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Impacts of organic enrichment on macrobenthic production, productivity, and transfer efficiency: What can we learn from a gradient of sewage effluents?

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Abstract:	We studied the macrobenthic invertebrate biomass (B), production (P), productivity (P/B̄-ratio), and transfer efficiency (TE) influenced by sewage effluents discharge in a diffusion zone. Our results indicated a clear distribution pattern of macrofauna communities along the sewage discharge gradient where biological factors (B, P, P/B̄, and TE) were driven by changes observed in community structure, composition, and the influence of environmental variables. The lowest B, P, and P/B̄ were observed at the stations sampled close to the pipelines. Abundance, biomass, production, and productivity increased with increasing distance from the pipelines toward stations placed at 100 m distance and then decreased toward the stations placed at 200 m, where there was a negative relationship between TE and B of benthic macrofauna at sampling stations. Overall, there was a clear influence of the sewage discharge on macrofauna communities, but not overly negative, and indicates surrounding environments are influenced by moderate organic impact.

OGS
National Institute
of Oceanography
and Applied
Geophysics

March 15th, 2022

To Francois Galgani, Gui-Peng Yang, Michel Boufadel
Editor-in-Chief of Marine Pollution Bulletin

Dear Editors,

We would like to submit our manuscript for consideration of publication in the journal of Marine Pollution Bulletin. The manuscript is entitled "Impacts of organic enrichment on macrobenthic production, productivity, and transfer efficiency: What can we learn from a gradient of sewage effluents?". It answers to scopes and aims of the journal. The manuscript has not been published elsewhere and it has not been submitted simultaneously for publication elsewhere. We think that our study deserves to be published, because it is the first attempt to investigate the variations in the production (P), productivity ($P/\bar{B}$ yr^{-1}), and transfer efficiency (TE) of coastal macrobenthic communities subjected to a sewage discharge. In this study, our results showed a clear pattern of macrofaunal community distributions along the sewage gradient and among station positions. Our results showed minimum biomass (B), P, and $P/\bar{B}$ nearby the pipelines (stations located at <5 m from the pipelines) and maximum ones for intermediate distance (stations located at 100 m from the pipelines). Besides, this study displayed a negative relationship between TE and B of benthic macrofaunal at the sampling stations. Therefore, this suggests that the stations placed at 100 m distance from the source of organic matter most probably could be considered as the middle of the transition zone, where species numbers and biomass are usually higher, while close to the pipelines, there is an ecotonal zone where the exclusion of sensitive species is no already occurred but the presence of some opportunistic species start to attest. However, the effects of the sewage pipeline were not so dramatic and in fact, compared to other sites indicates an average of contribution to productivity, and local (proximity) effects were never too negative. However, due to the lack of information on TE of macrobenthic communities, not only in stressed conditions, we strongly recommend applying this approach in further studies to better understand the behaviour of TE and the related role of the energy fluxes among the macrobenthic trophic webs concerning to different environmental conditions and macrofaunal compositions.

All authors have approved the final article.

As requested, we include a list of potential reviewers:

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We are looking forward to receiving the reviewers’ comments.

Yours sincerely,

Dr. Seyed Ehsan Vesal (On behalf of the other authors), ehsan.vesal@ias.cnr.it

Consiglio Nazionale delle Ricerche, Istituto per lo studio degli impatti Antropici e Sostenibilit`a in ambiente marino (CNR-IAS).

Dear MPB editorial manager,

there is no response to reviewers since there are no previous reviewers comments, only for Language Editing Changes, that we addressed.

Best regards,

Seyed Ehsan Vesal

Highlights:

- Community features were affected by moderate organic impact;
- Production and productivity displayed higher values in the intermediate distance;
- Analyses distinguished a negative relationship between transfer efficiency and biomass;
- Biomass shows the lowest values at the high impacted stations;
- The highest values of transfer efficiency were observed at the high impacted stations.

Impacts of organic enrichment on macrobenthic production, productivity, and transfer efficiency: What can we learn from a gradient of sewage effluents?

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Abstract

We studied the macrobenthic invertebrate biomass (B), production (P), productivity ($P/\overline{B}$-ratio), and transfer efficiency (TE) influenced by sewage effluents discharge in a diffusion zone. Our results indicated a clear distribution pattern of macrofauna communities along the sewage discharge gradient where biological factors (B, P, $P/\overline{B}$, and TE) were driven by changes observed in community structure, composition, and the influence of environmental variables. The lowest B, P, and $P/\overline{B}$ were observed at the stations sampled close to the pipelines. Abundance, biomass, production, and productivity increased with increasing distance from the pipelines toward stations placed at 100 m distance and then decreased toward the stations placed at 200 m, where there was a negative relationship between TE and B of benthic macrofauna at sampling stations. Overall, there was a clear influence of the sewage discharge on macrofauna communities, but not overly negative, and indicates surrounding environments are influenced by moderate organic impact.

