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RESEARCH

Soft bottom benthic communities under potentially anthropic and natural pressures in Terra Nova Bay (Ross Sea, Antarctica)

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Abstract

Benthic communities have a key function in the Antarctic marine ecosystem since they play a central role in the food web pathway and represent the main "environmental biological memory". The goal of the study is to evaluate differences in macrozoobenthic community structure, along the Antarctic coasts of Tethys Bay (Ross Sea), potentially affected by two types of pressures: anthropic, due to the presence of a scientific station, and natural, due to the presence of a penguin colony. In the austral summer of 2017/2018 and 2018/2019, four sites were sampled in order to detect the effects of these two types of pressure (anthropic *vs*. natural *vs*. two control non-impacted areas). The Road Bay site, near the Italian Station, is rich in macro- and micronutrients and NO^{3-} and Cd^{2+}; the Adélie Cove site, characterized by organic loads from a close penguin colony, is rich in PO_4^{3-}. Lower values of NO^{3-} and PO_4^{3-}, as well as Cu^{2+} and Pb^{2+} heavy metals occurred in control sites, between Road Bay and Adélie Cove. The benthic community showed different qualitative–quantitative dominances of species among sites, mainly at 50 m depth. The results clearly show the dominance of different opportunistic species of macrobenthos where penguins or humans' presence occurred. Therefore, benthic communities may be considered good biological indicators of the overall environmental conditions.

Keywords Biodiversity · Community structure · Environmental parameters · Shallow water · Bathymetrical zonation · Ross Sea

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Introduction

The biodiversity is generally related to the controversial concept of latitudinal diversity gradient (LDG) at a global scale, according to which, both in freshwater and marine or terrestrial environments, there is an increase of taxa richness from poles to tropical regions (Fischer 1960; Stehli and Wells 1971; Gallardo 1987; Hillebrand 2004; Cairns 2007; Coll et al. 2010; Fautin et al. 2013). It is worth noting, that not all marine taxa show this correlation between diversity and latitude. Concerning the marine benthic communities, Silva (1992) found that macroalgae reach their acmes in temperate latitudes, while Coates (1998) detected no clear trend in species diversity or density between tropical and temperate areas for rocky intertidal assemblages. It is widely known that benthic communities distribution is strongly influenced by key environmental factors, such as depth, light, water movement, salinity, oxygen availability and organic enrichment, producing heterogeneous communities of both soft and hard substrates, rapidly responding to

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changes in environmental conditions. Along the coasts of the Antarctic continent, but also in Arctic areas, seawater temperature and sea ice formation represent the major drivers of marine species and ecosystem functioning (Eicken 1992). The former represents a biogeographic barrier that strongly separates the polar benthic communities from those occurring in temperate and tropical waters. Cold water conditions produce not only a reduction of biodiversity, if compared with that of non-polar zones, but also an increase of species size (Antarctic giant) and a higher percentage of endemic species (Dayton 1990; Arntz et al. 1997; Chapelle and Peck 1999; Gutt et al. 2021). The second major driver, sea ice, represents an additional important physical stressor producing a shift below the shallow zones of available substrata for macro- and megazoobenthic species (Lenihan and Oliver 1995; Clarke 1996; Barnes and Conlan 2007, and references therein). Sea ice is very important also for primary production at the base of the food web of coastal benthic communities (Garrison and Close 1993; Loeb et al. 1997).

The main components of the macrozoobenthos are polychaetes, mollusks and crustaceans. They reach about 92% of soft-bottom Antarctic communities (Clarke 1996). These three taxonomic groups are characterized by very high species diversity (Gambi et al. 2000; Griffiths 2010) and are widely known as the key components of marine biodiversity, showing several adaptations to different physico-chemical conditions and food sources (Clarke 1979; Gambi et al. 1994; Peck and Conway 2000; Ellingsen 2002). This is particularly true for the nearshore communities that are in a transitional boundary between the terrestrial and open sea ecosystems, more related to natural and anthropic pressure, such as high sedimentation rate, pack-ice melting, and organic and heavy metal enrichment. Taking into account the human activities along the coasts of the Antarctic continent, even though confined, they strongly increased in the last decades, primarily due to the fishing and research activities, that produce naval traffic, oil spillage, heavy metals and sewage pollution (De Moreno et al. 1997; Griffiths 2010; Lim et al. 2021). These anthropogenic disturbances may strongly affect the benthic community around the Antarctic research stations (Lenihan and Oliver 1995; Stark et al. 2003; Conlan et al. 2004, 2010; Martins et al. 2012; Palmer et al. 2021). Mario Zucchelli Italian Station (MZS) was established along the Terra Nova Bay (Ross Sea) during the Austral summer of 1985 (Manzoni and Zucchelli 1991), and represents a relevant opportunity to assess the potential anthropic pressure on the nearshore benthic communities characterizing the soft bottoms.

A few kilometers away from the MZS, an Antarctic Special Protected Area (ASPA) was established in 2003. It is an extended marine zone aimed to prevent or reduce anthropic disturbances on the biodiversity of benthic communities. A large and old Adélie penguin (*Pygoscelis adeliae* [Hombron & Jacquinot, 1841]) colony is present along the coast of ASPA (Gao et al. 2022). Penguins represent non-negligible bio-vectors of heavy metals and of organic pollutants in sediment (Nie et al. 2012; Santamans et al. 2017). However, very less attention has been devoted to exploring the effect of this pressure on macrozoobenthic communities. Thereby, the primary goal of this study is to investigate the structure of macrozoobenthic communities and their ecological distribution in two shallow areas, one close to the MZS, exposed to potential anthropic pressure, and the other one close to the Adélie Cove penguin colony, exposed to potential natural pressure. After a first step focused on meiobenthos (Semprucci et al. 2021), this study represents the second step of data analysis, focused on macrobenthic communities sampled during the 33rd and 34th Italian Antarctic expeditions (austral summers 2017/2018 and 2018/2019).

Materials and methods

Study area and sampling design

The study area was chosen within Terra Nova Bay (Ross Sea), along a coastal marine area between Tethys Bay and Adélie Cove. Taking into account different types and levels of pressure (anthropic *vs*. natural *vs*. two control non-impacted areas), a sampling program was performed in four sites (Fig. 1), during two Italian expeditions in the Austral summer of 2017/2018 and 2018/2019. The four sites were: Rod Bay (RB, 74° 42′ S–164° 07′ E), potentially impacted by effluents from the sewage system of MZS (anthropic pressure); Adélie Cove (AC, 74° 42′ S–164° 07′ E), within ASPA n°161, influenced by excrements of a wide penguin colony (natural pressure); two control sites between Rod Bay and Adélie Cove, in ASPA n°161, namely Control Rod Bay (CRB, 74° 42′ S–164° 07′ E) and Control Adélie Cove (CAC, 74° 42′ S–164° 07′ E). At each site (RB, AC, CRB and CAC), two stations were sampled at 20 m and 50 m depth, respectively. At each station, four replicates were performed by a 12 l van Veen grab to collect benthos and sediments. One replicate was used to analyze sediments (*i.e.*, interstitial water chemistry, grain size and total organic carbon [TOC]) and the remaining three to analyze the macrobenthic community.

