river flow (Horvat et al., 1999; Notar et al., 2001). Idrija, situated 50km west of Ljubljana (Slovenia), was the second largest Hg mine in the world, operating for nearly 500 years until its definite closure in 1995 (Faganelli et al., 2003; Covelli et al., 2012). During this period, over 5 million tonnes of Hg ore were mined and much of the residues were spread around the town and its vicinity (Miklavčič, 1999). Most of the Isonzo riverine input of Hg is in particulate form (1500kg/yr), followed by dissolved Hg at 8.6kg/yr (Faganelli et al., 2003). Dissolved mercury is biogeochemically reactive and tends to accumulate in certain seafood from mariculture, presenting social and economic problems for the local population (Faganelli et al., 2003).

3. Methods

3.1. Sampling

Three sediment cores, two for sedimentological and one for foraminiferal analyses, 1.6m long with a diameter of 9cm, were acquired using an UWITEC piston corer with hammer action (Gallmetzer et al., in press) from a research vessel in summer 2013. The drilling station is located in the central part of Panzano Bay (45°44,122’ N; 13°36,029’ E) at a water depth of 12.5m. The uppermost 20cm

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of each core were sliced into 2cm thick intervals in order to attain high resolution data. The rest of the core was sliced into 5cm thick samples. For analytical purposes and in order to improve compatibility with the lower part of the core, the uppermost 2cm thick samples were merged into 4cm thick intervals (reducing the number of samples from 36 to 31). Sediment samples were used to determine grain size, the content of major, minor and trace elements, nutrients and persistent organic pollutants. Core chronology is based on molluscan shells dated by ^{14}C calibrated amino acid racemisation.

3.2. Sediment parameters

The grain size of 36 samples was analysed using a sedigraph (SediGraph III 5120 Particle Size Analyzer) for the small fractions (<63µm) and dry sieving for fractions from <63µm to >1mm. The sediments were classified according to the Shepard's classification (1954).

The concentrations of elements, nutrients and pollutants were determined at specific core sections: 1cm, 5cm, 9cm, 24cm, 46cm, 69cm, 85cm, 105cm, 126cm and 151cm core depth. Geochemical analyses included the content of major (Fe, Al), minor (Mn, P) and trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn), nutrients (total organic carbon – TOC and total nitrogen – N tot), persistent organic pollutants (polycyclic aromatic hydrocarbons – PAH, polychlorinated biphenyls – PCB) and total inorganic carbon (C tot).

To analyse elemental concentrations, each sediment sample was gently squeezed to break down aggregates and screened through a PE sieve to remove particles bigger than 1mm. A part of the screened sediment was dried in an oven at 105°C until reaching a constant weight (to measure water content). The dried sediment was ground to powder using an agate mortar and pestle before further analysing the contents of heavy metals and As. The sample (about 0.4g d.w.) was digested with 8ml HNO_3 in a microwave oven (Multiwave 3000, Anton Paar, Austria). The digested material was left to cool at room temperature and then filtered through a 0.45µm nitrocellulose membrane filter. The filtered digestates were diluted with distilled deionised water to 40ml in a volumetric flask (USEPA,

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1994A). The concentrations of the elements (Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn) were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Optima 2100DV, Perkin Elmer, USA) (USEPA, 1994B). Mercury analyses were carried out using atomic absorption spectrophotometry with cold vapour (Analyst 100, Perkin Elmer, USA) (USEPA, 1976).

The quality acceptance protocols required that one blank sample or one certified reference material (BCR-277r estuarine sediment, Community Bureau of Reference) were digested and analysed with each batch of fifteen samples. The blank results indicated that the analytical procedure was free from contamination because the concentrations of all metals were below the respective method detection limits. Mean recovery from the certified material ranged between 84% (Zn) and 103% (Hg), except for Al (40%) because the extraction method was not strong enough to break crystalline aluminosilicates. The analytical precision, determined using five replicates of homogenized samples, was estimated to be better than 10% for all elements. Calibration for ICP-AES and AAS analysis was achieved with prepared external standards via the standard curve approach. Full calibration was performed after every set of 48 samples. The method detection limit for element analysis was defined as 3 times the standard deviation of 10 blank measurements.

Carbon and nitrogen determination was performed following the method of Hedges and Stern (1984), using an elemental analyzer (CHN 2400, Perkin Elmer, USA). The total concentration (TC and TN) was determined on an aliquot of the sample as is (about 10mg of dry sediment); the organic fraction of carbon (OC) was determined after treatment of another aliquot of the sample with acid vapors. The inorganic fraction (C tot) was calculated by the difference between the total and organic concentrations. For the instrument calibration, before each daily series of analyzes, three replicates of Acetanilide standard were performed. For the quality acceptance protocols one blank sample every five samples was analyzed.

To analyse the concentrations of persistent organic pollutants, sediment samples were thoroughly mixed, sieved through a 1mm mesh to remove any debris, and subsequently air dried in the dark at room temperature for 48h on hexane rinsed aluminum foil. The dry samples were finely ground in an agate mortar. The extraction was performed using a Microwave Sample Preparation System (Multiwave 3000, Anton Paar Graz, Austria), in accordance with the EPA recommendation (method 3546). Two grams of dried sediments were weighed into lined microwave extraction vessels. Then, a 25ml 1:1 acetone/hexane solvent mixture was added. The vessels were then assembled as instructed by the manufacturer and the extraction was conducted during 15 min at 110°C and 6-10 bars. At the end of the oven program, vessels were cooled to room temperature and the extracts were filtered and rinsed with the same solvent mixture.

The samples were concentrated in a rotating evaporator (Rotavapor-R Buchi, CH), and the sulphur compounds were removed by soaking the extracts with activated copper powder. Purification and fractionation were performed by eluting extracts through chromatography glass columns packed with Silica gel/Alumina/Florisil (4+4+1gr). The first fraction, containing PCBs, was eluted with 25ml of n-hexane, whereas the second fraction, containing the PAHs, was eluted with 30ml of 8:2 n-hexane/methylene chloride solvent mixture (Fossato et al., 1996, 1998). After concentration with a rotary evaporator, the samples were ready for the instrumental analysis.

The identification of PAHs and PCBs was based on matching retention time, and the quantification was obtained from calibration curves established for each compound by analysing four external standards. Average determination coefficients R^2 of the calibration curves exceeded 0.99 for both PAH and PCB, and the relative standard deviations of the calibration factors were always less than 20% (average 10%). The detection limits were 0.05-0.1ng/g and 0.05ng/g for PAHs and PCBs, respectively. Blanks were run for the entire procedure. Recovery and accuracy were validated with IAEA-417 and IAEA-159 sediment sample certified reference materials. Laboratory methods were also validated by intercalibration activities (IAEA, 2001, 2007, 2012).

Raw concentrations of Hg, Cr, Pb, As, Cd and PCB were compared to Italian sediment quality guidelines (SQG), following the directive D. L.vo n. 172 of 13/10/2015, whereas PAH and Ni

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threshold concentrations were taken from directive DM 367/2003. Additionally, raw concentrations were compared to two sediment quality criteria used around the world: effects range low (ERL), representing the threshold level below which effects to benthic organisms rarely occur, and effects range medium (ERM), above which effects are likely to occur (Burton, 2002). Finally, trace elements were noirmalised to a reference element (Al) in order to compensate for grain size and mineralogical effects on the metal variability in samples (Covelli et al., 2006).