Keywords: Benthic ecology; Sewage discharge; Production; Productivity; Transfer efficiency; Marine coastal areas.

1. Introduction

Coastal environments host a wide diversity of benthic macrofauna species that represent a fundamental food source for higher trophic levels (Kabat et al., 2012). Benthic macrofauna invertebrates hold key ecological functions (Reiss and Kröncke, 2005; Bolam and Eggleton, 2014) and macrozoobenthos communities are often used for monitoring marine systems (Nasi et al., 2018; El Asri et al., 2021; Oselladore et al., 2022). Changes in the composition of macrozoobenthic communities can also affect the food webs, with potential repercussions on the entire ecosystem functioning (Gray et al., 2006; Tillin et al., 2006; Schratzberger et al., 2007). Consequently, defining the potential role and estimating the benthic invertebrates interactions on food webs can contribute to the assessment of marine ecosystems and evaluating possible anthropic impacts.

Benthic secondary production, which indicates the assimilation of organic matter's energy per unit of time and area (Cusson and Bourget, 2005), is an important ecological parameter, considered as an overall indicator of ecosystem functioning summarizing in traits of populations (biomass, life span, and body size) (Dolbeth et al., 2012), effects of biotic interactions (Hall et al., 2006; Kimmerer, 2006), as well as other environmental variability and anthropogenic impacts (Waters and Crawford, 1973; Dolbeth et al., 2012).

The information on benthic biomass and production helps understand ecosystems dynamics (Nilsen et al., 2006; Libralato and Solidoro, 2010) and knowing how benthic production and biomass are distributed through trophic levels (TLs; Odum and Heald, 1975; Pauly and Watson, 2005) is important for understanding energy pathways, transfer efficiency as well as potential availability to upper TLs (Eddy et al., 2020).

Furthermore, the pyramid of biomass and production over TLs has long been used to represent the structure of the ecosystem (Lindeman, 1942; Baumann, 1995), since it provides an understanding of energy flows (Stergiou and Karpouzi, 2002) and can be applied as an empirically based synthetic index to compare species feeding habits (Badalamenti et al., 2000). The continuous description of biomass and production pyramids (Gascuel et al., 2005; Libralato and Solidoro, 2010) also favored the development of novel analyses and definitions of ecosystem perturbation indicators (Link et al., 2015). Another important concept related to the TLs is the transfer efficiency of energy (TE), which is calculated as the ratio between production at two successive trophic levels (Libralato et al., 2008). In an ecosystem, the TE between trophic levels is a central concept related to the mean individual growth efficiency and standard metabolism (Kerr, 1974; Andersen et al., 2008); even intangible changes in TE can pool across trophic levels, and cause intense differences moving in the upper TLs until it affects the top predator abundances (Ryther, 1969; Stock et al., 2017; Moore et al., 2018; Link and Watson, 2019).

Stress effects on benthic assemblages have already been evaluated, especially in the last 40 years (e.g. Pearson and Rosenberg 1978; Souza et al., 2013; Nebra et al., 2016; Gomes and Bernardino, 2020). Many authors found that changes in abundance of small-sized species and dominance of opportunists were caused by stressors, and have led to a reduction in macrofauna species richness and diversity (Gray et al., 1990; Hyland et al., 2005; Magni et al., 2022). To date, the focus has mostly been on changing in terms of structural features of benthic communities (e.g., abundance, biomass, and species composition) but little is known about the production (P), productivity (P/$\overline{\mathrm{B}}$), and transfer efficiency (TE) of macrofauna communities.

Studies on benthic macrofauna communities have traditionally focused on species diversity and composition (e.g., Washington, 1984; Mouillot et al., 2006; Ieromina et al., 2016), and little attempts have been made to assess the ecological importance of benthic invertebrate productivity and TE (e.g., Pranovi et al., 2005) also because of inherent difficulties in their quantification. In brief, while biomass is classically sampled directly (e.g., Llopis-Belenguer et al., 2018), the macrobenthic production (P) can be measured using empirical models for the production-to-biomass (P/$\overline{\mathrm{B}}$-ratio) (yr^{-1}), namely also productivity (Dolbeth et al., 2005). The benthic P/$\overline{\mathrm{B}}$-ratio patterns are universally recognized as being mostly influenced by life-history characteristics such as population density, body mass, recruitment, age, and trophic conditions (Waters, 1977, Rigler and Downing, 1984). The P/$\overline{\mathrm{B}}$-ratio for a given species was proposed by Sanders, (1956) as a proxy of the population turnover time of an organism and lifespan has been recognized as its main predictor (Robertson, 1979). Recent studies have successfully predicted P/$\overline{\mathrm{B}}$ values using empirical models (Cusson and Bourget, 2005; Zhang et al., 2011; Bolam and Eggleton, 2014). Brey's (2012) model is an assumption to better estimate than any other empirical one the prediction of P/$\overline{\mathrm{B}}$ or P values and also takes into consideration further requirement inputs such as feeding mode and motility (Fuhrmann et al., 2015).

