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First Application of the AMBI Index to the Macrobenthic Soft-Bottom Community of Terra Nova Bay (Ross Sea, Southern Ocean)

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Abstract: The assemblages of marine benthic organisms and sediment characteristics were investigated in the coastal area between the Mario Zucchelli Antarctic Research Station and Adelie Cove in Terra Nova Bay (Ross Sea, Southern Ocean) during the 2015 summer season. Sediment samples were taken from 11 stations at depths between 25 and 140 m. The dominance of sand characterised sites, and the biochemical composition of the sedimentary organic matter resulted in very variable between the different sites. A total of 142 taxa were identified, with Annelida (68 taxa) and Arthropoda (35 taxa) constituting the main macrobenthic groups. The benthic community at deeper stations showed higher species richness and lower dominance compared to the shallower stations. For the first time in Antarctica, we also investigated the response of the AZTI's Marine Biotic Index (AMBI) to the organic gradient. Of the 142 taxa found, 97 were not listed in the AMBI library, and we were able to assign as many as 88 taxa to an ecological group. All of these new species were added to the new AMBI species list. AMBI showed a good response to the organic gradient.

Keywords: antarctic benthos; AMBI; ecological quality assessment; Ross Sea

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1. Introduction

Remote areas such as Antarctica are historically less disturbed by human activity. There are 64 major facilities south of 60° with peak populations approaching 4000 annually (www.comnap.aq/operations/facilities/, accessed on 30 July 2022). Sewage outfall from those facilities may contribute to an excessive organic input to the benthic community, for instance, McMurdo Station reported [1]. As a matter of fact, organic enrichment in the marine environment is one of the most documented forms of disturbance. Pearson and Rosenberg [2] established a model and a biotic classification in response to disturbance related to organic enrichment. Many biotic indices based on this succession model have been developed and implemented for macrobenthic invertebrate communities: among them, AZTI's Marine Biotic Index, AMBI [3], has received significant attention due to its wide applicability and efficiency in different habitats (coastal waters, transitional areas). Although first developed in Europe, AMBI has also been successfully applied in other geographic areas, from the tropics to the poles [4]. Even if marine benthic communities were observed to change along a gradient of organic pollution in Antarctic waters [1], no attempts have been made to adapt the AMBI index to the Antarctic waters.

Considering this, this research aims to test the efficiency of AMBI for ecological assessment in Antarctic macrobenthic communities.

2. Materials and Methods

2.1. Study Area and Sampling

Terra Nova Bay (Ross Sea) is a coastal marine area encompassing 29.4 km^2 between Adélie Cove and Tethys Bay, where the Stazione Mario Zucchelli (SMZ), a scientific research centre and a strategic logistics node for other bases in Antarctica, is located. The SMZ is active only during the austral summer, when it can accommodate up to a maximum of 120 people. The human impacts within Terra Nova Bay are believed to be minimal and confined to those arising from the nearby SMZ and scientific work conducted within the area [5]; however, Adelie Cove hosts an important rookery of Adelie penguins (*Pygoscelis adeliae* Hombron & Jacquinot, 1841), whose activities and faeces probably enrich the surrounding environment with organic matter [6].

In the austral summer of 2015, during the 30th Antarctic Expedition (PNRA, Italian Research Program in Antarctica), sediment samples were taken from Terra Nova Bay. The sampling program was carried out aboard the MS "Malippo" in January 2015, and sediment samples were collected with a Van Veen grab (surface 0.18 m^2) from 11 stations at 25, 70, and 140 m depth (Table 1) at increasing distance from the SMZ (Figure 1). In particular, one station was located in front of the Stazione Mario Zucchelli (SMZ25), three stations at Rod Bay (2 km south of the base; RB25, RB70, RB140), three stations at Camp Icarus (4 km south of the base; CI25, CI70, CI140), one station at Central Bay (7 km south of the base; CB25), three stations at Adelie Cove (10 km south of the base, AC70, AC25, AC140). At each station, the sediments were collected in three independent replicates, except for the stations CB25 and SMZ25, where adverse weather conditions allowed the collection of only two replicates. Three Plexiglas cores were retrieved from independent grab casts in each station for texture and biochemical analysis and immediately stored at −20 °C. At SMZ, the sediment grabs were sieved through 0.5 mm mesh, fixed in ethanol, stored at −20 °C, and sent to Italy by ship at the end of the campaign.

Table 1. Coordinates, location and depth of the 11 sampling stations.