3.3. Foraminiferal analyses

A total of 36 sediment samples were washed with water through a set of sieves with 63, 125, 250 and 500µm meshes. Each obtained subsample was split with a microsplitter in order to yield around 300 specimens. Individuals were identified under a binocular microscope following the classification of Loeblich and Tappan (1987) and Cimerman and Langer (1991). Foraminiferal species were categorised into different ecological categories: according to their substrate relation (infaunal, epifaunal, epiphyitic, epifaunal/infaunal and an epiphytic/infaunal group for ambiguous literature data) and to their tolerance to different types of pollution, namely organic or chemical (hereafter referred to as stress tolerant taxa). This classification was based on a total of 84 sources, including studies carried out in the Adriatic (Jorissen, 1987, 1988; Van der Zwaan & Jorissen, 1991; Jorissen et al., 1992; Barmawidjaja et al., 1992, 1995; Hohenegger et al., 1993; Vaniček et al., 2000; Donnici and Serandrei Barbero, 2002; Albani et al., 2007,; Frontalini and Coccioni, 2008, 2011; Di Bella et al., 2008, 2013; Vidović et al., 2009, 2014; Coccioni et al., 2009; Popadić et al., 2013; Melis and Covelli, 2013; Langlet et al., 2013, 2014). Additionally we used important primary and secondary literature about foraminiferal ecology (Murray, 1991, 2006; Langer, 1993), the most recent studies about improved tools and methods when using benthic foraminifera in environmental monitoring (Barras et al., 2014; Alve et al., 2016; Dimiza et al., 2016) and Paleobiology Database (Behrensmeyer and Turner, 2013). A full list of used sources is given in the Supplement: Table S1.

3.4. Statistical analyses

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Before further statistical treatment, 18 environmental variables (grain size and raw concentrations of nutrients and organic and inorganic pollutants) were checked for normality, log transformed when non normal distribution was detected, and z standardized to account for different units and scales. Pearson correlations among environmental variables and principal component analysis (PCA) based on these 18 variables were performed to assess their collinearity and stratigraphic distribution. Only clay content was used in the multivariate analyses because other grain size fractions correlate with the percentage of clay.

The total foraminiferal assemblages were used in all analyses by pooling all mesh size fractions for each sample. Species diversity was measured using species richness, the exponential of Shannon entropy, and Fisher's α. The exponent of the Shannon index (H) corresponds to the number of equally abundant species that would produce the given value of H (Hill, 1973; Jost, 2006). As all three diversity measures strongly depend on the number of sampled individuals, we rarefied our abundance data down to the size of the smallest sample (240 specimens). This procedure was repeated 1000 times and the mean values of species richness, exp(H) and α with corresponding 95% confidence intervals were computed across all iterations.

Species relative abundance data were square root transformed before multivariate analyses. Non metric multidimensional scaling (NMDS) based on Bray-Curtis distances was used to visualize gradients in community composition. Rescaling the NMDS space according to the underlying dissimilarity matrix and rotating it with the principal component analysis maximized the compositional variation among samples along the first ordination axis (Oksanen et al., 2015). NMDS axis 1 scores thus correspond to the relative position of samples along the main gradient in species composition. The Pearson correlation was used to measure the association between the environmental variables and NMDS axis 1 scores for the subset of samples with available values of elemental concentrations.

Redundancy analysis (RDA) combined with the forward model selection approach was employed to quantify variation in the multivariate composition of foraminiferal assemblages explained by environmental variables. The effects of environmental variables were first tested in single regressions. Most environmental variables, however, show some degree of collinearity, and the forward model selection approach was thus employed to find a subset of factors that maximizes the explanatory power of environmental variables. At each step of the model building algorithm, an environmental variable with the highest partial R^2 was added while considering the effects of the already selected variables, and the significance of the additional contribution was evaluated through a permutation test (10 000 permutations) (Blanchet et al., 2008).

To identify the timing of the major shifts in assemblage composition, we performed chronological clustering, a type of constrained cluster analysis that takes into account the temporal sequence of samples (Birks, 2012), by using the CONISS algorithm (constrained incremental sum of squares agglomerative clustering) implemented in “chclust” function from the “rioja” package (Juggins, 2015). The number of significantly distinct temporal bins was determined by comparing the amount of variance accounted for by a given number of clusters to a random expectation based on the broken stick model (Bennett, 1996). Clustering was performed on the Bray-Curtis distance matrix based on relative abundance data. All statistical analyses were performed in R 3.2.1 (R Core Team, 2015) using “vegan” (Oksanen et al., 2015) and “rioja” (Juggins, 2015) packages.

3.5. Chronological framework

Core chronology is based on the radiocarbon calibrated amino acid racemization dating of the bivalve species *Varicorbula gibba* (Tomasovych et al., submitted). First, 13 shells of *V. gibba* were selected for ^{14}C dating and analysed at the Poznan Radiocarbon Laboratory. Radiocarbon ages were converted to calendar years using Calib7.1 (Stuiver and Reimer, 1993), the Marine13 data (Reimer et al., 2013), and a regional marine reservoir correction (ΔR) in the northeastern Adriatic equal to = -61 years (standard deviation = 50 years) (Siani et al., 2000). The extent of amino acid racemization (AAR) in 329 shells was analysed at Northern Arizona University using reverse phase

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high pressure liquid chromatography (RP-HPLC) and the procedures of Kaufman and Manley (1998). Thirty specimens of *V. gibba* were randomly selected from eleven, more or less evenly spaced, 4cm or 5cm thick intervals covering the whole core thickness. The rate of AAR was calibrated based on the13 shells dated with ^{14}C and three live collected individuals with the Bayesian model fitting according to Allen et al. (2013). The time dependent reaction kinetic model with the initial D/L value estimated from data and lognormal uncertainty showed the best calibration between D/L values of aspartic acid and calendar ages. AAR data in 18 shells did not pass screening criteria, and ages of 311 specimens in total were used for core chronology (Fig. 2).

4. Results

4.1. Sediment parameters and geochemistry

The grain size distribution is rather homogeneous throughout the Panzano Bay core, with only a slight increase in the contribution of the >1mm fraction in the uppermost part (up to 8.9%). The sediment in the lowermost part of the core is composed of silty clay (50.4-54.5% clay). Starting from 135cm toward the upper section of the core, the amount of clay decreases to 43.5-50% and the sediment changes into clayey silt (Fig. 3).

Principal component analysis (PCA) based on raw elemental concentrations illustrates the correlation between elements, with the first two axes explaining 75.8% of the variance of the data (Fig. 4). This approach distinguishes two major groups of elements with different vertical distribution trends (Fig. 3, Table S2), and three elements (Hg, As, C tot) that do not fall into this grouping and have distinct position in the ordination space. The first group comprises trace (Cr, Cu, Ni, Cd, Mn) and conservative elements (Fe, Al), characterized by positive mutual correlations (Table S3) and a pronounced decrease in the upper 35cm. The second group includes organic and inorganic pollutants and nutrients whose raw concentrations are stable (Pb, Zn, PCB) or increase only slightly (PAH, N tot, TOC, P) in the lower part of the core, but sharply increase in the uppermost 35cm. Normalisation to Al reveals two pronounced peaks in the concentrations of the

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elements from the first group: at 125-130cm core depth and in the uppermost 10cm. The latter peak is also visible in normalised Pb and Zn values (Fig. 3). The concentration of Hg sharply increases at 100-130cm, from 12.74mg/kg to 44.7mg/kg. The Hg values then decrease upcore to a minimum in the surface sediment (8.22mg/kg). Concentrations of As vary in the lower core (2.14-9mg/kg) but gradually decrease to 4.3mg/kg in the surface sediment (0-20cm). Normalisation to Al reveals one concentration peak of As in the upper 10cm; it coincides with the peak of all other trace elements. Total carbon remains constant throughout the core (8-9.35%), except for the lowermost part (3.7% C tot).