Some studies evidenced the variation of P/$\overline{\mathrm{B}}$ for benthic communities due to temperature and depth (e.g., Degen et al., 2015), while others are linked to areas at higher latitudes (e.g., Nilsen et al., 2006) and also estuary influences (e.g., Bissoli et al., 2018). However, less attention was paid to P/$\overline{\mathrm{B}}$ and TE estimations in coastal areas due to anthropogenic impacts. In this regard, several studies have noted that coastal eutrophication, in particular sewage discharges (Nixon, 1995; Dell'Anno et al., 2002; Yeleliere et al., 2018), significantly impacts the structural features of the macrozoobenthos community, causing notable variations in species composition and a biodiversity decrease (Short and Wyllie-Echeverria, 1996; Patrício et al., 2009; Tadir et al., 2017).

In this study, we investigate the local variations in the production (P), productivity (P/$\overline{\mathrm{B}}$ yr^{-1}), and transfer efficiency (TE) of coastal macrobenthic communities subjected to a sewage discharge. We hypothesized that the macrobenthic communities respond to the sewage discharge with spatial variations, not only in terms of species composition and numbers but also in terms of biomass, production, P/$\overline{\mathrm{B}}$, and TE. More specifically, we answered the following questions: 1) What are the spatial differences in the biological factors of macrobenthos (biomass, production, P/$\overline{\mathrm{B}}$, and TE) along a gradient of sewage effluents discharge? 2) Is there any relationship between the spatial variability of biological factors with the environmental variables (grain-size, TN, Corg, C/N, and Eh)? 3) Does TE reflect the variations in benthic community compositions and structure along the gradient of sewage discharges?

Overall, the results can shed light on the impacts of the organic and nutrient loads on the ecosystem, as well as the contribution of the benthic community to the reduction of their effects.

2. Material and methods

2.1. Study area

The Gulf of Trieste is a shallow basin (average depth 17 m, maximum depth 25 m) along the northeastern side of the Adriatic Sea, Italy (Fig.1), covering a total area of 750 km^2 from the Tagliamento River mouth in the north-west to Savudrija/Punta Salvore (Croatia) in the south east and has a coastline of approximately 100 km (Celio et al., 2002; Fonda Umani et al., 2012; Barago et al., 2020). Bottom temperatures vary from 6°C to >20°C, whereas the temperatures range from 5°C to >27°C at the surface.

Sedimentation within the Gulf is mainly controlled by river inputs rather than marine currents; in particular, Isonzo River, the main contribution of freshwater and sediments, leads to a pycnocline which increases during summer due to mixing with the high temperature of the surface layer, whereas the sedimentation rate reaches about 2.5 mm y^{-1} in front of Isonzo stream and a rate up to 1 mm y^{-1} in the central part of the Gulf (Malačič, 1991; Covelli et al., 1999).

The Servola disposal plant is the main urban sewage discharge plant of Trieste city. It is a mixed type plant, collecting and treating both meteoric and wastewaters, serves up to 200,000 inhabitants and has a maximum flow of 6000 L sec^{-1} (Solis-Weiss et al., 2007). Since 1992, the Servola pipelines dispose of sewage after mechanical and chemical treatments. The sewage discharge flow is released through two submarine pipelines of 6.5 and 7.5 km in length, which includes several sewage diffusion towers at the end of both pipes, leading to the sea to a depth of 22 m. These diffusion towers are located in the last 500 m of the shortest pipe and the last 1000 m in the longest one, for a total of 1.5 km diffusion zone. Moreover, the pipelines have a capacity varying from 206 L/s during the dry to 618 L/s during the rainy season, respectively (Novelli, 1996).