Analytical procedure

To sample the interstitial water of sediments for chemical analyses, a syringe (20 ml) was introduced into the sediment and the collected water was kept frozen until analyses. For grain size and TOC analyses, sediment was collected by a cylindrical corer with a 2.8 cm diameter and 5 cm down into the grab.

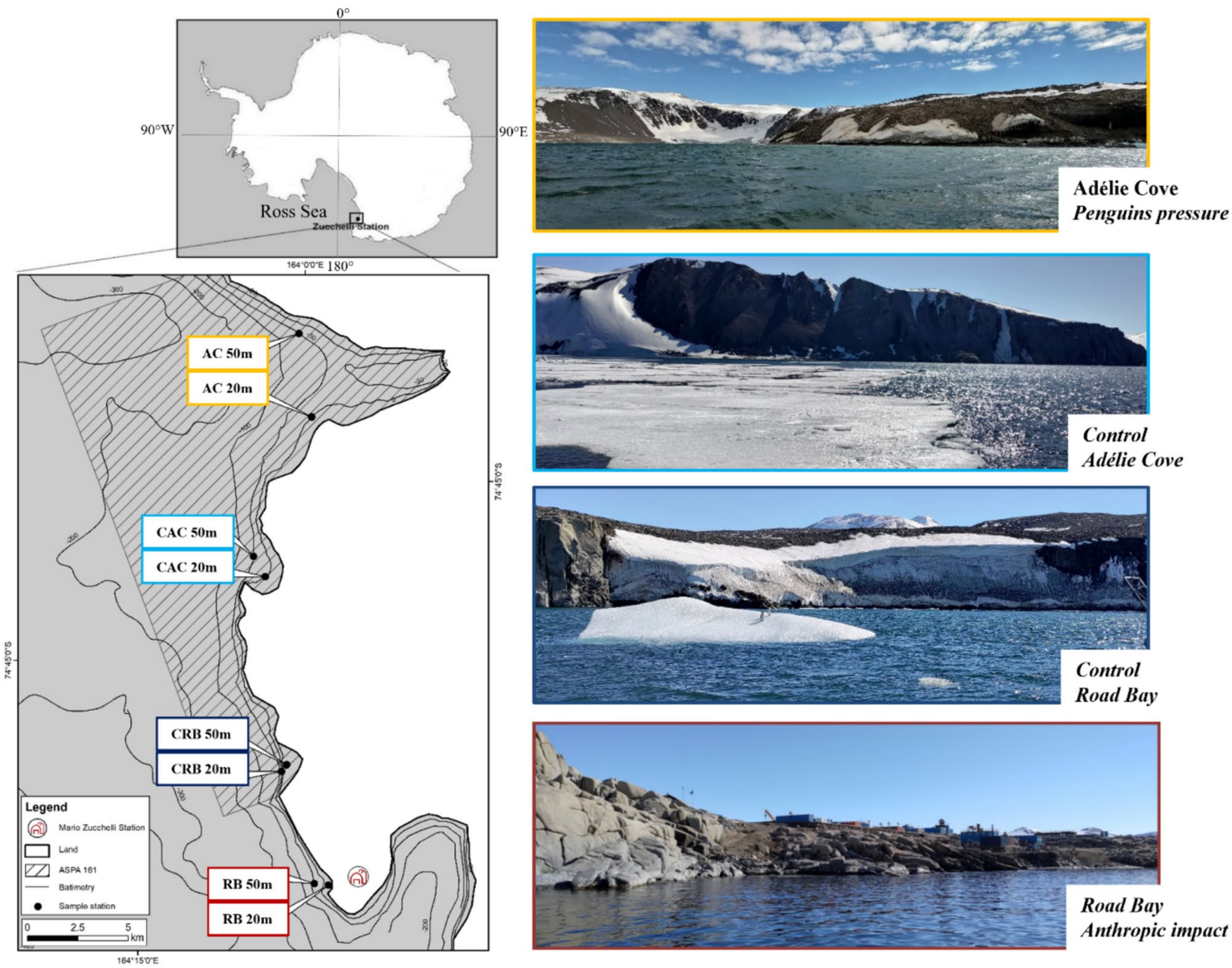

Fig. 1 Study area showing the four sampling sites in Terra Nova Bay (Ross Sea): *RB* Road Bay (Anthropic impact), with the Italian Mario Zucchelli Station (MZS) on the coast, *AC* Adélie Cove (Penguin colony area), *CRB* Control Road Bay, *CAC* Control Adélie Cove Photographs by L. Donnarumma

Prior to chemical analysis of interstitial water, the samples were thawed, filtered directly from the syringes using 0.20 µm porosity filters, and treated with H_2O_2 (100 µl in 10 ml of sample) for organic carbon digestion; samples were then fractioned in two aliquots for ions and metals determinations.

The analysis of major and minor ions concentration was performed using a Dionex ICS1100 apparatus; anions (Cl^-, F^-, Br^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-} as inorganic species, and $HCOO^-$, CH_3COO^-, and $C_2O_4^{2-}$ as organic species) were determined with a system equipped with an ASRS 300-4 mm suppressor, an AS22 column working with a cell volume of 100 µl and a buffer solution with 3.5 mM of sodium carbonate/bicarbonate as eluent (flow rate of 1.20 ml/min). Cations (Li^+, Na^+, K^+, NH_4^+, Ca^{2+} and Mg^{2+}) were determined using a system equipped with a CERS 500-4 mm suppressor, a CS12A column working with a cell volume of 25 µl and 20 mM methanesulfonic acid solution as eluent (flow rate of 0.25 ml/min). Calibration curves for anions and cations quantification were defined using certified multistandard solutions.

Metals determination was carried out using voltammetric analyses with a Metrohm 797 VA Computrace (Mercury electrode). To limit the matrix effects, the standard addition procedure was applied as a calibration method. All elements were quantified using linear regression based on the height of voltammogram peaks; this method allows the determination of zinc, cadmium, lead and copper. Details on the analytical method's accuracy were reported in Chianese et al. (2019).

According to Eleftheriou and McIntyre (2008) methodology for grain size evaluation, sediments were overdried at 60 °C for 48 h and sieved over a series of sieves, with mesh sizes ranging from 1 to 0.25 mm; three main sediment fractions were considered: coarse sand (sediment fraction $\O \geq 1$ mm), medium sand (1 mm $< \O <$ 0.25 mm), and fine sand ($\O <$ 0.25 mm), while TOC was determined according to Schumacher (2002), combusting the sediment matter content in an oven at 500 °C and the difference in weight loss between initial and final sample was calculated and expressed as TOC % in the analyzed sediment.