4.2. Trends in foraminiferal assemblages

A total of 69 benthic foraminiferal species were identified in the sediments from Panzano Bay, with raw species richness varying between 29 and 41 species in individual samples (26.2-36.4 species after rarefaction; Table S1). The highest percentage of individuals belongs to the suborder Rotaliina (63-89%), followed by Miliolina (8-29%) and Textulariina (1.5-11%). Relative abundances of suborders are generally stable throughout the core and vary notably only in the uppermost 20cm (Fig. 5). Diversity is high throughout the core and increases only in the second half of the 20th century. Values of Fisher α index vary from 7.5 in the lower core to 12 in the uppermost sample; the exponential of Shannon index ranges from 14.2 to 23 and shows the same vertical trend (Fig. 6A). Epifaunal/infaunal and infaunal taxa dominate the assemblages, having variable abundances in the lower core (late 17th and 18th century) and more stable abundances in its upper part (Fig. 6D). In contrast, the number of infaunal species increases distinctly during the 20th century (Fig. 6E). Foraminiferal species tolerant to both chemical and organic pollution dominate the assemblages (40-60%), with maximal abundances in the 18th, 19th and the second half of the 20th century. Species known to tolerate only organic pollution make up 18.5 to 41.7% of the assemblage and have opposite temporal trends than the organic/chemical group, with decreasing trends in the above mentioned time intervals (Fig. 6F, Table S1).

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NMDS ordination and chronological cluster analysis of the assemblages reveal two main groups of samples, with the major shift in relative species abundances starting around 35cm (Figs. 6B, 6C and 7). This depth approximately corresponds to the late 19th century, ~1860 AD (Fig. 2). In the NMDS space, samples from the lower core (150-35cm) are tightly grouped, indicating relatively homogeneous faunal composition. These assemblages are characterised by dominance of *Valvulineria* sp., *Nonionella* sp., non keeled elphidiids, *Ammonia* sp., *A. tepida* and *Haynesina depressula* (Fig. 5). The 130-135cm sample (latest 17th century) represents an outlier with unusually low abundance of *Ammonia* sp. and an increased share of epiphytic species. In contrast, the samples from the upper 35cm of the core are widely distributed in the ordination space, with gradually decreasing axis 1 scores. This suggests a continuous, but strong shift in the assemblage composition in the uppermost core. Here, the major drop in the abundance of *Valvulineria* sp. and non keeled elphidiids is accompanied by a growing share of *Miliolinella* sp., *Triloculina* sp., *Haynesina* sp. and *Nonion* sp. (Fig. 5).

Within each of the two major groups of samples, further clusters are recognizable, defined by the breaks at 85 and 20cm (Fig. 6C). The lowermost part of the core (150-85cm) corresponds to the period from ~1600 to ~1800 AD and has variable foraminiferal distribution trends. The middle part of the core (85-35 cm, ~1800 to ~1860 AD) is characterised by more stable foraminiferal abundances and a pronounced decline of the genus *Valvlulineria*. At 35-20cm (~1860 to 1950 AD) the diversity of the foraminiferal assemblages starts to increase, as do the abundances of epiphytic species. The uppermost sediment (20-0cm, 1950 until today) is characterised by a further increase in biodiversity and in the abundance of textulariids (Figs. 5 and 6A).

4.3. Relationship between foraminiferal assemblages and geochemical proxies

NMDS axis 1 scores are positively correlated with concentrations of Cu, Ni, Cd, Mn, Fe, Al and negatively with total nitrogen and PCB (Table S3). The amount of clay does not correlate with axis 1 scores (Table S3).

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Total nitrogen content explains the highest proportion of variation in assemblage composition (42.4%) and is the only explanatory variable included in the RDA analysis following the forward model selection procedure (Table S4). Nonetheless, other elements that closely (positively or negatively) correlate with total nitrogen content explain a significant amount of variation in single RDA analyses (Table S4), including TOC, organic pollutants (PAH and PCB), and trace elements (Mn, Fe, Ni, Cu, Cd, Zn). The assemblages from the topmost sediment layers (20^{th} century) are clearly separated from the middle core assemblages and from assemblages at the base along RDA axis 1. This reflects the stratigraphic increase in the content of nitrogen, organic carbon and pollutants and the stratigraphic decline in several trace elements (Fig. 8).

5. Discussion

5.1. The effects of agricultural and maricultural activities

The agricultural use of pesticides and of organic or inorganic fertilizers releases considerable amounts of pollutants into the environment (Campos et al., 2003; He et al., 2005; Finch et al., 2014). Pesticides contain pollutant elements such as As, Hg, Cr and Pb (Campos, 2003), while fertilizer contamination includes the discharge of macronutrients (N, P, K) and trace elements, including Co, Cu, Fe, Mn and Zn (Finch et al., 2014). Maricultural activities, in turn, disperse organic matter (waste feed and faeces) and nutrients (Mantzavrakos et al., 2007), leading to elevated concentrations of P, N and TOC in the sediment (Holby and Hall, 1991; Hall et al, 1990, 1992; Mook et al., 2012).

In the Panzano Bay sediments, only few elements (Cd, Cr and Pb) sporadically and slightly exceed the limits imposed by the Italian SQG (Fig. 3, Table S2). Only Ni has elevated values throughout the core, even when compared to the standards evaluating the effects of trace elements to benthic organisms (ERL and ERM). Ni, Cd and Cr have a high positive correlation with the major constituents of clay minerals (Al and Fe), the main scavengers of trace elements (Romano et al., 2013). This points to a possible grain size and mineralogical effect on the accumulation of these elements throughout the core because the sediment in Panzano Bay is composed of silt and clay fractions (Fig. 3).

In order to account for such natural processes, to identify background levels and to determine excess trace elements related to anthropogenic contamination, normalised values (trace elements/Al ratios) are usually applied (Covelli et al., 2006; Romano et al., 2013). Normalised concentrations of Cr, Cu, Ni, Cd, Zn and Mn in Panzano Bay are low before the 1950s and, together with As and Pb, increase only in the last 30 years (Fig. 3). Such an increase can reflect the rapid development of technology and the intensification of agricultural activities during the 20th century.

Similar vertical trends have been recorded in the Marano Lagoon, located 20km west of Panzano Bay (Covelli et al., 2013). The Ni concentrations are almost the same in the two areas, while Pb values are slightly higher in Panzano Bay (starting from 1980 until today). The additional source of Pb here could come from industrial or port activities (see below).

The responses of foraminiferal assemblages to elevated trace element concentrations generally include declining species abundance and diversity as well as altered taxonomic composition because more sensitive species die off and more tolerant taxa prevail (Debenay et al., 2000; Coccioni et al., 2009). Foraminifera can assimilate potentially toxic elements by ingesting contaminated detritus or algae, but also by incorporating these elements during test crystallization, leading to test abnormalities (Le Cadre and Debenay, 2006; Frontalini et al., 2009; Martinez-Colón et al., 2009). In foraminiferal assemblages from Panzano Bay, however, no test abnormalities occurred, indicating that the threshold of elemental concentrations for such an impact was never reached during the last 400 years.