Station	Latitude	Longitude	Location	Depth (m)
SMZ25	−74, 41.335	16, 407.098	Stazione Mario Zucchelli	25
RB25	−74, 41.831	16, 407.532	Rod Bay	25
RB70	−74, 41.918	16, 407.896	Rod Bay	70
RB140	−74, 41.972	16, 408.208	Rod Bay	140
CI25	−74, 43.037	16, 406.908	Camp Icarus	25
CI70	−74, 43.078	16, 407.757	Camp Icarus	70
CI140	−74, 43.101	16, 408.399	Camp Icarus	140
CB25	−74, 44.925	16, 405.243	Central Bay	25
AC70	−74, 46.390	16, 357.977	Adelie Cove	70
AC25	−74, 46.467	16, 400.266	Adelie Cove	25
AC140	−74, 46.617	16, 402.798	Adelie Cove	140

2.2. Sample Processing

At our laboratory, the sorting of the macrofauna was carried out under a stereomicroscope and organisms were identified at the lowest possible taxonomic level.

Sediment texture was analysed by sieving the gravel-sandy fraction and with a Micromeritics SediGraph III Particle Size Analyzer for the muddy fraction. Organic matter content was measured as weight loss of dry samples at 500 °C for 24 h.

Chlorophyll-a and phaeopigment were extracted (12 h at 4 °C, in the dark) from triplicate superficial (0–1 cm) sediment samples (ca. 1 g), using 90% (v/v) acetone as the extractant. These extracts were analysed fluorometrically to estimate chlorophyll-a, and after acidification with 200 µL of 0.1 N HCl, to estimate the phaeopigments [7].

Chloroplastic pigment equivalents (CPE) were expressed as the sum of chlorophyll-a and phaeopigments concentrations. The protein, carbohydrate, and lipid analyses were carried out on the sediment samples using photometric protocols [7]. The protein, carbohydrate, and lipid contents were converted to C equivalents using the conversion factors of 0.49, 0.40, and 0.75 mg of C per milligram, respectively. Their sum was referred to as the biopolymeric carbon, BPC [7].

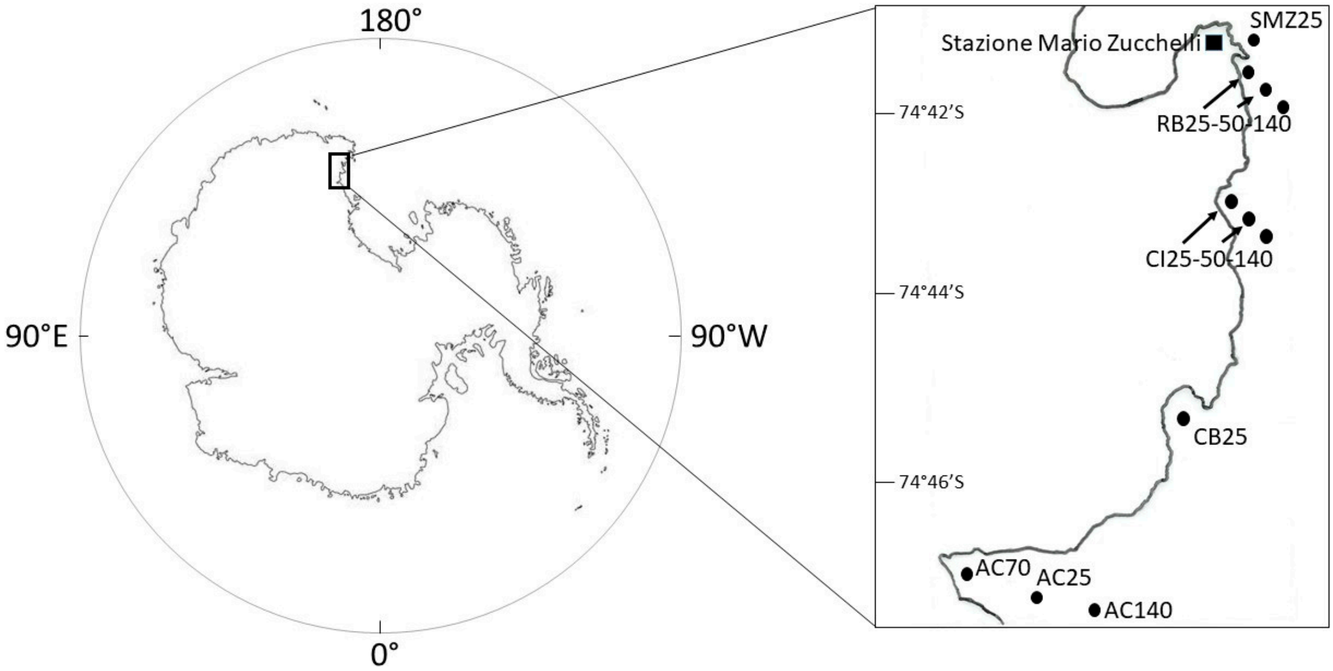

Figure 1. Map of the studied area and sampling stations.