To analyze the zoobenthic community, the samples were carefully washed through a mesh sieve (0.5 mm) and the retained fraction was fixed in 70% ethanol and seawater solution. Macrofauna was then sorted under a stereomicroscope, and identification was obtained to the lowest taxonomic level, when possible. The name of the species was cross-checked with the World Register of Marine Species (WoRMS 2023).

Statistical analyses

Multivariate patterns of normalized data of chemical and sedimentological variables among sites, related to time (I—2017/2018 and II—2018/2019 austral summers) and stations (20–50 m for each site), were visualized by principal component analysis (PCA).

The macrozoobenthic community was analyzed through synecological indices: the abundance (N), species richness (SR), Shannon–Weaver diversity index (H′) and Pielou evenness (J). Community data were subject to statistical analyses using permutational multivariate analysis of variance (PERMANOVA, Anderson 2001), based on Euclidean distance of transformed data (log $x + 1$), to test differences among the following factors: years (Y, fixed, 2 levels: I *vs*. II), sites (Si, fixed, 4 levels, Anthropic [RB]; Natural [AC]; Controls [CRB; CAC]) and stations (St, fixed, 2 levels, 20 m *vs*. 50 m). With the same computational design, PERMANOVA analysis, based on Bray–Curtis similarity of transformed data, was performed to assess differences in the structure of community assemblages [Clarke and Warwick 2001]). Post hoc pair-wise comparisons, using the PERMANOVA t-statistic, were also applied when needed. To visualize patterns of multivariate analyses, Canonical Analyses of Principal coordinates (CAP) will be also used. In addition, the similarity percentage (SIMPER) was calculated to identify those species that contributed more to the similarity among sites, as well as the species that better characterized each site. Finally, chemical and sedimentological data were related to community assemblages using distance-based linear modeling (DistLM) and visualized through redundancy analysis (dbRDA) (Legendre and Anderson 1999). All multivariate analyses were performed using the PRIMER-PERMANOVA + v.6 software package (Clarke and Gorley 2006; Anderson et al. 2008).

Results

Chemical and sedimentological parameters

Sediment and interstitial water chemistry are summarized in Table 1. Sediment's grain size analyses showed high percentages of medium sand and/or fine sand at all sampling sites (Fig. 2), with overall mean values of 46.37 (± 25.16)% and 31.13 (± 25.07)%, respectively. Coarse sand was less represented, with an overall mean value of 20.50 (± 12.82)%. In particular, at each site, medium sandy sediment mainly occurred at 20 m depth, while it decreases of about 40% at 50 m depth. On the contrary, at each site, fine sandy sediments were abundant at 50 m depth, showing a decrease of about 34% at 20 m depth. The coarse sand fraction occurred with higher percentages at 20 m in RB (39.10%) and CRB (26.84%) compared to 50 m depth, while occurred with higher percentages at 50 m depth in CAC (37.73%) and AC (15.46%) compared to 20 m depth. As for TOC concentrations (Table 1), the highest values were detected at CRB, both at 20 m (4.04%) and 50 m (3.02%) depths, while the lowest values were detected at CAC, both at 20 m (1.26%) and 50 m (1.15%) depths.

As for ions of interstitial water (Fig. 3a; Table 1—Ions_µMol), Mg^{2+} was the most abundant ion, followed by SO_4^{2-}, K^+ and Ca^{2+}, occurring with quite similar concentrations at each station, while PO_4^{3-} and NO_3^- occurred with lower values. As for as heavy metals (Fig. 3b; Table 1—Metals_nMol), Zn^{2+} was the most abundant, followed by Cu^{2+}, Pb^{2+} and Cd^{2+}.

Given 100 the total concentration of each parameter at all sites/stations, their percentage contribution was individually calculated (per each sites/station). As for ions (Fig. 3c), at 20 m depth, the highest percentage value of NO_3^- (20.58%) occurred at RB, while the highest value of PO_4^{3-} (70.53%) occurred at AC, while the lowest values of NO_3^- (17.48%) and PO_4^{3-} (21.20%) occurred respectively at 20 m in CRB and at 50 m in CAC. As for heavy metals (Fig. 3d), the highest percentage value of Cd^{2+} occurred at RB at 50 m depth, with a concentration of 40.55%, while the highest value of Zn^{2+} (31.72%), Pb^{2+} (32.53%) and Cu^{2+} (26.48%) was recorded in AC at 20 m depth. Conversely, the two control sites (CRB and CAC) showed heavy metals with low mean concentration values.

The above sediment and interstitial water variables, in both years of study, were also processed through multivariate PCA analysis (Table 2) and graphically shown in a PCA plot (Fig. 4). In the plot, two separated clusters occurred according to the depth factor (20 m *vs*. 50 m), for both sampling years. PC1 accounted for 31.3% of variation among sites and PC2 for 22.8%. Along the first axis,

Table 1 Chemical and sedimentological parameters of the study area expressed as mean value (±S.D.) among all replicates of two sampling years (2017/2018–2018/2019) for each site (RB; CRB; CAC; AC) and station (20 m; 50 m)

Variables	RB		CRB		CAC		AC	
	20 m	50 m	20 m	50 m	20 m	50 m	20 m	50 m
Sediment variables								
TOC/100 g (%)	1.76 (±0.26)	1.82 (±0.11)	4.04 (±0.47)	3.02 (±0.45)	1.26 (±0.20)	1.15 (±0.20)	2.35 (±0.03)	1.91 (±0.34)
Coarse sand (%)	39.10	23.83	26.84	25.43	7.80	37.73	3.81	15.46
Medium sand (%)	53.71	28.82	73.14	29.89	89.74	29.08	50.01	16.54
Fine sand (%)	7.19	47.35	0.01	44.68	2.46	33.19	46.17	68.00
Interstitial water variables								
Ions (µMol)								
NO_3^-	0.31 ± 0.07	0.21 ± 0.04	0.11 ± 0.12	0.14 ± 0.15	0.13 ± 0.14	0.27 ± 0.05	0.14 ± 0.15	0.22 ± 0.02
PO_4^{3-}	< lod	< lod	< lod	0.56 ± 0.01	< lod	0.22 ± 0.24	1.87 ± 0.01	< lod
SO_4^{2-}	25.56 ± 1.49	25.16 ± 1.95	23.73 ± 5.88	25.4 ± 1.64	23.52 ± 3.42	26.78 ± 2.54	25.37 ± 1.30	25.78 ± 1.57
K^+	10.47 ± 0.43	8.78 ± 1.07	10.79 ± 0.53	10.01 ± 1.46	10.66 ± 0.24	9.90 ± 1.51	8.02 ± 2.14	7.74 ± 2.47
Mg^{2+}	55.57 ± 3.25	43.22 ± 8.65	48.32 ± 5.65	49.23 ± 5.43	48.38 ± 5.52	48.01 ± 5.98	39.63 ± 14.40	38.41 ± 17.06
Ca^{2+}	6.92 ± 1.50	6.4 ± 0.92	6.35 ± 1.06	6.07 ± 0.62	5.38 ± 0.57	6.88 ± 0.48	5.90 ± 0.05	5.26 ± 1.34
Metals (nMol)								
Zn^{2+}	20.70 ± 1.80	18.65 ± 1.09	13.84 ± 2.17	7.72 ± 1.97	25.87 ± 4.80	8.16 ± 1.52	49.24 ± 6.91	11.06 ± 2.85
Cd^{2+}	0.03 ± 0.03	4.97 ± 1.14	1.05 ± 0.28	2.45 ± 0.66	0.49 ± 0.54	0.60 ± 0.26	2.64 ± 1.17	0.03 ± 0.04
Pb^{2+}	1 ± 0.91	9.09 ± 1.19	12.55 ± 1.20	0.44 ± 0.49	7.37 ± 2.84	0.63 ± 0.71	15.47 ± 1.64	0.71 ± 0.78
Cu^{2+}	2 ± 1.08	9.94 ± 1.20	15.22 ± 1.31	2.4 ± 0.65	10.73 ± 1.70	1.19 ± 0.62	15.73 ± 2.16	2.19 ± 0.77