2.2. Sampling design and processes

The sediment and macrobenthic monitoring were carried out in April 2018 through 18 sampling stations placed in such a way as to take into account the distance from the diffusion zone, the distance from the ending part of the pipelines, and the direction to the average annual bottom current in the area (SSE 170 °). Thus 15 sampling stations were distributed along 3 transects: Proximal-P (at the end of the shortest pipeline); Medial-M (in the middle part of the 1km sewage diffusion area); Distal-D (at the end of the longest and main pipeline). For each transect, one station (0P, 0M, 0D) was placed nearby the diffusion area at <5 meters, other stations were located over current and undercurrent (indicated with "-") at 100 (1P, 1M and 1D; -1P, -1M and -1D) and 200 meters from the pipelines (2P, 2M and 2D; -2P, -2M and -2D). Two additional stations were placed in front of the main outfall at 100 and 200 m (1FD, 2FD, respectively). Additionally, a reference station was located at the same depth as the others, 2 km from the distal end of the pipeline in the opposite direction to the average annual bottom current (station RS) (Fig. 1 and Table 1). In this study, we considered groups 0, 1 and 2 of stations for those stations placed at <5, 100, and >200 meters away from the pipes, respectively.

In each sampling station, we estimated the water column temperature at the bottom using CTD Probe (SBE 16plus V2 SeaCAT). Sediments for physical and chemical analyses (grain-size, Total Organic Carbon-Corg, Total Nitrogen-TN contents, and redox potential-Eh) and macrofauna communities were collected by a Van Veen grab (0.1 m^2). The macrofauna communities were sampled in three replicates for each station and sieved through a 1 mm mesh. The retained sediment and organisms were immediately fixed in ethanol 70°. In the laboratory, taxonomic identification of benthic macrofauna was carried out to the lowest possible taxonomic level and species abundance was counted. Species names were cross-checked against the World Register of Marine Species (https://marinespecies.org/).

2.3. Environmental variables

The grain-size analysis, including sand, silt, and clay fractions (%), was determined by sieving sediments at 2 mm; it was then first pre-treated with 10% hydrogen peroxide ($60^{℃}$ for 24 hours) and afterward analyzed with a Malvern Mastersizer 2000 equipped with Hydro 2000s. Total organic carbon (Corg) and nitrogen (TN) were also measured on freeze-dried sediment samples which were milled using a pestle and mortar and a fraction > 250 μm was isolated from the rest of the specimen.

Subsample triplicates (~8–12 mg) were straightforwardly weighed in capsules (5×9 mm) on a micro-ultra balance Mettler Toledo model XP6 (precision of 0.1 μg). Tin and silver capsules were utilized for TN and Corg measurement, sequentially. The values of Corg and TN (represented as mg g^{-1}) were estimated utilizing an elementary analyzer CHNO-S Costech model ECS 4010. Before Corg quantification, based on Sharp methods (1974), subsamples were treated with expanding HCl concentrations (0.1 and 1 N) to eliminate the carbonate (Nieuwenhuize et al., 1994).

The redox potential (Eh) allows inferring the depth of oxygen permeation from surface sediments (Hargrave et al., 2008), determines the physico-chemical state of marine sediments, and indicates the amount of organic matter. Eh measurements were estimated on the cores on board. Estimations were made utilizing electrodes from the undisturbed superficial layer (0-1 cm) (Pearson and Stanley, 1979). The platinum electrode was standardized (CRISON 5265) in a light solution and then the analysis was carried out with Metrohm 704 voltimet (Clesceri et al., 1996).

2.4. Estimation of community biomass, production, and $P/\overline{B}$ ratio

For biomass measurements, individuals were blotted dry for about 30 seconds (Nilsen et al., 2006) and then wet weight (WW) was measured using a digital laboratory scale with high precision and accuracy. In case of the presence of tubicolous polychaetes, the tubes were removed before weighing. Subsequently, to obtain the Dry Weight (DW), samples were placed in an oven at 100°C for 24 hours, cooled in a lab desiccator to normal room temperature, and then weighed. To obtain the ash quantity of the organisms, they were placed in an oven at 500°C for 24 hours, cooled to room temperature in a lab desiccator, and then weighed. To obtain Ash Free Dry Weight (AFDW), ash-weight was subtracted from DW (Wetzel et al., 2005).

A modified multi-parameter model based on Artificial Neural Network (ANN) and developed by Brey, (2012; Version 01-2012 at http://www.thomas-brey.de/science/virtualhandbook), was used to estimate the Production-to-Biomass ratio (P/$\overline{\mathrm{B}}$) of sampled invertebrates.