2.3. Data Analyses

Each station calculated the average and standard deviation (SD) for each physicochemical parameter. To test whether they varied significantly among depths, a Chi-square test was applied to Kruskal–Wallis ranks test, and a Wilcoxon–Mann–Whitney statistic was used for pairwise comparisons. Spearman (rs) correlations were used to search for correlation among the physicochemical variables.

The taxa richness (S), abundances (N), Shannon diversity index on a log2 basis (H′log2) and Pielou equitability index (J′) were calculated for each sample. Then, the averages and SD were calculated for each station. To test whether the indices varied significantly among sampling depths, a Chi-square test applied to Kruskal–Wallis ranks was run, and Wilcoxon–Mann-Whitney statistic was used for the pairwise comparisons.

To analyse the differences in community structure, k-dominance curves [8] were built for each sampling station. In order to check whether there were significant differences in structure among stations, depth and percentage of organic matter, the DOMDIS routine [8] was used. This method starts from a species per samples matrix. It calculates, separately for each sample, the cumulative relative abundances of species ranked in decreasing order, exactly as for the k-dominance plots. The Manhattan distance was then used to calculate the distance of every pair of cumulative curves, generating a dissimilarity matrix for all pairs of samples. On this matrix permutational multivariate analysis of variance, the PERMANOVA (Plymouth, UK) [9] was run.

A CCA analysis was used to investigate the relationship between environmental variables and macrobenthic species. The data were log-transformed, and seven environmental variables (chlorophyll a, phaeophytins, organic matter, sand, gravel, silt, and clay) were chosen to reduce collinearity problems previously detected through pairwise Spearman correlations.

Univariate and multivariate analyses were performed using the PRIMER v6 + PERMANOVA (Plymouth, UK) software package and the vegan package for R 3.5.2.

2.4. AMBI Application

Before the application of AMBI, many Antarctic species were not present in the AMBI library and needed to be assigned to an ecological group. The criteria followed to assign those species to one of the five ecological groups (I to V) required for the calculation of AMBI were those described by Borja et al. [3]. The supporting data (listed in Table S1) were obtained from the literature [10–17] and combined with the present work results. Those data regarded the presence and the abundance of species in relation to different concentrations of organic matter, and biological traits, in particular feeding modes. Only rare, very poorly known species were present with a few individuals in a few samples, did not show a clear distributional pattern, and were left not assigned (NA).

The percentage of invertebrates belonging to the different ecological groups was then calculated for each station. The AMBI values were calculated using the corresponding software (http://ambi.azti.es; accessed 30 June 2019). The stations were classified according to the following thresholds for status classification: "undisturbed" if AMBI < 1.2, "slightly disturbed" if 1.2 < AMBI $\leq$ 3.3, "Moderately disturbed" if 3.3 < AMBI $\leq$5, "heavily disturbed" if 5 < AMBI $\leq$ 6, and "extremely disturbed" if AMBI $\geq$ 6 [3].

3. Results

The sites were characterised by the dominance of sand (Table 2), ranging from 81% (RB25) to 99% (AC25). The only exception was represented by station RB70, where gravel predominated (56.4%), followed by sand (42.5%). Table 2 shows the physico-chemical characteristics of the sediment at the 11 sampling stations. Organic matter concentration ranged from 0.26% (RB25) to 2.34% (AC70). The amount of organic matter was positively correlated with the finer fractions of sediments (silt: rs = 0.93, $p < 0.05$; clay: rs = 0.92, $p < 0.05$). Total phytopigments ranged from 15.3 $\pm$ 6.98 µg g^{-1} (AC25) to 126.85 $\pm$ 23.86 µg g^{-1} (AC70), with phaeophytins representing the largest fraction (75–95%) of total sedimentary pigments. The protein concentration was high at each sampling station (53–70%), followed by carbohydrates (17–32%) and lipids (12–25%).

A total of 142 taxa (Table S1, Supplementary Material) were identified within the following groups: Annelida (68 taxa), Arthropoda (35 taxa), Mollusca (22 taxa), Echinodermata (6 taxa), Nemertea (5 taxa), Cnidaria (4 taxa), Sipuncula (1 taxon), and Cephalorhyncha (1 taxon).