Interstitial water parameters are expressed as µMol and nMol

lod limit of detection

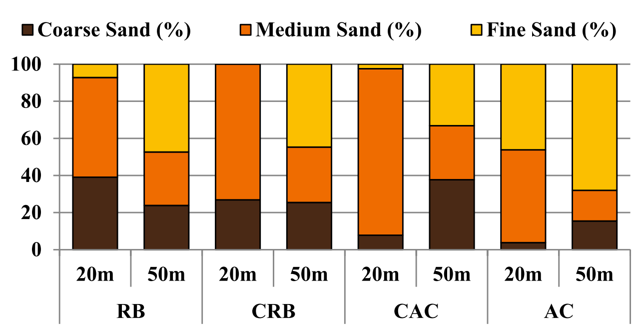

Fig. 2 Particle size analyses. Contributions of each class size percent values at each site (RB; CRB; CAC; AC) and station (two sampling depths: 20 m; 50 m)

the ion PO_4^{3-} and the heavy metals Zn^{2+}, Pb^{2+} and Cu^{2+} were mainly correlated to AC at −20 m, for both years, while coarse sand showed a high correlation to the −50 m sites, always for both years (Fig. 4—PC1). Along the second axis, medium sand was mainly correlated to first year control sites (CRB and CAC) −20 m, and fine sand to first year AC −50 m, while Mg^{2+} and K^+ were mainly correlated to second year RB and CAC (Fig. 4—PC2).

Benthic community assemblages

During the two Antarctic sampling periods, a total of 3264 individuals, belonging to 3 major benthic taxonomic groups (polychaetes, crustaceans and mollusks) were collected, analyzed and identified. A total of 31 species (or higher taxon) and 26 families (Supplementary material: Table S1) have been found. The polychaete taxon showed the highest number of individuals (1996), belonging to 13 species and 10 families, followed by crustaceans (813 ind., 6 sp., 5 families) and mollusks (455 ind., 12 sp., 11 families). The most abundant species for each taxonomic group were the polychaete *Leitoscoloplos kerguelensis* (McIntosh, 1885) (1014 individuals), the amphipod *Lysianopsis subantarctica* (Schellenberg, 1931) (626 ind.) and the gastropod *Subonoba turqueti* (Lamy, 1906) (178 ind.). These three species alone account for 55.70% of total quantitative dominance and show a frequency among samples of 87.50%, 81.25% and 41.67% respectively (Table S1). They were dominant species in both depths (20 m and 50 m), but their abundance drastically increases with the increasing depth, with a percentage of 57.94 (±24.85)% for *L. kerguelensis*, 81.90 (±7.41)% for *L. subantarctica* and 31.77 (±17.50)% for *S. turqueti* (Table S1). Other abundant species were the polychaete *Aglaophamus trissophyllus* (Grube, 1877) (475 ind.)

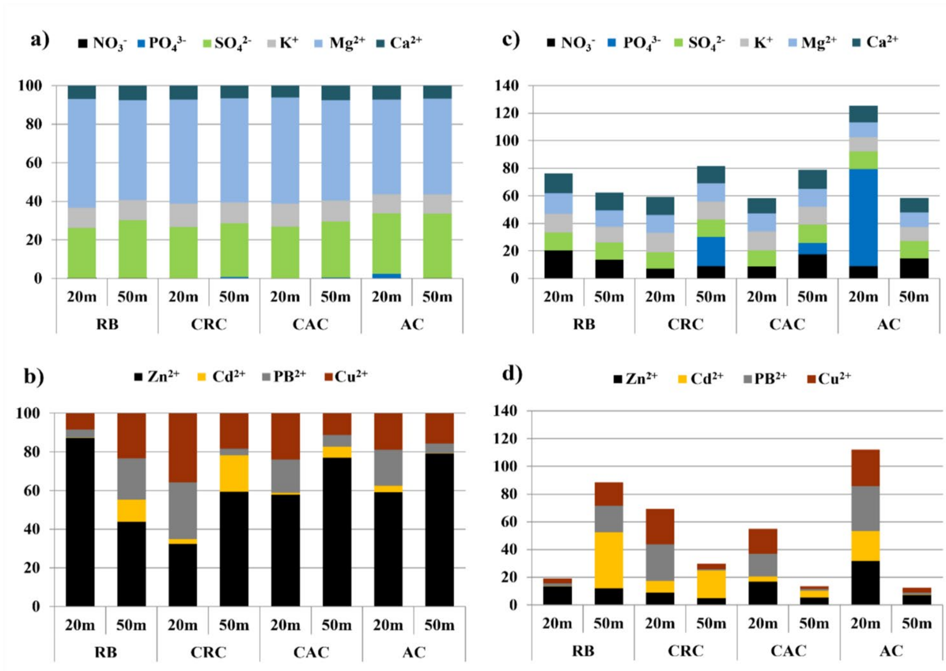

Fig. 3 Interstitial water parameters. Contribution of each parameter percent value at each site/station (**a**, **b**). Percent contribution of each parameter at all sites/stations, considering as 100 the total cumulative concentration of each parameter in the sampled area (**c**, **d**)

Table 2 PCA of sediment and interstitial water variables for potentially impacted and control sites sampled at Terra Nova Bay

PC	Eigenvalues	%Variation	Cum. %Variation
1	**4.38**	**31.3**	**31.3**
2	**3.19**	**22.8**	**54.1**