During the period 1600-1900 AD, foraminiferal assemblages in Panzano Bay are characterized by stable diversity indices and a high, variable abundance of stress tolerant genera and species, including *Valvulineria* sp., *Ammonia* sp., *A. tepida* and non keeled elphidiids (Figs. 5 and 6F). The only major discrepancy of this trend occurred in the latest 17th century, when species richness and

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Fisher's α decrease, following an abrupt, short term decline of *Ammonia* sp. (Figs. 5 and 6A). This event approximately coincides with a peak of Cr, Cu, Ni and Cd recorded just above this interval (Fig. 3). The genus *Ammonia* (and especially *A. tepida*) is usually described as being tolerant to all kinds of stress conditions, including organic and heavy metal pollution (Jorissen, 1988; Coccioni et al., 1997; Armynot du Châtelet et al., 2004; Ferraro et al., 2006; Frontalini and Coccioni, 2008). However, some species of this genus (namely *Ammonia parkinsoniana*) are known for their poor tolerance to high levels of trace elements (Jorissen, 1988; Frontalini and Coccioni, 2008; Coccioni et al., 2009). Such a sensitivity to increased trace elemental concentrations of certain *Ammonia* species may have caused the decline of *Ammonia* sp. in this interval.

Non keeled *Elphidium* species prefer an infaunal mode of life (Murray, 2006) and can be associated with food enrichment of the sediments (Donnici and Serandrei Barbero, 2002; Vidović et al., 2009, 2014). This is similar to the infaunal genus *Valvulineria,* which is adapted to large seasonal variability of organic matter and periodic hypoxic conditions (Jorissen, 1987; Donnici and Serandrei Barbero, 2002; Piva et al., 2008). Moreover, *Valvulineria* is considered to be representative of environmental conditions prevailing during the "Little Ice Age" (LIA), that include enhanced rainfall, increased fluvial runoff and increased turbidity (Piva et al., 2008). Interestingly, the distinct peak of *Valvulineria* in Panzano Bay in the early 19th century (Fig. 5) coincides with the maximal abundances of this genus in sediments from the central and south Adriatic. This peak is attributed to one of the coldest and most humid phases of the LIA, characterised by substantially increased river discharge (Piva et al. 2008).

High abundances of the above three genera during the 17th to 19th century suggest strong seasonal variations of river runoff and organic matter input in Panzano Bay,. Accordingly, food availability was the primary controlling factor for foraminiferal community composition during this time. This conclusion is supported by the RDA results, which show high correlation between organic enrichment (N tot) and assemblage composition (Fig. 8, Table S4). Similar correlations between

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community composition/diversity and the type of substrate/food availability (higher abundance of infauna in organic rich muddy sediments) have been reported for modern foraminiferal assemblages in the northern Adriatic (Jorissen, 1987; Donnici and Serandrei Barbero, 2002). The foraminiferal community from Panzano Bay is also highly correlated with several trace elements (Table S3) that naturally accumulated in fine grained sediments during this period (as discussed above). Although the assemblages show no effects of elevated trace element concentrations in terms of decline of species abundance or diversity, they remain dominated by taxa tolerant to both chemical and organic pollution (Fig. 6F). These results imply that the community in Panzano Bay has a long history of adaptation to elevated trace element concentrations.

With the onset of the 20th century, the diversity of foraminiferal assemblages starts to increase (mainly with the increase of infaunal taxa, as reported in Naeher et al., 2014), but this trend becomes pronounced only from 1950 AD onwards (Figs. 6A and 6E). Nutrient concentrations (N tot, TOC and P) display the same dynamics of increase during this period (Fig. 3), and we interpret these to have caused the observed increase in foraminiferal diversity. The nutrient increase can be attributed both to intensifying agricultural and maricultural activities. Moreover, total N and P in Panzano Bay sediments are similar to the values measured in sediments beneath adjacent mussel farms (Rampazzo et al., 2013; Franzo et al., 2014). Mussel farming here became an important activity by the middle of the 20th century, reaching peak production in 1990 (Melaku Canu and Solidoro, 2014). Intense mussel biodeposition enriches surface sediments underneath the farms in organic matter, causing anoxic conditions (Rampazzo et al., 2013). Nonetheless, the impact of this farming does not significantly alter the overall coastal marine system (Danovaro et al., 2004; Vidović et al., 2009, 2014). Rather, strong winds disperse and resuspend surface organic rich sediments over the broader area of the gulf (Franzo et al., 2014).

Besides the increase in diversity, the 20th century is marked by a taxonomic change in foraminiferal assemblages: the abundances of *Valvulineria* sp., *Ammonia* sp. and non keeled elphidiids decrease, whereas *Haynesina* sp. and epiphytic genera (*Miliolinella* sp., *Triloculina* sp.) become more abundant (Figs. 5 and 6D). Relatively higher abundances of these epiphytic, herbivorous genera during this period suggest the presence of seagrasses or macroalgae meadows near the sampling station (e.g., Langer, 1993; Mateu-Vicens et al., 2010). Furthermore, a slight shift in the trophic mode of foraminiferal species in the 20th century (increase of herbivorous taxa) indicates enhanced phytoplankton, probably reflecting higher nutrient levels. The distribution of the genera *Miliolinella* and *Triloculina* in the Gulf of Trieste has already been related to their feeding preference for diatoms in addition to organic detritus and bacteria (Hohenegger et al., 1993).

Haynesina, another genus commonly found in the studied sediments, is also herbivorous, known to be tolerant to high concentrations of organic matter (Debenay et al., 2001; Armynot du Châtelet et al., 2004; Murray, 2006; Romano et al., 2008). Higher abundances of *Haynesina,* together with the increase in overall foraminiferal diversity, may be related to the 20th century nutrient enrichment (Fig. 3) because the representatives of this genus indirectly benefit by feeding on enhanced microalgal biomass (Ward et al., 2003). The faunal shift in dominance of *Valvlulineria* to *Haynesina*, together with higher abundance of epiphytic species, suggests milder seasonal variations of river discharge and enhanced microalgal biomass as a consequence of nutrient enrichment. These conclusions are supported by the RDA analysis, pointing to organic enrichment as a key factor controlling the composition of foraminiferal communities in Panzano Bay (Fig. 8, Table S4).

Finally, the increase in abundance of the suborder Textulariina in this uppermost part of the core may be the result of taphonomic processes: agglutinated taxa are susceptible to postdepositional degradation, and the destruction of their tests explains the downcore reduction of their relative abundances (Diz and Francés, 2009).

5.2. Idrija mercury mine

The activity of the Idria mercury mine is well recorded in Panzano Bay sediments. The Hg

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concentrations during the last 400 years are high and significantly exceed the limits imposed by the Italian SQG, but also ERL and ERM standards (Fig. 3, Table S2). Interestingly, there are some distinct trends: the concentrations are considerably higher during the 18th century and decrease in the 19th and 20th century (Fig. 3), corresponding to the history of the mine: the onset of its significant impact on Panzano Bay occurred in the 18th century, when mining activity sharply increased (Covelli et al., 2012). In the early 19th century, metal recovery from the mine improved, thus releasing less Hg into the river (Covelli et al., 2006).

Foraminiferal assemblages in Panzano Bay remained mostly unaffected by these elevated Hg values throughout the observed period. This implies that speciation of mercury and the bioavailability of its species are more relevant than its total concentration (Martinez-Colón et al., 2009; Aquavita et al., 2012). Most of the Hg enters Panzano Bay in particulate (unreactive) form, with only a small fraction of dissolved Hg (Faganeli et al., 2003). This suggests that the mercury species found here are not accessible to foraminiferal assemblages or, if they are bioavailable, their concentrations do not reach values sufficient to produce toxic effects.