The K-dominance curves showed that the stations with higher percentages of organic matter were better structured than the stations with lower percentages [8] (Figure 2A). At the same time, the deeper stations showed a better-structured community, with higher richness and lower dominance compared to the most shallow ones (Figure 2B). Significant differences were observed in the dominance structure among different stations, depths and ranks of organic matter (PERMANOVA, $p < 0.05$).

Some macrobenthic species showed a correlation with the following environmental variables: Chlorophyll-a, Phaeophytins, organic matter, sand and clay (CCA, $p < 0.05$). Species showing a preference for higher organic matter concentration were: *Diastylis enigmatica* Ledoyer, 1993, *Echiurus antarcticus* Spengel, 1912, *Capitella capitata* (Fabricius, 1780), *Parougia furcata* (Hartman, 1953), *Ophryotrocha notialis* (Ehlers, 1908), *Monoculodes curtipediculus* Hendrycks & Conlan, 2003, *Syllidia inermis* (Ehlers, 1912), and *Eudorella gracilior* Zimmer, 1907. Conversely, species showing a preference for lower organic matter concentrations were: *Harmothoe fuligineum* (Baird, 1865), *Neobuccinum eatoni* (E.A. Smith, 1875), *Adamussium colbecki* (E.A. Smith, 1902), *Uromunna nana* (Nordenstam, 1933), *Austrofilius furcatus* Hodgson, 1910, *Harmothoe magellanica* (McIntosh, 1885), *Austrosignum glaciale* Hodgson, 1910, and *Scolelepis eltaninae* Blake, 1983.

Table 2. Grain size (%) and chemical characteristics of sediment at the 11 sampling stations. OM = organic matter (%), Chl-a = chlorophyll-a (μg g^{-1}), Phaeo = phaeophytins (μg g^{-1}), CPE = total phytopigments (μg g^{-1}), PRT = proteins (mg g^{-1}), CHO = carbohydrates (mg g^{-1}), LIP = lipids (mg g^{-1}), BPC = biopolymeric carbon (mg g^{-1}).

Station	Gravel	Sand	Silt	Clay	OM	Chl-a	Phaeo	CPE	PRT	CHO	LIP	BPC
SMZ25	ND	ND	ND	ND	ND	2.74 ± 0.32	26.57 ± 1.55	29.31 ± 1.24	2.69 ± 0.14	1.78 ± 0.74	0.41 ± 0.18	2.34 ± 0.37
RB25	18.3	81.0	0.3	0.3	0.26	5.83 ± 0.47	19.72 ± 3.55	25.55 ± 4.02	2.84 ± 0.82	0.94 ± 0.54	0.33 ± 0.09	2.01 ± 0.68
RB70	56.4	42.5	0.6	0.6	0.41	1.48 ± 0.41	27.86 ± 7.45	29.35 ± 7.85	3.08 ± 1.00	1.40 ± 0.25	0.46 ± 0.07	2.41 ± 0.60
RB140	0.0	96.9	2.0	1.1	0.68	8.15 ± 1.98	49.39 ± 8.88	57.54 ± 10.85	3.90 ± 1.14	2.83 ± 0.29	0.86 ± 0.10	3.75 ± 0.56
CI25	6.9	91.2	1.6	0.2	0.29	7.17 ± 6.96	26.00 ± 17.13	33.16 ± 24.09	1.67 ± 1.15	1.12 ± 0.89	0.40 ± 0.05	2.07 ± 0.15
CI70	2.5	91.8	3.6	2.2	1.05	6.02 ± 4.89	24.83 ± 13.74	30.85 ± 18.63	2.16 ± 0.69	1.44 ± 0.06	0.33 ± 0.07	1.88 ± 0.40
CI140	0.0	87.5	8.6	3.9	1.20	14.78 ± 3.48	58.61 ± 8.33	73.40 ± 11.44	3.58 ± 0.87	1.90 ± 0.41	0.80 ± 0.14	3.12 ± 0.67
CB25	ND	ND	ND	ND	ND	6.41 ± 2.72	29.49 ± 24.64	35.91 ± 27.34	0.70 ± 0.05	0.39 ± 0.23	0.23 ± 0.12	0.67 ± 0.21
AC70	0.0	81.6	12.5	6.0	2.34	5.81 ± 1.26	121.04 ± 22.69	126.85 ± 23.86	8.46 ± 1.21	4.00 ± 0.83	1.55 ± 0.40	6.91 ± 0.56
AC25	0.0	99.0	0.8	0.3	0.50	3.82 ± 1.45	11.48 ± 5.55	15.30 ± 6.98	0.61 ± 0.10	0.24 ± 0.08	0.15 ± 0.04	0.51 ± 0.04
AC140	0.0	82.8	12.9	4.3	1.37	5.20 ± 0.06	37.79 ± 1.12	42.98 ± 1.17	4.14 ± 1.28	2.28 ± 0.31	0.73 ± 0.06	3.49 ± 0.62

ND—Not detected.