To implement the model and as one of the required input data, the biomass of each species (AFDW) for each station was converted to energy Joule values using energy densities (kJ g^{-1}) referring to a global "Conversion factors" data published by Brey et al., (1988, 2010). If no conversion factors were available at the species level, a factor was used from the next highest taxonomic rank. The model allows determining estimates of annual P/$\overline{\mathrm{B}}$ ratios for each taxon at each station with a 95% confidence interval based on three main input parameters: individual body mass (Joules), the average temperature (°C), and the depth of the sampling station (meters). We added the average depth and temperature of the sampling area in April (21 m and 9.5°C, respectively) and considered this month as the right sampling period for obtaining annual estimates of P/$\overline{\mathrm{B}}$. Furthermore, the ANN uses functional traits which consisted in motility classes (infauna, sessile, crawler, and facultative swimmer), taxon (Mollusca, Annelida, Crustacea, Echinodermata, and Insecta), feeding type (herbivore, omnivore, and carnivore), habitat (lake, river, marine, subtidal, and exploited). Since the species were collected in a marine coastal environment with no commercial exploitation in our study area, the other indices were always zero. The functional traits are described by binary inputs (0 or 1) to indicate belonging to categories. Additionally, we measured the productivity for each station by summing the P/$\overline{\mathrm{B}}$ values of the species found in each station.

We obtained the information on data inputs and biological traits from literature (i.e. Giangrande, 1997; Rouse, 2000; Jumars et al., 2015), databases (https://www.itis.gov/; http://www.polytraits.lifewatchgreece.eu; https://www.marinespecies.org) and expert knowledge. We also calculated the average production for each station by multiplying P/$\overline{\mathrm{B}}$ and biomass of all taxon found at each station.

2.5. Trophic spectra and transfer efficiency (TE) calculation

Data on AFDW biomass, P/$\overline{\mathrm{B}}$-ratio, trophic levels (TL_i; species i), and dispersion of the TL_i (OI_i; quantified as the variance) for each species at sampling stations, were used for obtaining trophic spectra of production based on the dispersion-based method proposed by Libralato and Solidoro (2010). We collected the data on TL_i and OI_i for each species from the database (https://www.sealifebase.ca/search.php). When no information was available, we used the data for the family level.

The trophic spectra of production determined based on macrobenthic community data allowed calculating the transfer efficiency (TE; see Libralato and Solidoro, 2010) as a measure between productions at two adjacent integer trophic levels and varies between 0 to 1 (Lindeman, 1942). We used the trophic spectra in the range between 2<TL<4 to estimate TE values.

2.6. Data analysis

The dissimilarity matrixes from square root transformed abundance and productivity data were calculated using the Bray–Curtis coefficient. This transformation aimed to accentuate the effect of species with low values in the sampling stations. Then a non-metric multidimensional scaling analysis (nMDS) plot was performed on abundance data to visualize the ordering of the samples in reduced (2D) space.

To test for macrobenthic community differences along the gradient of sewage discharge, an ordered one-way ANOSIM test was performed on both matrices using the 'distance from the diffusion zone' as factor. Stations placed at <5, 100, and 200 meters were gathered in group 0, group 1 and group 2, respectively. The reference station was included in group 2.

Furthermore, RELATE analysis was used to test matched resemblance matrices to determine whether results from the combination of abundance and $P/\overline{B}$ values of macrofauna were significantly correlated. These analyses were performed using PRIMER 7 (PRIMER-E Ltd. Plymouth, UK) (Clarke et al., 2014).

Multivariate analysis (Principal Component Analysis, PCA) on log-transformed and normalized data was used to investigate the spatial variations in biological factors (biomass, production, $P/\overline{B}$, and TE) and abundance of macrofauna community among sampling stations. Additionally, redundancy analysis (RDA) was performed to determine the relationships between biological and environmental data. Both the PCA and RDA analyses were conducted using R version 3.6.0 (R Development Core Team, 2018). Statistical tests in SPSS v. 20 were also carried out to indicate the significant levels of observed environmental variables and biological factors. A Spearman–rank correlation test was performed to investigate relationships between the biological factors and environmental variables, and Jonckheere-Terpstra test was used to detect significant differences in biological factors and environmental variables among the stations along the sewage effluents discharge gradient.

3. Results

3.1. Spatial variation in environmental variables

The variation of environmental variables measured at each station e.g. sediment grain size, TN (Total Nitrogen); Corg (organic carbon); C/N (carbon and nitrogen ratio); Eh (redox potential), are shown in Table 1. The sediments of the whole study area were mainly characterized by the high percentage of fine particles. A higher value of silt was observed at stations 2M, whereas a higher percentage of clay was noticed at stations RS. The mean value of sand fraction (%) was 15.7±11.6 and the highest percentages were detected at the stations 0D and 0M. As corroborated by the Jonckheere-Terpstra test, significantly higher sand fractions were detected at stations nearby the ducts ($z= -3.60$, $p<0.01$). On the contrary, silt ($z=1.90$, $p<0.05$) and clay ($z=3.60$, $p<0.01$) values significantly increased toward the farther stations.