Variables	PC1	PC2
NO_3^-	−0.279	−0.054
PO_4^{3-}	**0.303**	0.095
SO_4^{2-}	−0.187	−0.041
K^+	−0.191	**−0.464**
Mg^{2+}	−0.241	**−0.396**
Ca^{2+}	−0.205	−0.218
Zn^{2+}	**0.352**	−0.095
Cd^{2+}	0.155	0.055
Pb^{2+}	**0.4**	−0.212
Cu^{2+}	**0.394**	−0.26
Coarse sand	**−0.380**	−0.066
Medium Sand	0.181	**−0.438**
Fine sand	0.013	**0.474**
TOC	0.135	−0.147

Bold values were considered high (≥|0.300|)

and the bivalve *Aequiyoldia eightsii* (Jay, 1839) (127 ind.), that together with the three previously mentioned species, account for 75% of total individuals. Also for these latter two species (*A. trissophyllus* and *A. eightsii*), an increase in abundance with the depth was observed: 11.32 (±7.44)% for the first and 93.02 (±2.97)% for the second species. This increase in abundance regards all the species of the community (Fig. 5) and was constant over time (Two sampling years—PERMANOVA pairwise comparison: $F=1.6585$, $p=0.2114$).

When comparing each synecological index between the two stations of a site (*t* and *p* values in Table 3), some

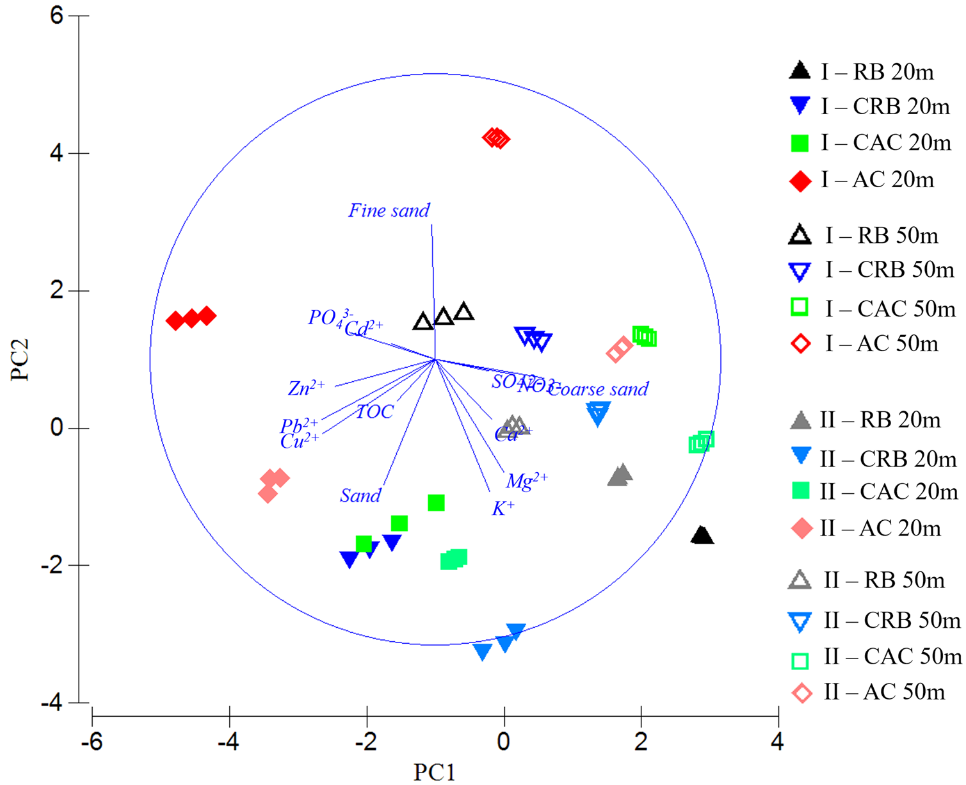

Fig. 4 PCA ordination of sediment and interstitial water variables for the two sampling years (I–II), at the four sites (RB; CRB; CAC; AC) and two stations (20 m; 50 m) at Terra Nova Bay

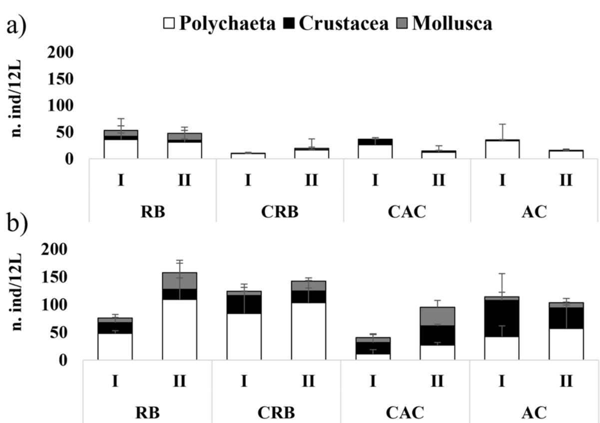

Fig. 5 Mean values (± S.D.) of polychaetes, crustaceans and mollusks abundances at 20 m (**a**) and 50 m (**b**) depths, for each site and sampling year (I and II)

observations can be made: in all sites, the abundance (N) significantly increases from the shallowest to the deepest station (20 m *vs.* 50 m); species richness (SR) do not significantly differ between 20 and 50 m depths at RB, with an average of about 9 species, while increasing with depth in the other sites. Pielou's evenness (J) and Shannon (H′) indices do not show significant differences between the two bathymetries except for RB and CAC, respectively. Comparing all the synecological indices of stations at the same depth in the four sites (Table 3, F and p values), a significant difference was only detected for SR at 20 m depth (PERMANOVA analyses: $F = 4.0122$; $p = 0.00102$).

Through the PERMANOVA analysis of the community structure, related to taxonomic composition and community distribution pattern, significant differences were found among sites ($F = 4.2567$; $p = 0.0002$) and between stations ($F = 14.897$; $p = 0.0002$). Instead, the factor year (2017/2018 *vs.* 2018/2019) was not significantly different ($F = 1.0426$; $p = 0.4108$). The pairwise comparisons of sites with the depth factor (Table 4) showed significant differences at 20 m depth only between RB with CRB and AC, while all sites were statistically different from each other at the 50 m depth. All these differences are highlighted by the plot of CAP analysis (Fig. 6): 20 m stations are mainly clustered in the negative sectors of the two axes, whereas the 50 m stations CAC, CRB and AC are clustered in the positive sector of CPA1 and RB 50 m in the positive sector of CAP2. These stations, occurring at 50 m depth, were characterized by few clustered specie related to each station. By applying a moderate Pearson's correlation ($p > 0.5$), the benthic species are vectorized along the 50 m depth (Fig. 6). This aligns with the previously mentioned results, indicating that dominant species are consistent across both depths but exhibit a significant increase in abundance at the deepest stations (− 50 m). The dominant gastropod *S. turqueti* and amphipod *L. subantarctica* characterized the community at RB 50 m and AC