5.3. The port of Monfalcone

Panzano Bay is also affected by the industrial and port activities of the City of Monfalcone. Although the first port features were established in the early 19th century, the port as it is known today was designed and built in the 1930s ("CPM"). In 1965, a thermoelectric plant powered by coal and fuel oil was opened in the industrial area ("The Monfalcone Thermoelectric Plant"). One of the main byproducts of coal and oil combustion are persistent organic pollutants: polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyl (PCB), contaminants that potentially form highly carcinogenic and mutagenic derivatives (Notar et al., 2001; Pozo et al., 2009). Moreover, the use of antifouling paints in ports produces trace elements as residues, namely Cu and Zn, but also Cd, Cr, Ni and Pb (Singh and Turner, 2009).

The presence of PAH and PCB in Panzano Bay sediments is probably related to industrial activities in the port. Their concentrations are low throughout the core and start to increase from the middle of the 20th century on (Fig. 3), corresponding to the opening of the thermoelectric plant. However not even the highest measured concentrations exceed Italian SQG, or ERL and ERM values. In contrast, the concentration peaks of As, Cr, Cu, Ni, Cd, Zn and Pb in the late 20th century reflect not only agricultural sources (see above) but also intensifying port (antifouling paints) and industrial activities (coal and oil burning).

Certain changes in foraminiferal taxonomic composition correlate with the concentrations of persistent organic pollutants (as detected by RDA in Table S4). These include the decrease of the genus *Valvulineria* and the increase in the abundance of taxa tolerant to chemical pollution (Fig. 6F), primarily the genus *Ammonia* (Fig. 5). Nevertheless, as the genus *Ammonia* is also known to tolerate organic enrichment, a synergistic interaction of both processes (chemical and organic pollution) may have caused such community change.

5.4. The chronology of environmental changes in Panzano Bay over the last 400 years

Integration of foraminiferal and geochemical proxies, combined with a robust chronological framework based on extensive radiometric dating of mollusc shells, reveals four major phases in the recent history of Panzano Bay.

During the 17th and 18th century, the effects of port activities, as well as of agriculture in the surrounding area on the composition of foraminiferal assemblages are negligible. In the early 18th century, the release of high amounts of mercury into the environment is related to increasing activity at the Idrija mercury mine (Faganelli et al., 2003). These high inputs, however, did not affect foraminiferal communities because the dominant particulate form of Hg is not bioavailable. Environmental conditions in Panzano Bay during this period are characterized by strong seasonal variations of Isonzo River runoff and variable organic matter input: the foraminiferal community was therefore composed predominantly of stress tolerant species adapted to such unstable conditions.

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During the 19th century, metal recovery at the Idrija mine improved (Covelli et al., 2006) and less mercury was released into the bay. The onset of maricultural activities here area dates back to this period, when bivalve farming was established along the eastern coast of the Gulf of Trieste (Melaku Canu and Solidoro, 2014). This also marks the construction of the port of Monfalcone. The effects of bivalve farming as well as of agricultural and port activities remain negligible during this period.
The first half of the 20th century, however, is marked by rapid technological development. In Panzano Bay, agricultural, maricultural and port activities intensified. The associated slight increase of nutrients caused an increase in foraminiferal diversity and a shift in the trophic mode of the species.
In the second part of the 20th century, the Monfalcone thermoelectric plant, powered by coal and fuel oil, became operative. This slightly increased the concentrations of persistent organic pollutants caused a minor change in the foraminiferal community composition. The nutrient increase that started in the early 20th century extended to this period. As a consequence, the trend of increasing foraminiferal diversity continues until today.

6. Conclusions

The chronology of changes in the geochemical composition of sediments and foraminiferal assemblages in shallow and sheltered marine embayments of the northern Adriatic reflects agricultural and industrial development, coastal eutrophication and natural variations. Mercury is a major pollutant in the area, whose concentrations during the last 400 years have significantly exceeded Italian sediment quality guidelines, ERL and ERM. Surprisingly, these high concentrations have not affected the ecosystem because the mercury species are not bioavailable to foraminifera.
The impact of agricultural, maricultural and industrial activities intensified during the second half of the 20th century and is ongoing. This is reflected in increasing concentrations of trace elements and persistent organic pollutants (PAH, PCB), as well as in progressive nutrient enrichment.

The foraminferal response to such anthropogenic impacts are shaped by their long history of adaptation to naturally elevated trace element concentrations. Consequently, the shift in community composition during the 20th century reflects a combination of factors, including the recorded increase of pollutants, varying natural conditions, but also a natural, preindustrial predisposition of foraminifera here to tolerate trace elemental pollution.

This combination of factors - and therefore our results - are clearly applicable to many other shallow coastal areas impacted by human activities, which are largely synchronised ony a global scale. Finally, our approach points to the importance of using long term baseline data for evaluating the environmental and ecological status of present day marine ecosystems.

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Figure 1. Study area and location of sampling site in Panzano Bay.

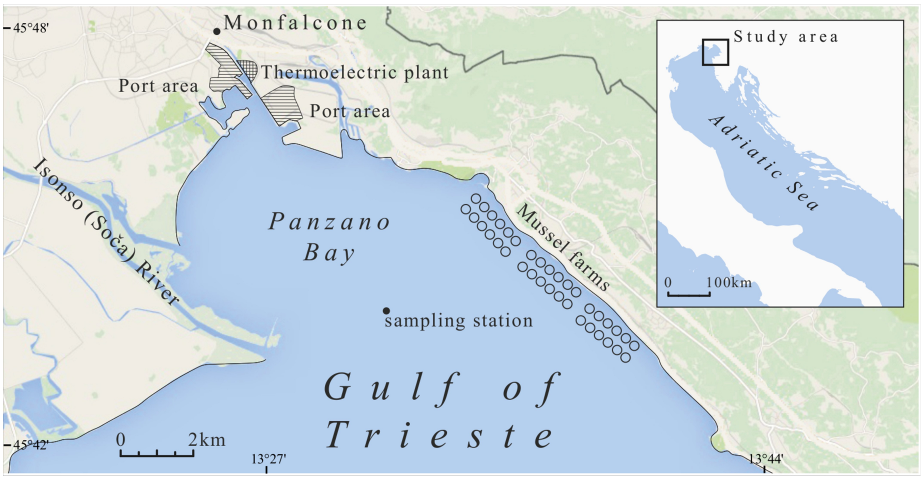

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Figure 2. Radiocarbon calibrated AAR dating of shells of the bivalve species *Varicorbula gibba*.