Furthermore, Corg significantly increased nearby the pipelines (Jonckheere-Terpstra test, $z= -2.23$; $p<0.05$). For both Corg and TN, the highest contents were observed at 0D (60.5 and 3.8 mg g^{-1} respectively), whereas the lowest ones were measured at RS (10.7 and 1.2 mg g^{-1} respectively). Also for C/N ratio, the highest values were observed in the sewage diffusion zone compared to RS. Notably, higher ratio values were estimated at stations close to the two outfalls (18.6 and 13.0 at 0D and 0P, respectively).

Lower Eh values were observed in the whole sewage diffusion area (average: -105.0±97.1 mV) compared to RS (54 mV). In particular, the lowest values of Eh were observed in the D and P transects, both influenced by the two sewage pipeline outfalls with an average of -177.0±63.8 mV and -101.6±71.4 mV, respectively.

3.2. Macrobenthic community distributions along the gradient of sewage effluents discharge

The total abundance ranged between 343 ind. m^{-2} to 3436 ind. m^{-2} at sampling stations 0P and -1D, respectively. Species composition varied following the gradient of distance from the main pipeline (Fig. 2). The nMDS plot divided the station close to the pipes (group 0) at the left side of the plot, to those farthest away at the right side (group 1 and group 2). The global ordered one-way ANOSIM test confirmed what was highlighted by nMDS analysis. The species composition significantly differed among groups of stations (R=0.52; P<0.01). In addition, the pairwise tests evidenced the similarly higher-range values of R (0.49 and 0.84) for the group 0 *vs* group 1 and group 0 *vs* group 2 comparisons respectively, while with a lower value (of 0.18) for group 1 *vs* group 2. These results implied that the explanation for the global test results in group 0 differed from group 1 and group 2, but the latter ones were less distinguishable. Therefore, the pairwise test mirrored a clear pattern of decreasing differences in the macrofauna community composition with the increasing distance from the pipelines.

3.3. Biomass, production, and productivity ($P/\overline{B}$-ratio)

The biomass (B) of the macrofauna communities in the whole sampling area was rather variable among the sampling stations, measured in 344.2±14.4 g m^{-2} based on wet weight. The average biomass, expressed in energy content, was 14.2±7.9 kJ m^{-2}, while production (P) and productivity ($P/\overline{B}$) were 21.7±17.3 kJ m^{-2} yr^{-1} and 1.3±0.4 yr^{-1}, respectively (Table 2). The highest biomass occurred at the station -1P, with a value of 28.1 kJ m^{-2}, whereas the lowest one was found at the station 0D with a value of 5.8 kJ m^{-2}. The production values ranged from a minimum of 3.9 kJ m^{-2} yr^{-1} (0D) to a maximum of 61.7 kJ m^{-2} yr^{-1} (1M), whereas the $P/\overline{B}$ varied from a minimum of 0.6 yr^{-1} (0D and 0P) to a maximum of 2.2 yr^{-1} (1M) (Table 2). Additionally, B, P, and $P/\overline{B}$ values followed the same pattern. The highest values were observed at the stations placed 100 m away from the pipelines (B, P, and $P/\overline{B}$: 16.7±10.5 kJ m^{-2}, 29.5±22.9 kJ m^{-2} yr^{-1} and 1.5±0.4 yr^{-1}, respectively) compared to farther ones (B: 13.4±6.6 kJ m^{-2}; P: 18.5±11.8 kJ m^{-2} yr^{-1}; $P/\overline{B}$: 1.2±0.3 yr^{-1}), whereas lower values were calculated at stations nearby the pipes (B: 10.3±5.3 kJ m^{-2}; P: 12.3±13.0 kJ m^{-2} yr^{-1}; $P/\overline{B}$: 0.93±0.5 yr^{-1}) (Fig. 3).

The estimated average $P/\overline{B}$ for the main taxonomical groups Polychaeta, Crustacean, Mollusca, Echinodermata were 0.72, 0.38, 0.20, 0.05 yr^{-1}, respectively. Polychaeta was the only group present in high number and high biomass, despite the low individual medium weights, at the majority of stations. A high dominance of the polychaete *Capitella capitata* with low estimated $P/\bar{B}$ ratios and biomass resulted at the stations located nearby the main underwater outfall (0D), indicating a lower contribution of this species to the total production compared to biomass at these stations (Table 2). Polychaeta showed the highest value of $P/\bar{B}$ at stations 1FD (69.3%) and -1D (67.6%) (0.87 and 0.99 yr^{-1}, respectively), whereas Crustacea and Mollusca were the major contributors to $P/\bar{B}$ at 1M. Echinodermata generally had a small contribution to total productivity due to their low abundance and biomass. Moreover, comparing the macrofauna species abundance and $P/\bar{B}$ matrices, they were very significantly related (RELATE test: rs=0.92; p<0.01). Spearman's rank correlation coefficient tests indicated that there was a positive significant correlation between B and P (rs=0.923, p<0.01), as well as a positive significant correlation between B and $P/\bar{B}$ (rs=0.617, p<0.01). Moreover, $P/\bar{B}$ was positively correlated with production (r_s=0.923, p<0.01). The result showed that there was no significant correlation between TE and other biotic factors (i.e., biomass, production, and productivity) (Table 3).