Table 3 Mean values (±S.D.) of abundance (N), species richness (SR), Pielou's evenness (J) and Shannon–Weaver diversity (H′) calculated at each site (RB; CRB; CAC; AC) and stations (20 m; 50 m), and their comparisons respectively among sites (PARMANOVA analysis: *F* and *p* values) and between stations (pairwise analysis: *t* and *p* values)

	Stations (depth)	Sites				*F–p(perm)*
		RB	CRB	CAC	AC	
N	20 m	50.50 ± 36.72	15 ± 15.97	25.83 ± 17.29	25.83 ± 21.75	2.2833–0.0954
	50 m	116.67 ± 61.20	133.33 ± 50.24	68 ± 34.50	108.83 ± 44.08	1.9661–0.1474
	t − p(perm)	2.6175–**0.0178**	5.0377–**0.003**	4.3453–**0.0022**	3.812–**0.0064**	
SR	20 m	8.50 ± 3.89	4 ± 2.68	5.17 ± 2.14	3.67 ± 1.51	4.0122–**0.0102**
	50 m	8.83 ± 2.48	8.83 ± 3.92	7.67 ± 1.37	8.17 ± 2.48	0.26118–0.8548
	t–p(perm)	0.18334–0.8486	3.6537–**0.011**	2.3146–**0.0234**	3.429–**0.0128**	
J	20 m	0.81 ± 0.06	0.76 ± 0.17	0.61 ± 0.31	0.70 ± 0.35	0.70578–0.5856
	50 m	0.73 ± 0.06	0.64 ± 0.08	0.77 ± 0.04	0.71 ± 0.15	2.1072–0.1112
	t–p(perm)	2.3547–**0.0314**	1.8185–0.1048	1.2144–0.2498	0.11578–0.9408	
H'	20 m	2.40 ± 0.39	1.41 ± 0.84	1.57 ± 0.81	1.42 ± 0.74	2.6358–0.082
	50 m	2.25 ± 0.25	1.92 ± 0.40	2.25 ± 0.23	2.15 ± 0.65	0.83528–0.493
	t–p(perm)	0.79812–0.4822	1.5422–0.1588	1.9233–**0.0342**	1.693–0.1232	

Significant differences were reported in bold

Table 4 PERMANOVA pairwise comparisons of stations among sites, using 4999 permutations

Sites	20 m depth		50 m depth	
	t	*p*(perm)	*t*	*p*(perm)
RB, CRB	1.9002	**0.0134**	2.4465	**0.0028**
RB, CAC	1.4322	0.051	3.8918	**0.002**
RB, AC	1.8103	**0.0186**	1.8634	**0.0104**
CRB, CAC	1.0567	0.3114	2.9648	**0.0018**
CRB, AC	0.79871	0.6612	1.7347	**0.0228**
CAC, AC	1.2617	0.2024	2.5245	**0.003**

Significant differences were reported in bold

50 m stations respectively, while the amphipod *Parapeliculodes* spp. were dominant only at CAC 50 m. Finally, the community multivariate similarity composition was 44.40% among stations at 20 m depth and 53.72% among stations at 50 m depth. At both depths, the polychaetes *A. trissophyllus* and *L. kerguelensis*, and the crustacean *L. subantarctica* are the main responsible for these similarities (Table 5).

Interaction of benthic communities and chemical and sedimentological parameters

To identify which chemical and sedimentological parameters were the most important in explaining the distribution pattern of benthic assemblages among sites and between depths, distance-based linear modeling (DistLM) and redundancy analysis (dbRDA) were performed by combing environmental and biological data. All sediment variables significantly explained variations in benthic community distribution when separately tested (Table 6a—Marginal test), but the best source of variation is explained by medium sand and TOC (Table 6a—Sequential test). As for interstitial water variables, when separately tested, the ion PO_4^{3-} had a significant effect on community distribution (Table 6b—Marginal test), while the ion K^+ showed the greatest influence in the sequential test (Table 6b). All heavy metals represent the most important driving factor affecting benthic assemblage distribution when assessed both through the separated and the combined DistLM analysis (Table 6b—Marginal and Sequential tests). The influence of chemical and sedimentological parameters on the benthic community, clearly divided into two clusters according to depth (20 m and 50 m), is explained by the magnitude and direction of vectors shown in the dbRDA plot (Fig. 7). The chemical and sedimentological parameters were found to explain a total of 34.80% of community variation (dbRDA axis 1–24.1%; dbRDA axis 2–10.7), while for the fitted model, the first dbRDA axis explained 44.9% and the second dbRDA axis explained 20%.

Discussion

In the investigated shallow soft bottoms of Terra Nova Bay, as expected, the substrate differs in grain size along the depth gradient, with a decrease in grade, from medium sand to fine sand. Among the main ions analyzed, nitrate (NO_3^-) shows high values at Road Bay, the site close to the Italian Antarctic base, while phosphate (PO_4^{3-}) at Adélie Cove, the site close to the penguin colony.