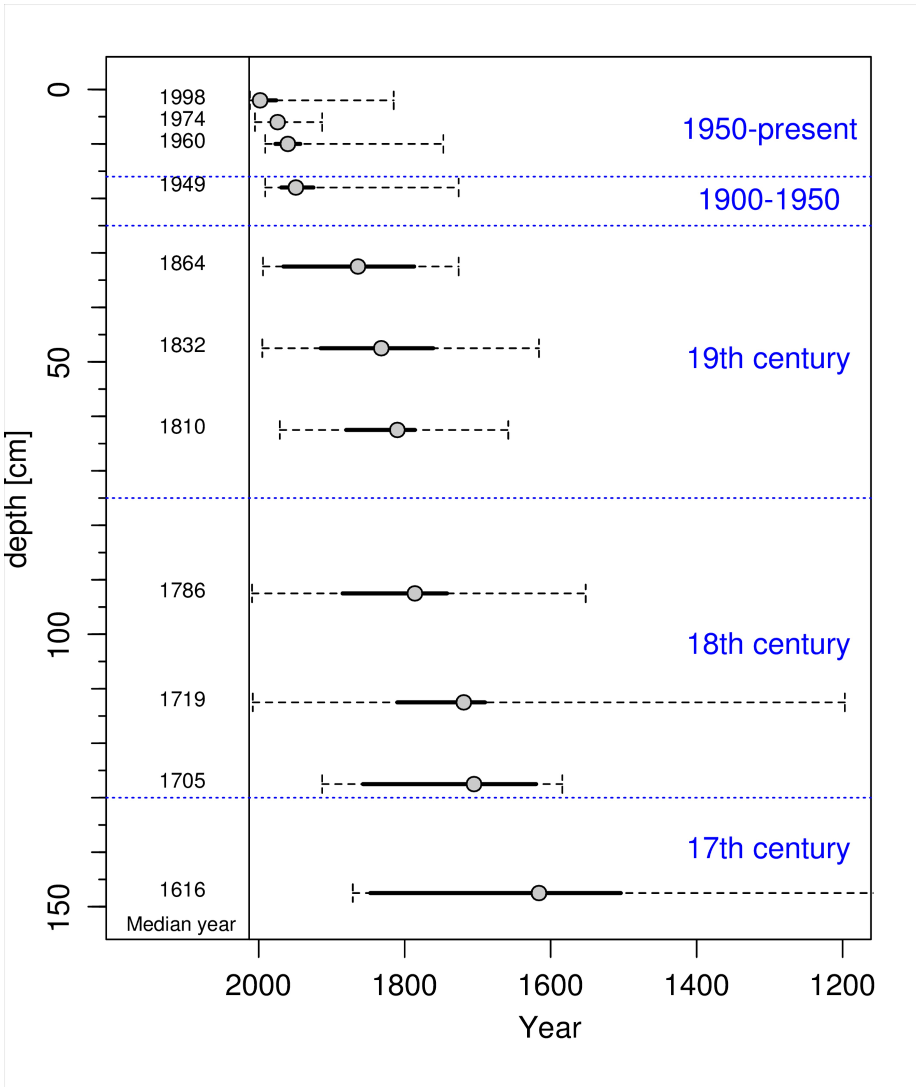

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Figure 3. Vertical changes in grain size, major, minor and trace elements, nutrients and persistent organic pollutants.

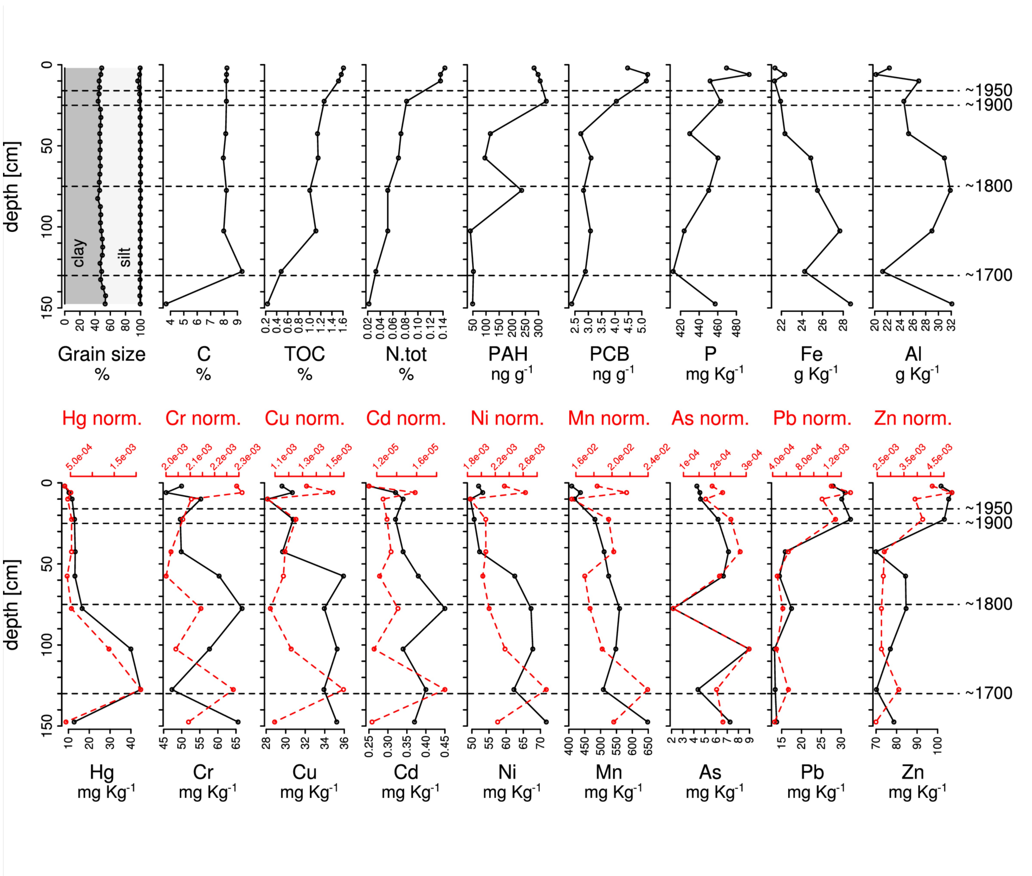

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Figure 4. Principal component analysis (PCA) plot of geochemical data.

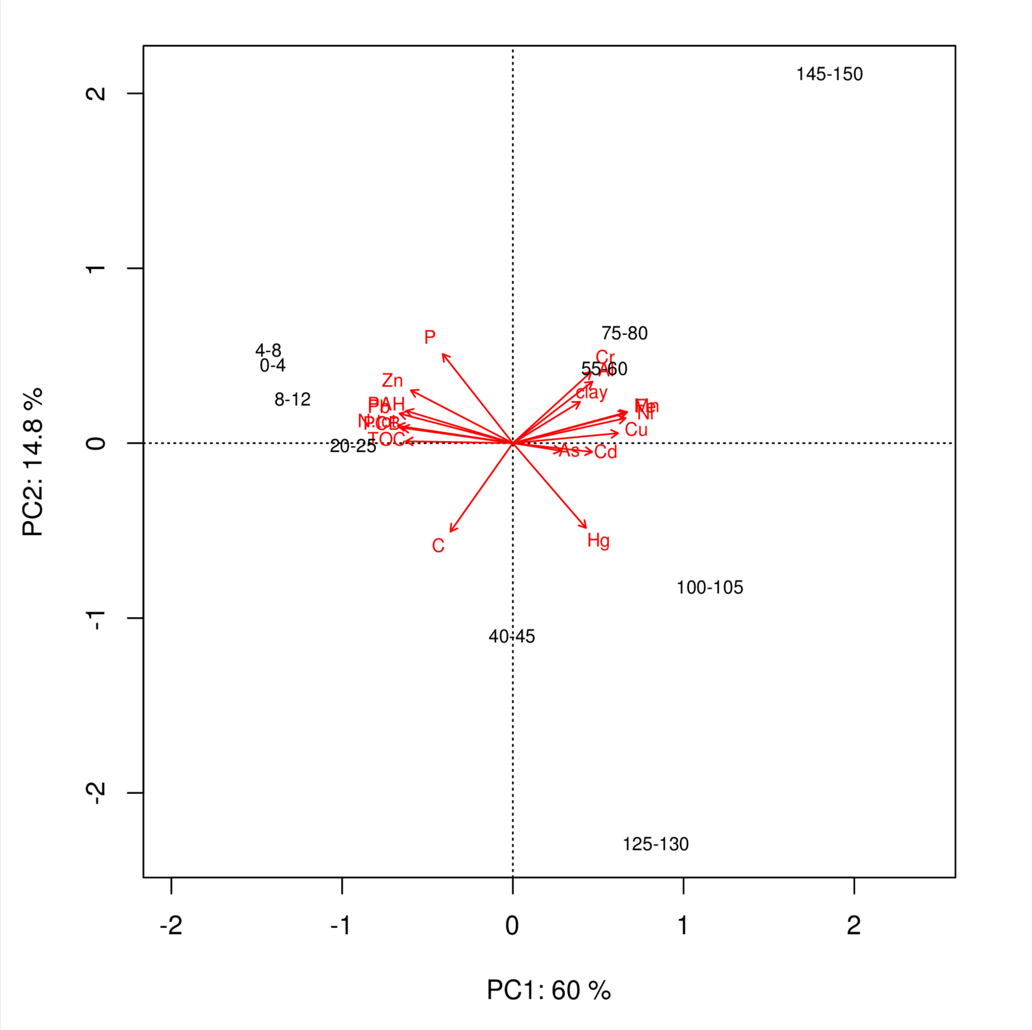

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Figure 5. Temporal trends in the relative abundance of foraminiferal suborders (following suprageneric classification of Loeblich and Tappan, 1987) and dominant genera and species (represented by >2 % of individuals in the pooled data).