3.4. Transfer efficiency (TE)

TE showed the lowest values at the stations located 100 meters away from the pipes (group 1), whereas the highest TE value was observed at stations placed <5 m away from the pipelines (group 0) (Fig. 4). Moving away from the group 0 to group 1, TE decreased (17.6±3.3% and 11.8±0.2%, respectively), whereas biomass increased (10.3±1.7 and 16.7±1.4 kJ m^{-2}, respectively) (Fig. 4). However, in group 0, 0M showed a high TE value compared to other stations in front of the two outfalls (i.e. 0P and 0D) (Table 2).

In the principal component analysis (PCA), all stations were distributed along the gradient of sewage effluents discharge. The cumulative variance was 78%, which showed stations plotted along the PCA1 (54.2%) were separated by distinctions in B, P, and $P/\bar{B}$. The PCA2 (23.8%) separated stations correspond to differences in TE and abundance. Stations on the right side of the plot with higher values of B, P, and $P/\bar{B}$ were noted (i.e. the stations located at 100 m distance from the pipelines), whereas the stations draft on the left side of the plot showed lower ones. Further, stations plotted at the top side of the plot showed high values of TE and low values in B such as 0D and 0M. Lastly, stations located on the bottom side showed high B values and low TE values (Fig. 5).

3.5. Relationships between benthic macrofauna biological factors and environmental variables

The RDA, performed on biological factors and environmental variables of the sampling stations, showed 88.7% of the total variance, accounting for the 1st (64%) and 2nd (24.7%) axes (Fig. 6). TN, Corg, and C/N were correlated with the negative part of the first axes and plotted on the left side, nearby the station in front of the outfalls (0D and 0P). The sand was positively related with TE, where 0M, 0D, 2FD, and RS were plotted in the top part of the plot, while silt and clay had a negative correlation with TE, where 1M, 1P, and -1P were plotted on the bottom side of the plot. The Eh vector points in the opposite direction of TN, Corg, and C/N contents, showing that stations with higher Eh tend to have the higher B, P, and $P/\bar{B}$ with lower TN, Corg, and

C/N values (i.e., -1D, -1M, 1M, -1P, 1P, 2P and 2M). Moreover, Corg, and C/N values were positively associated with TE. RDA analysis showed that the distribution of stations nearby the pipelines in the plot (i.e. 0D, and 0M) was mainly related to the high values of TE and sand fraction. According to Spearman's rank correlation coefficient, P, $P/\overline{B}$ and TE showed a positive correlation with Eh (r_s=0.593, 0.632 ($p<0.01$) and 0.582 ($p<0.05$), respectively). Furthermore, the results highlighted that B, P, and $P/\overline{B}$ were negatively correlated with C/N (rs=-0.565 ($p<0.05$), -0.656 and -0.630 ($p<0.01$), respectively) (Table 3).

4. Discussion

In this study, we evaluated the influence of underwater sewage discharges on P, $P/\overline{B}$, and TE and considered the variation of these biological factors related to physico-chemical (grain-size, TN, Corg, C/N, and Eh) variables. The sampling design based on stations placed at increasing distance from the main source of contamination allowed assessing the spatial extent of increased organic enrichment, and biological effects on structure, production and efficiency of macrobenthic communities along the enrichment gradient.

Our results showed that the sand fraction and organic carbon was higher at stations nearby the pipelines compared to farther ones and lower redox potential with negative Eh values were observed at the stations gathered in D and P transects close to pipeline end. On the contrary, we found increasing patterns for silt and clay percentages toward the station placed 200 meters away from the pipes. All the above, was to be expected. For instance, higher sand fraction and Corg are due to the high value of deposit discharge by sewage pipelines, which also includes meteoric water that leads sand size clasts to the sea coming from the washout of land and streets (Diaz et al., 1995, Melis et al., 2019), while the low redox potential results from the lack of oxygen and hypoxic conditions at sediments nearby the end of the two pipes. The high microbial activity in the sediments for decomposing organic matter severely deplete dissolved oxygen, thus explaining the negative Eh values which are a direct effect of wastewater discharge at the diffusion area (Matijević et al., 2007; Arend et al., 2011).