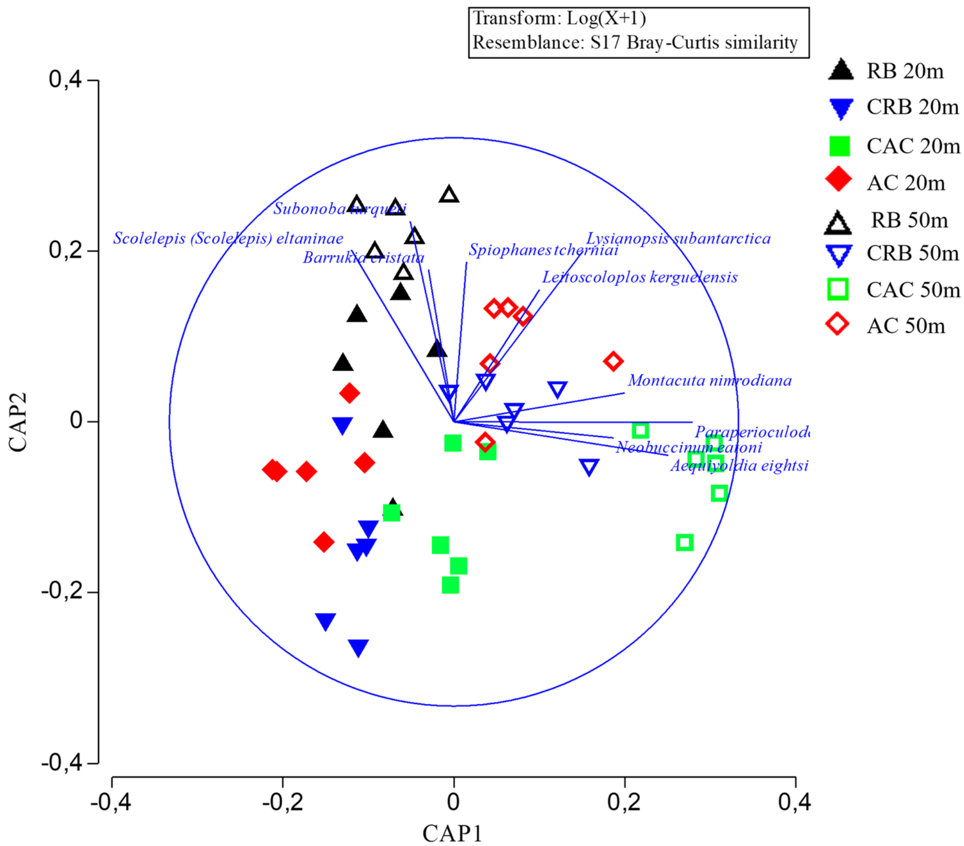

Fig. 6 Canonical Analysis of Principal coordinates (CAP) showing the 6 replicates (3 *per* sampling year) for each site and station of the benthic community, which is correlated with the Pearson coefficient ($p > 0.55$). Straight lines represent species vectors, with their orientation and length proportional to the most correlated sites. Circles indicate the 95% confidence interval

Table 5 Results of SIMPER similarity of percentages test, showing taxa that mostly contributed to similarity among stations

Stations	Taxa	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
20 m (Similarity 44.40%)	*Aglaophamus trissophyllus* (Grube)	2.04	23.39	1.91	52.69	52.69
	Leitoscoloplos kerguelensis (McIntosh, 1885)	1.51	9.25	0.91	20.82	73.51
	Lysianopsis subantarctica (Schellenberg, 1931)	0.85	4.02	0.71	9.05	82.56
	Scolelepis (*Scolelepis*) *eltaninae* Blake, 1983	0.60	3.05	0.46	6.88	89.43
	Laternula elliptica (P. P. King, 1832)	0.46	2.05	0.52	4.61	94.04
50 m (Similarity 53.72%)	*Leitoscoloplos kerguelensis* (McIntosh, 1885)	3.02	14.39	2.44	26.78	26.78
	Lysianopsis subantarctica (Schellenberg, 1931)	2.80	14.14	2.85	26.32	53.10
	Aglaophamus trissophyllus (Grube)	2.34	12.45	3.52	23.17	76.27
	Paraperioculodes spp.	1.21	2.90	0.54	5.40	81.67
	Aequiyoldia eightsii (Jay, 1839)	0.96	2.01	0.50	3.75	85.41
	Spiophanes tcherniai Fauvel, 1950	0.98	1.69	0.55	3.15	88.56
	Scolelepis (*Scolelepis*) *eltaninae* Blake, 1983	1.02	1.52	0.38	2.83	91.39

Abun abundance, *Sim* similarity, *SD* standard deviation, *Contrib%* contributed percent, *Cum%* cumulative percent

Nitrates are well-known to be released by anthropogenic activities and may act as a nutrient at medium concentrations but as a pollutant at high levels (Jiang et al. 2019; Clark et al. 2020; Patricia et al. 2022). Concerning the Antarctic continent, nitrates were mainly reported in the area interested by sewage of research bases (e.g., McMurdo Station, Crockett 1997; Scott Base, Williams 2012). Also in our case, among the four sites analyzed, the highest value of nitrate occurred close to the Italian Station, but in low concentration (0.31 μMol), so that it could be considered as a possible macronutrient. According to Capodaglio et al. (1998), additional nitrate of natural origin, due to the deep waters upwelling process occurring during the summer along the shallow coasts of Terra Nova Bay, cannot be excluded. This is also corroborated by literature data related to other nutrients or trace elements, which contribute to sustain the enrichment

Table 6 DistLM results comparing benthic community data and sedimentological and chemical variables (a; b)

Variables	Marginal tests			Sequential tests			
	Pseudo-F	*p*-value	Proportion of variation explained	Pseudo-F	*p*-value	Proportion of variation explained	Cumulative variation
(a) Sediment variables							
Coarse sand	2.5976	**0.0182**	5.345E−2	1.5826	0.1172	2.3713E−2	0.46059
Medium Sand	8.4593	**0.0002**	0.15533	2.3562	**0.0242**	3.4023E−2	0.49461
Fine sand	5.9992	**0.0002**	0.11537	0	1	3.203E−16	0.49461
TOC	2.7359	**0.0126**	5.6138E−2	3.1018	**0.005**	4.2252E−2	0.53686
(b) Interstitial water variables							
NO_3^-	1.5924	0.1204	3.346E−2	1.5924	0.1294	3.346E−2	3.346E−2
PO_4^{3-}	2.0507	**0.0456**	4.2678E−2	1.78	0.0806	3.6778E−2	7.0238E−2
SO_4^{2-}	1.4842	0.1636	3.1258E−2	1.4179	0.187	2.9026E−2	9.9264E−2
K^+	1.4565	0.1654	3.0691E−2	3.163	**0.0066**	6.1716E−2	0.16098
Mg^{2+}	0.88287	0.4998	1.8831E−2	1.4685	0.1744	2.8345E−2	0.18932
Ca^{2+}	0.55283	0.8142	1.1875E−2	0.80591	0.5876	1.5628E−2	0.20495
Zn^{2+}	5.5735	**0.0002**	0.10807	4.9547	**0.0004**	8.7627E−2	0.29258
Cd^{2+}	2.9779	**0.0088**	6.0801E−2	3.0347	**0.0058**	5.1073E−2	0.34365
Pb^{2+}	8.0689	**0.0002**	0.14923	3.5341	**0.0026**	5.5848E−2	0.3995
Cu^{2+}	9.2612	**0.0002**	0.16759	2.4558	**0.0186**	3.7376E−2	0.43688