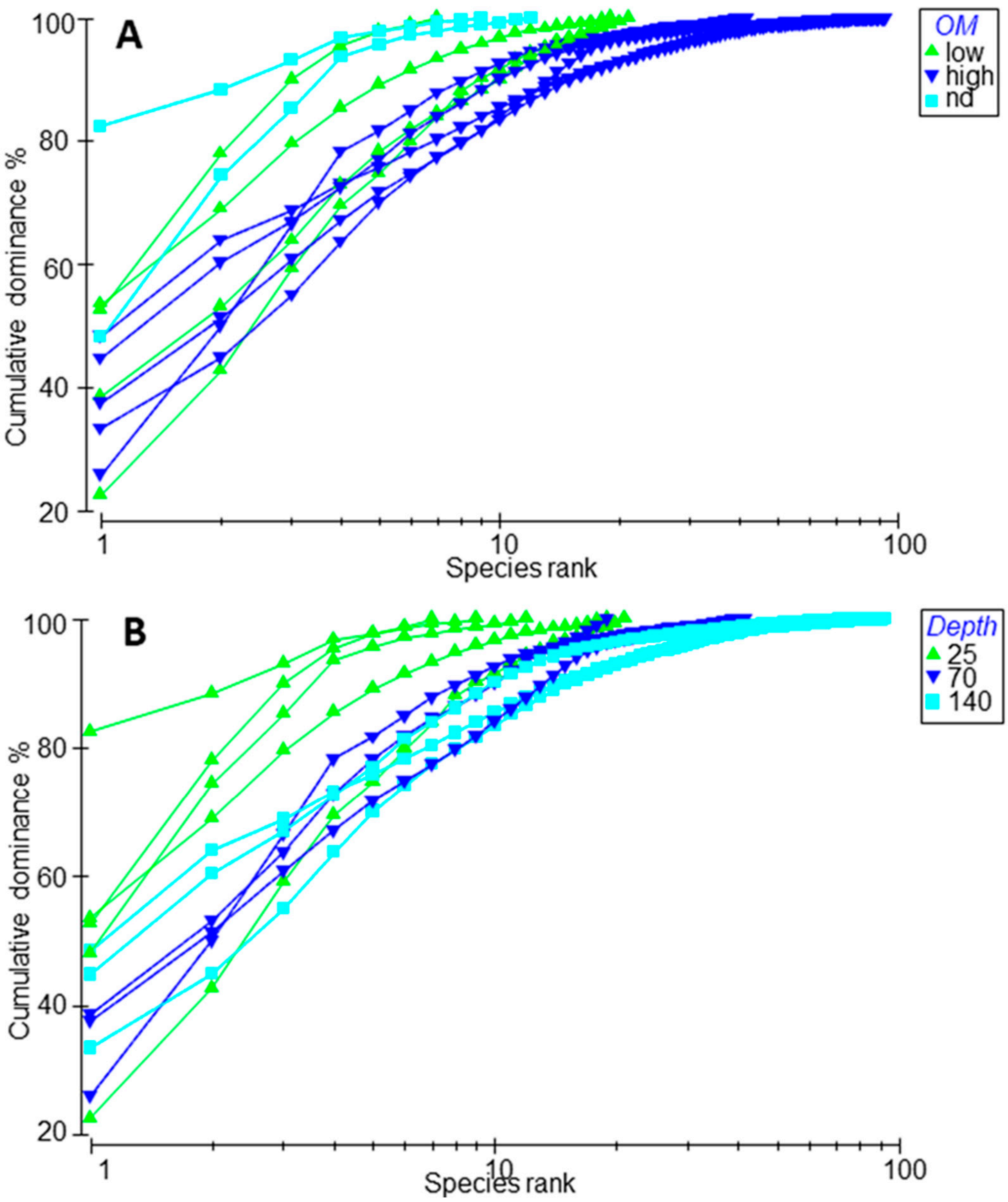

Figure 2. K-dominance curves for each analysed station show differences in OM (**A**) and depth (**B**) percentages.