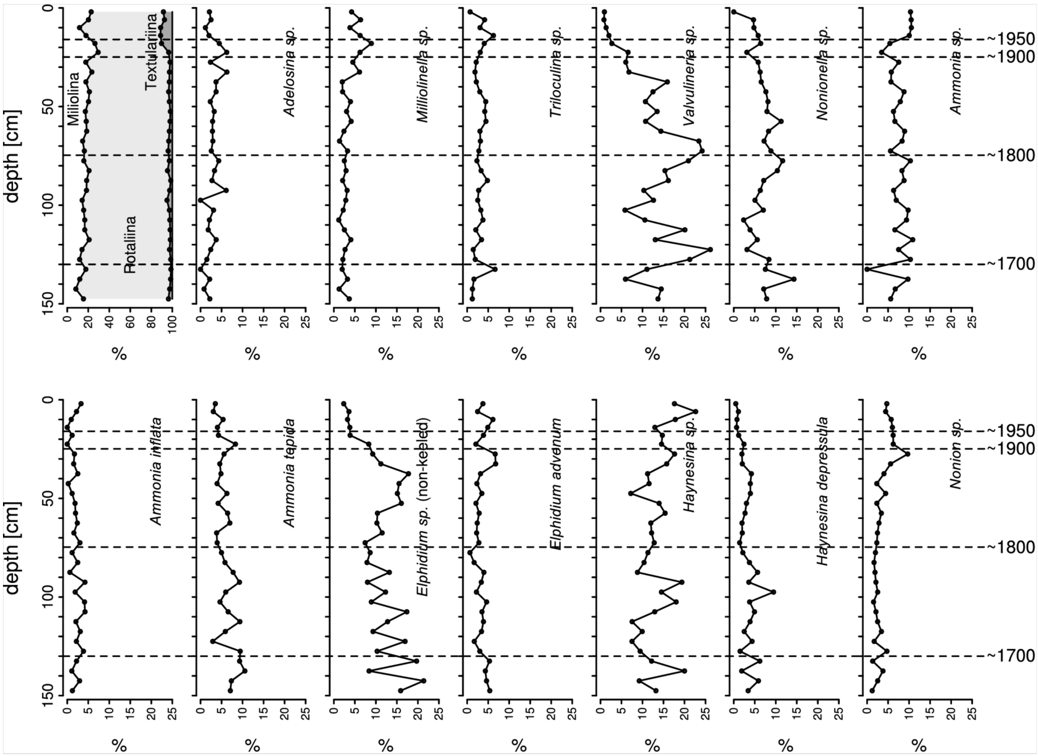

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Figure 6. (a) Temporal trends in species richness, Fisher's α index, the exponent of the Shannon index. Shown are mean values with 95% CI after rarefying to 240 individuals per sample. **(b)** Temporal trends in species composition summarized by NMDS axis 1 scores, **(c)** Chronological clustering of foraminiferal assemblages (different colours group samples separated by a major compositional shift, with the number of temporal bins determined by the broken stick model), **(d)** Temporal trends in the relative abundances of substrate relation groups, **(e)** Temporal trends in the rarefied species richness of each substrate relation group, **(f)** Temporal trends in the relative abundances of two foraminiferal groups according to their tolerance to different types of pollution (organic and organic/chemical). Figures D-F are plotted based on data in Table S1.

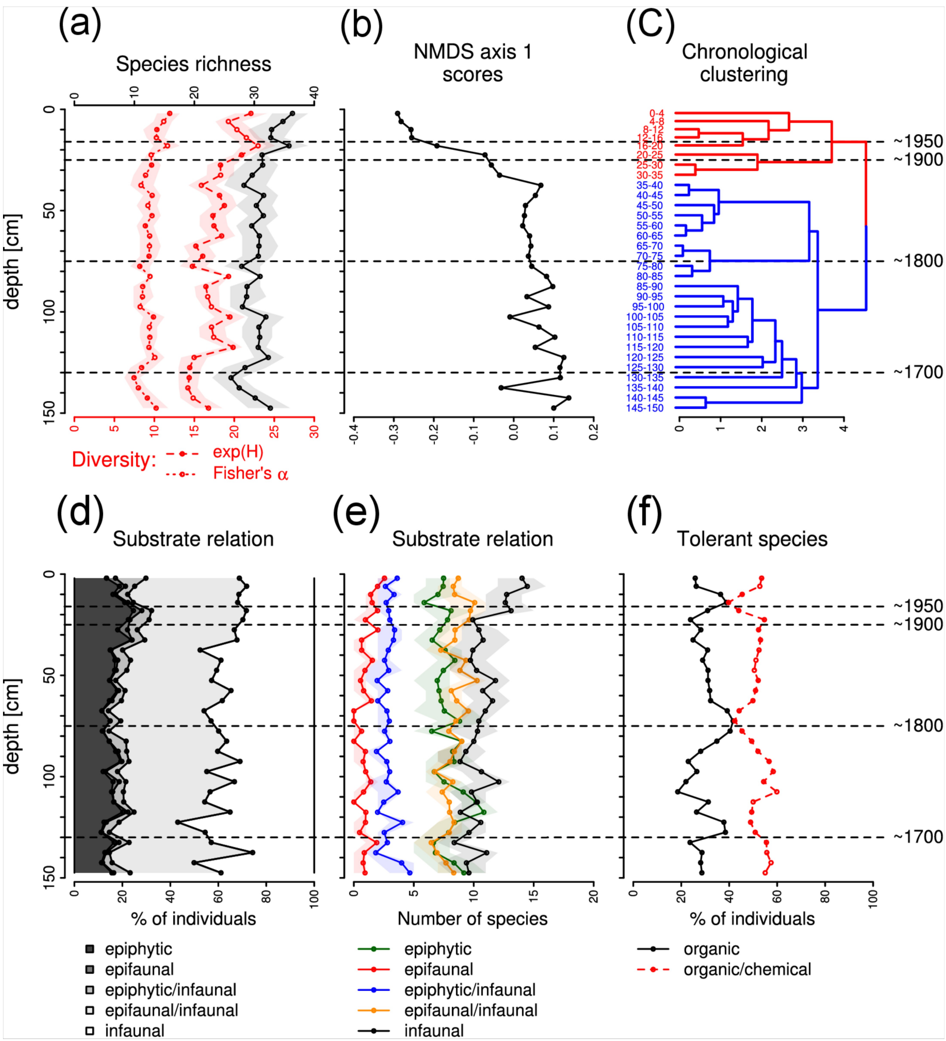

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Figure 7. Non metric multidimensional scaling ordination of the foraminiferal assemblages.