Bold data are significantly different at $\alpha \leq 0.05$

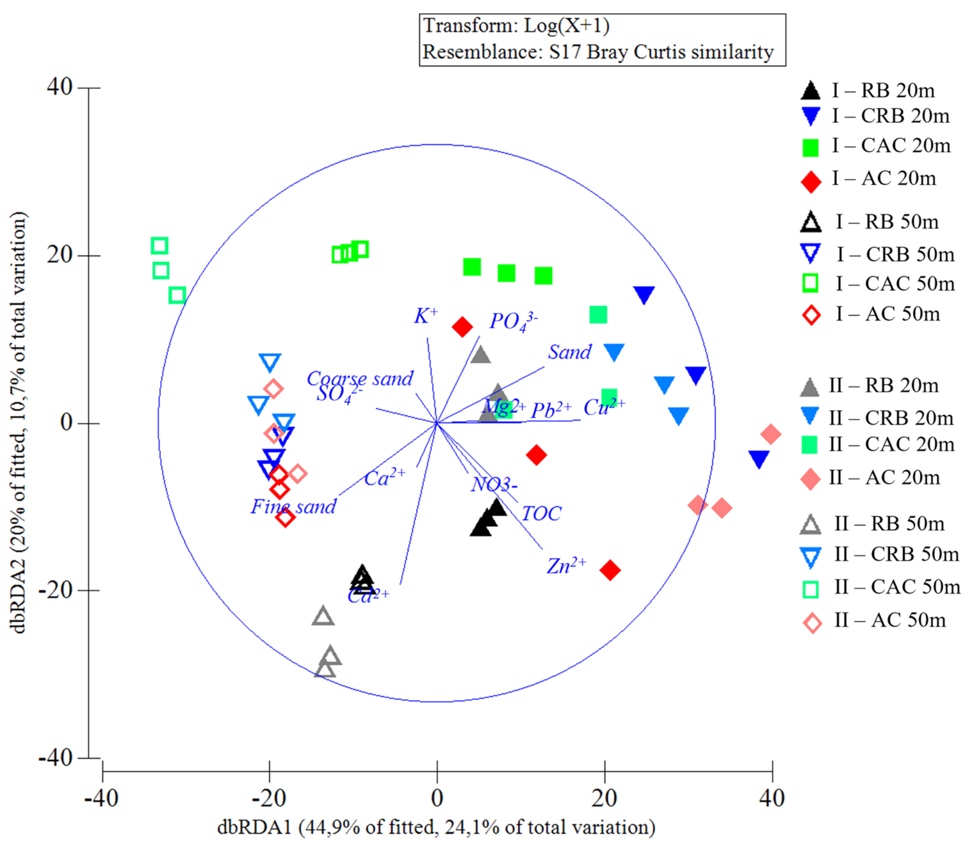

Fig. 7 dbRDA plot of benthic community structure fitted to sediment and interstitial water, showing each replicated site (RB; CRB; CAC; AC) *per* station (20 m; 50 m) and sampling year (I; II)

of organic matter in the investigated shallow coast by the upwelling process (Bargagli et al. 1996; Povero et al. 2001). The phosphate concentration in the interstitial water showed the highest percentage value close to the penguin colony. Such a phosphate enrichment was also observed in other shallow Antarctic soft bottoms near the nesting areas of penguins along the coasts, as in the Ross Sea region (Nie et al. 2012; Qin et al. 2014) and the Antarctic Peninsula (Espejo et al. 2017). Nevertheless, the upwelling process may represent one of the main drivers of phosphorous in marine sediments of Adélie Cove (Povero et al. 2001), as well as for heavy metals (Capodaglio et al. 1998). The highest values of nutrients (NO_3^- and PO_4^{3-}) are associated with the highest concentrations of some heavy metals: cadmium at Road Bay and lead and zinc at Adélie Cove. Capodaglio et al. (1998) detected dissolved cadmium, lead, and copper in the water column of Terra Nova Bay in the Antarctic summer of 1987/1988, 2 years after the first Italian expedition. For the same area, Bargagli et al. (1996) observed the absence of a relation between cadmium and anthropogenic input, as previously proposed by Capelli et al. (1989). Nevertheless, the penguin's presence, historically nesting along the coast of Adélie Cove (Gao et al. 2022), is widely reported as an important bio-vector of both macronutrients and trace metals (Espejo et al. 2017). However, according to Capodaglio et al. (1998) and Rivaro et al. (2012), the recorded amount of dissolved heavy metals has to be considered not detrimental to the biodiversity of the Southern Ocean.

The benthic biodiversity of the soft bottom was largely investigated in the past decades (Arrigo et al. 1998), while anthropogenic and natural pressures have been only recently considered as factors influencing the communities' structures. Our results revealed a marked depth zonation of the coastal benthic community of Terra Nova Bay. In fact, the benthic assemblage at the shallow bathymetry (20 m) was different from that of the deep (50 m), where a higher number of individuals and species richness occurred. A similar distribution trend of the benthic community, from the shallow to deeper zone, is in agreement with literature data (Dayton et al. 1970; Clarke 1996; Nonato et al. 2000).

Taking into account natural (penguins) and anthropic (base) pressures on soft-bottom benthic communities, no significant differences in abundance among sites have been detected. Instead, species composition is coherent with patterns of chemical and sedimentological parameters. This result confirms the role of benthic species as good environmental indicators. Taking into account the fundamental ecological perspective proposed by Pearson and Rosenberg (1978), based on species sensitivity or tolerance to organic enrichment, Road Bay and Adélie Cove are characterized by the dominance of few opportunistic polychaete species, such as the motile deposit feeder *L. kerguelensis* and the tube-dwelling *S. tcherniai*. This latter, together with the polychaetes *Ampharete kerguelensis* McIntosh, 1885 and *Scolelepis* (*Scolelepis*) *eltaninae* Blake, 1983 shows adaptations for environments disturbed by high organic matter levels (Munari et al. 2022), and consistently occurred both in human and penguin disturbed sites. The control site CRB, with the highest values of total organic carbon (TOC 40%), was dominated by the suspended-feeder bivalve *Adamussium colbecki* (E.A. Smith, 1902). This is the most common and large suspension-feeder bivalve of the Antarctic coasts, reaching densities of about 60 individuals per square meter at about 50 m depth (Cattaneo-Vietti et al. 1997; Chiantore et al. 2001). Due to its very high densities and the great shell size of adults, it forms a dense carpet of shells on the bottom. Probably, this could be the reason for the higher TOC content in the sediment of the control site CRB, where the high *Adamussium* coverage could reduce the bioturbation of sediment, limiting the aeration. The other control site CAC, with the highest percentage of medium sandy sediment (52%), was dominated by the facultative suspension-feeder *A. eightsii*. This borrowing species, occurring in the first centimeters of soft bottoms, is adapted to a wide range of sediment rates, thanks to its orientation ability in the sediment layers (Davenport 1988; Siciński et al. 2012).

Overall, our research represents a first study describing the biological structure of the macrobenthic community around Mario Zucchelli Station and Adelie Cove, *i.e.,* related to the anthropogenic and natural disturbances. The results clearly show the dominance of different opportunistic species of macrobenthos where penguins or humans' presence occurred. The pressure of the penguin colony on the benthic composition and community structure is rather clear and probably is also stable over time. As for increasing human presence, its pressure on benthic assemblages is almost negligible; therefore, it should be better investigated in future through monitoring plans, in order to assess possible impacts.

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Author contributions All authors contributed to the study conception. LD, LA conducted experiments. LD, LA, EC and FF performed formal analysis; LD and EC wrote the manuscript. R.S. and G.F.R reviewed, editing, and validated the manuscript. P.D.D. and R.S. managed resources and project administration. PDD acquired funding. All authors read and approved the manuscript.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval All applicable international, national, and institutional guidelines for animal testing, animal care, and use of animals were followed by the authors.

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