For the application of AMBI, 97 taxa were not listed in the AMBI library (Table S1). From them, 38 taxa were assigned to an Ecological Group based on the classification provided in the AMBI library for the same genus, 10 taxa were classified based on AMBI classification for higher taxa (subfamily, family), and 40 taxa were assigned from the result of our analyses and/or relevant literature. Finally, nine taxa were not attributed to any group (NA) without reliable information. All of these new species were added to the new AMBI species list, which was released in May 2019 (http://ambi.azti.es; accessed on 30 June 2019).

In AMBI, the percentage of sensitive species (EG I, Figure 3A) was relatively low at all stations, ranging from 0% at station AC25 to 9.3% at station CI25. Conversely, indifferent species (EG II, Figure 3A) were the dominant group in most stations but showed high variability: from 15% at station CI140 to 89.7% at station SMZ25. The tolerant species (EG III, Figure. 3A) varied from 0.3% at station SMZ25 to 61% at station CI140, and second order opportunistic species (EG IV, Figure 3A) varied from 3.7% at station AC140 to 45.5% at station CI70. First-order opportunistic species (EG V, Figure 3A) were absent at some stations (CB25, RB25, SMZ25, AC25, and RB70), and their percentages varied from 0.4 at station AC70 to 34.6% at station RB140.

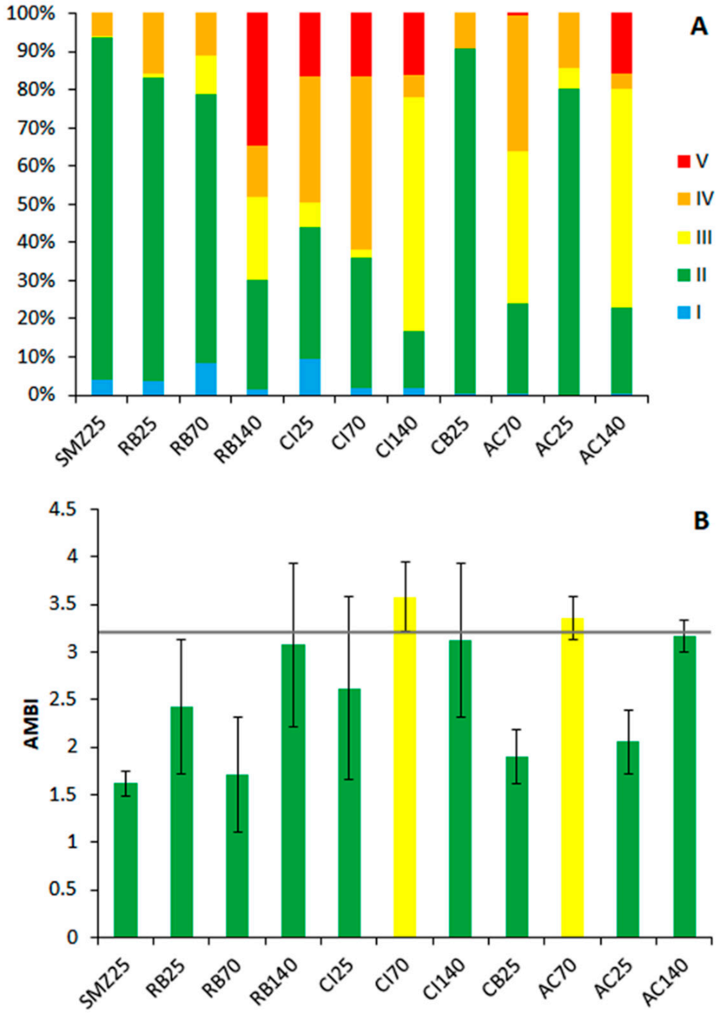

Figure 3. (**A**) Percentage of the different Ecological Groups (I–V). (**B**) The AMBI values at each station (horizontal line: the threshold between Good and Moderate status).

Most stations showed low AMBI values (<3.3) and therefore were classified as Slightly disturbed, except for stations CI70 and AC70 (AMBI = 3.58 and 3.35, respectively), classified as Moderately disturbed (Figure 3B). Station CI70 was dominated by second-order opportunistic species (EG IV = 45.5%, Figure 3A), in particular, the orbinid *Leitoscoloplos geminus* Mackie, 1987 and the cirratulid *Aphelochaeta palmeri* Blake, 2018; while station AC70 was dominated by tolerant species (EG III = 39.9%, Figure 3A), such as the cumacean *Eudorella gracilior* Zimmer, 1907, and second order opportunistic (EG IV = 35.7%, Figure 3A) species such as the orbinid *L. geminus*.