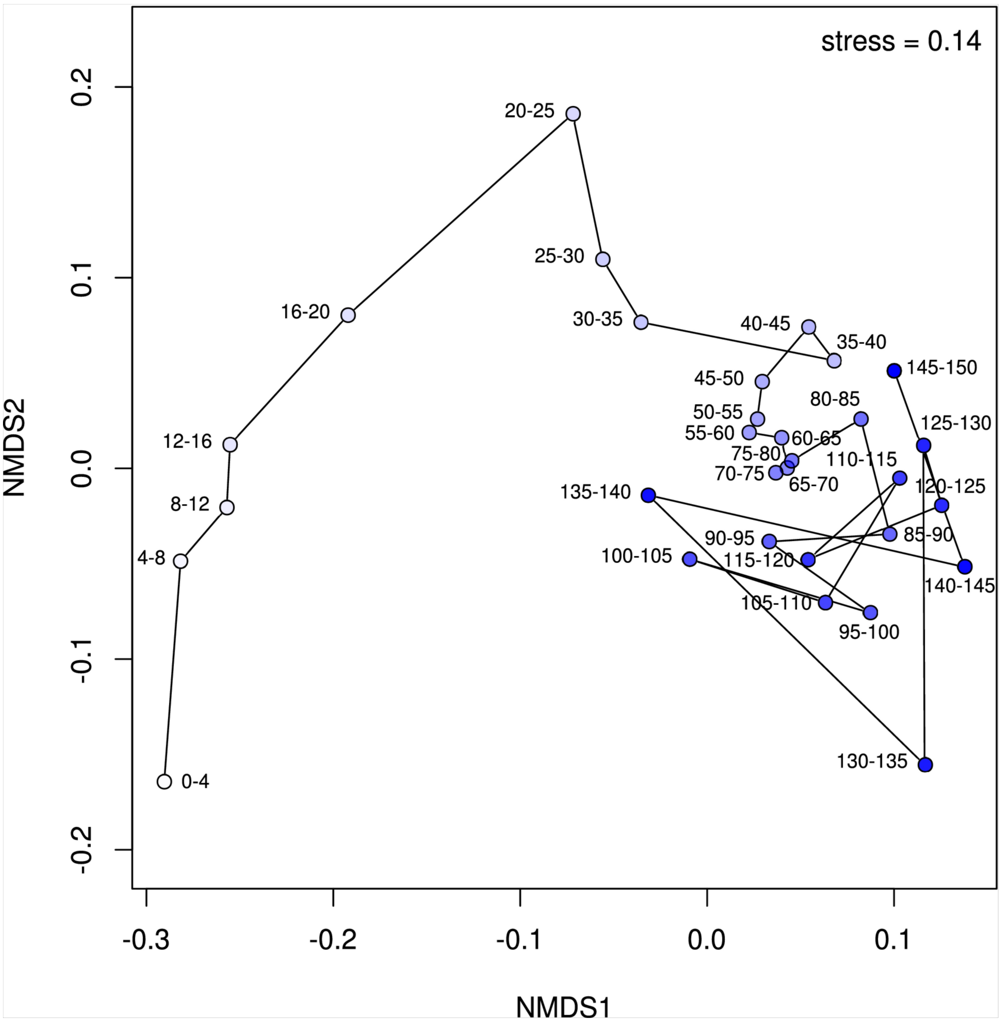

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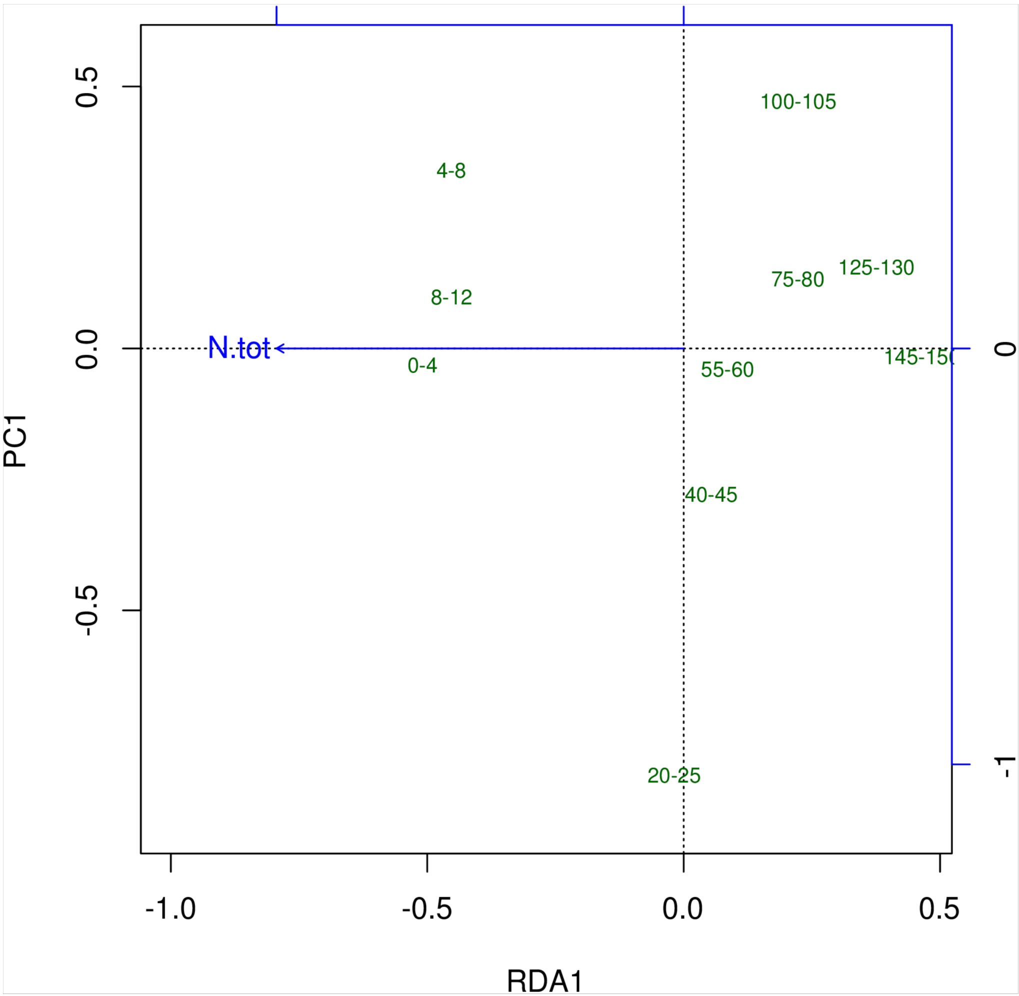
Figure 8. Redundancy analysis triplot of the foraminiferal and geochemical data based on the results of the forward model selection. Nitrogen concentrations were the only selected variable. The first canonical axis explains 42.4 % of the total variance in the data.



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Titles for supplementary tables

Table S1. (a) Absolute abundances of foraminiferal species with their ecological characteristics and tolerances to organic and chemical pollutants, **(b)** full list of all used sources; **(c)** diversity of foraminiferal community including rarefied species richness, Shannon index, the exponential of Shannon index and Fisher's α.

Table S2. Concentrations of major, minor and trace elements, Italian national sediment quality guidelines (SQG), ERL and ERM standards.

Table S3. Pearson's correlation coefficients between clay content, geochemical data, NMDS axis 1 scores and rarefied species richness in the subset of samples with measured elemental concentrations.

Table S4. Results of the forward model selection in the redundancy analysis. Proportion of variance explained in the community data (R^2), F-statistic and P-values from permutation tests are reported for (a) models with a single explanatory variable and (b) for the effects of a second variable added to the model already including total nitrogen.

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