The values of AMBI showed the best response to increasing concentration of organic matter (Figure 4A). Conversely, richness (Figure 4B), diversity (Figure 4C), and equitability (Figure 4D) did not show significant correlations with the concentration of organic matter.

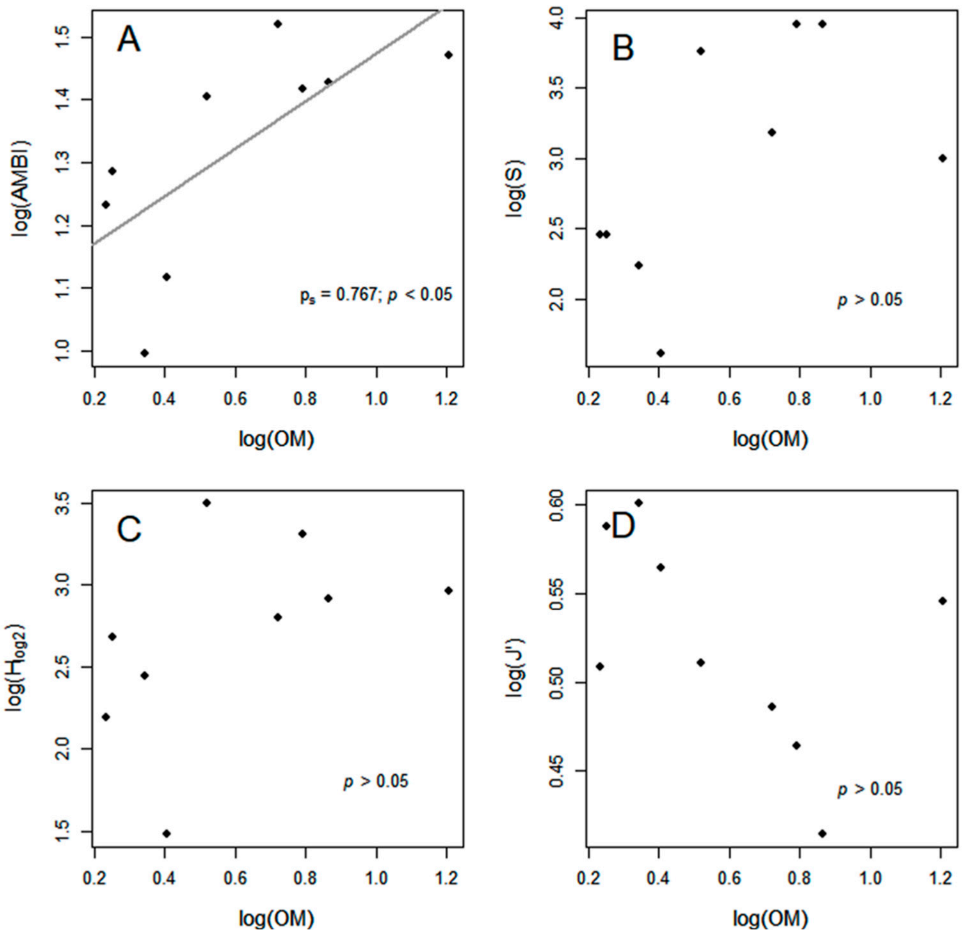

Figure 4. Relationship between concentration of organic matter (OM) and values of AMBI (**A**), richness (**B**), Shannon diversity index (**C**), and Pielou equitability index (**D**).

4. Discussion

The nature and magnitude of organic inputs are two of the most important factors in determining benthic community structure in Antarctica [18]. The strong seasonality of primary production and low degradation rates due to low temperatures and low microbial activities in the water column [19] determine the accumulation of organic matter in the sediment [20,21]. Benthic consumption of organic matter determines a general annual balance between organic matter production and degradation [22] and provides higher trophic levels with a more constant food supply, thus dampening seasonality [23]. Studying the benthic-pelagic coupling process in Adelie Cove, it was observed that the interaction between chemical, physical and biological processes in the water column, linked to katabatic wind patterns, led to a high build-up of materials in the sediments of the deepest part of the cove, contributing to the organic enrichment of the sediments [24]. The results from our study confirm those observations; furthermore, we observed that deeper stations, in addition to higher organic matter, also showed better-structured communities, with higher richness and lower dominance compared to the shallower ones, where organic matter was generally lower.

In this study, AMBI, considering the percentages of sensitive and opportunistic species, showed a good response to organic matter content, despite the differences in richness and dominance among the various stations. The results obtained, with most stations evaluated as “slightly disturbed”, and only two as “moderate”, indicated a slightly unbalanced benthic community, which is consistent with the mild organic content (OM = 0.26−2.34%) found in sediments. The very low density of the sensitive species suggests that communities are subjected to a certain disturbance. However, first-order opportunists were present with low density in most stations, and species showing a marked preference for higher organic matter content were not dominant or extremely abundant, as reported, for instance, in more polluted Antarctic zones [1,15].

The difficulties related to the application of AMBI to Antarctic macrobenthic communities were mainly related to the fact that most species were still not present in the AMBI library. The adequate classification of species in ecological groups is a key step in the computation of AMBI, which requires synthesizing a huge amount of information [25]. In most cases, it was based on taxonomical proximity with other taxa in the library, and published literature provided useful information to fill the gaps [17]. The preference of some species for certain levels of organic matter found in the present work enables us to confirm and refine the assignments. In fact, some species that showed a marked preference for high organic matter concentrations were known to have opportunistic habits, e.g., *Capitella capitata* (Fabricius, 1780), *Ophryotrocha notialis* (Ehlers, 1908) (AMBI library), or tolerant habits, e.g., *Amphicteis antarctica* Hessle, 1917, and *Eupraxillella antarctica* Hartmann−Schröder & Rosenfeldt, 1989 (AMBI library), while other showing a preference for low values of organic matter were already reported associated with referencing condition, e.g., *Austrofilius furcatus* Hodgson, 1910 [26], and *Austrosignum glaciale* Hodgson, 1910 [1], or showed traits usually associated with more sensitive species, such as carnivores, e.g., *Harmothoe fuligineum* (Baird, 1865), *H. magellanica* (McIntosh, 1885) [10], *Neobuccinum eatoni* (E.A. Smith, 1875) [11], or long-living with late sexual maturity, e.g., *Adamussium colbecki* (E. A. Smith, 1902) [27].

The data on feeding modes were considered as well. Carnivore and omnivore feeding behaviours were indicative of high-quality sediments [28], whereas a lack of suspension feeders indicates organic enrichment [1,2]. Nevertheless, our knowledge of Antarctic species is still limited, and many taxa are still taxonomically uncertain, with new species still to be described, so there will be the need to implement the AMBI library as long as new information is gained and new species are described. Notwithstanding those limits, AMBI was a good indicator of organic matter enrichment in Antarctic waters and robust in terms of variability of richness and diversity.

5. Conclusions

The data relating to the structure and composition of Antarctic benthic communities are extremely scarce, with most of them obtained by video transect, and therefore are not suitable for the application of benthic faunal indexes. With this study, we provide the quantitative sampling of the marine benthos and the first attempt to apply the AMBI index in the Ross Sea. The drawback of the use of AMBI is that the index cannot discriminate between natural enrichment, like those in Adélie cove (stations AC70, AC25, AC140) due to the presence of Adélie penguin colonies, and enrichment derived from anthropic activities, such as scientific investigations (stations SMZ25, RB25, RB70, RB140). Previous investigations have previously pointed out the difficulties in disentangling the effects of anthropogenic disturbance and the natural one in Antarctic waters [29]. Moreover, AMBI does not account for diversity and uses a single (i.e., independent of the studied data sets and habitats) scale to infer the ecological status. M-AMBI [30] was introduced to overcome this potential weakness. Unfortunately, to date, the application of M-AMBI for the Antarctic macrobenthic community is prevented by the limited basic knowledge of the dynamics of those communities. In fact, for the application of M-AMBI, the results of AMBI should be combined with the taxa richness and Shannon diversity—all of them require the reference conditions to be set, depending on the habitats and/or communities present in the area [31].

Our results are in line with the general understanding that a full range of benthic assemblages characterises Antarctic circumpolar benthos from extremely diverse to extremely meagre. An extremely low abundance of macrobenthic invertebrates was found in habitats, such as shallow depths with permanent disturbance, such as fresh iceberg scours [32], or under the ice shelf. High species richness was observed in the deep sea [33]. The high variability of those communities in terms of richness and diversity creates the need to establish new reference conditions and specific thresholds for Antarctic waters. However, the available data are still insufficient to establish robust, depth-specific or habitat-specific reference conditions and thresholds. Thus, additional investigations are required to widen our knowledge of macrobenthic community dynamics prior to M-AMBI application to Antarctic benthic coastal ecosystems.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w14192994/s1, Table S1: Taxonomic list, abbreviations and EGs assigned to each taxon.

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