Considering the faunal composition, the nMDS results (Figure 2) clearly indicated changes in benthic communities among stations sampled along the gradient of impacts of the sewage effluents. The monotonic changes in community compositions with increasing distance from the sewage ducts were also corroborated by the ordered ANOSIM analysis carried out in previous work (Auriemma et al., 2016). In particular, the stations nearby the diffusion zone (<5 meters) were clearly affected by the sewage, and the remarkable variations in species composition were observed, especially close to the two outfalls (stations 0P and 0D). Thus following the initial ‘disturbance effect’, when organic enrichment proceeds then reduced oxygen concentrations can lead to clear changes in species composition and abundance of the benthic organisms (Gray et al., 2002; Hyland et al., 2005; Magni et al., 2022).

4.1. Macrofauna production, productivity, and transfer efficiency role along the gradient of sewage effluents

Benthic biomass was an important factor in determining benthic production and this is consistent with other studies (Tumbiolo and Downing, 1994, Cusson and Bourget, 2005; Fuhrmann et al., 2015). In our study, stations belonging to group 1 showed higher abundance and number of species than other stations that have higher B and P: these findings confirm the classical Pearson and Rosenberg model, which is the prediction of responses of species richness, abundance, and biomass, for different levels of anthropogenic organic enrichment impacts on benthic communities (Pearson and Rosenberg, 1978; Rosenberg, 2001) (see Fig.3)

In accordance with Burd et al., (2012), our results showed that production of macrofauna communities increased with distance from the pipelines and reached a maximum at stations located 100 m away from the source of organic matter (group 1), probably due to the reduced organic matter flow. Production is affected by several anthropogenic disturbances to the seabed, such as dredged material disposal (Rhoads et al., 1978; Wilber and Clarke, 1998), fish farming (Kutti et al., 2008), and bottom fishing and trawling (Jennings et al., 2001a, 2002; Hiddink et al., 2006). This study illustrated that both biomass and production have a peak at intermediate distance and they stabilized at the group 2 at 200 meters from the pipelines. This result suggests that at 100 m (group 1) from the source of organic matter there is a transition zone, where, as postulated by Pearson and Rosenberg (1978), species numbers and biomass are usually higher also in accordance with the intermediate disturbance hypothesis (Connell and Slatyer, 1977). In this transition zone between the natural environmental condition, with reduced organic matter input and severe environmental conditions, with high organic matter and a huge reduction in species numbers, there is an ecotonal zone where the exclusion of sensitive species has not already occurred but the presence of some opportunistic species begins to be seen. Accordingly, our results show that stations close to pipelines (group 0) can most likely be considered at the end of ecotonal zone, due to the proximity to the maximum value of organic enrichment, where the macrobenthic community can almost fully utilize it before starting to decrease in species numbers and diversity. The findings of this study were similar to other studies regarding infauna species dominance concerning a gradient of organic enrichment, albeit on a much larger spatial scale (e.g. Brown et al., 1987; Weston, 1990).

According to the ANN model prediction, $P/\overline{B}$ increased along the gradient of sewage effluents discharge with increasing distance from the pipelines, where it was lower at group 0 than group 1 and group 2. Additionally, in the whole study area, polychaetes dominated the benthic macroinvertebrate communities in numbers, biomass, and $P/\overline{B}$ despite the low average individual weights, compared to the other macrofauna taxa. The dominance of polychaetes is well documented in silty-clay sediments, also in our study area (Nasi et al., 2017; Vesal et al., 2021). Previous studies have also reported that polychaetes were the most productive group, mainly due to their density combined with a high $P/\overline{B}$ ratio (e.g. Rhoads et al., 1978; Möller, 1985; Mistri and Ceccherelli, 1994; Nilsen et al., 2006). In agreement with our results, Lin et al., (2016) estimated higher productivity in the stations with lower Corg that were linked to modifications in the benthic community. In our study, the highest values of P/$\overline{B}$ were observed at stations placed 100 meters away from the pipes due to a higher proportion of polychaetes which are recognized as r-strategist species, i.e., with high P/$\overline{B}$ values (Möller, 1985). Therefore, our results support the pattern hypothesised by Pearson and Rosenberg (1978) in the classic model for biomass and numbers of species. Surprisingly, we found the low values of P/$\overline{B}$ ratios at the group 0 (close to the pipeline). We observed no peak of opportunistic species (e.g., Polychaeta *C. capitata*) at stations near the pipeline, but we did find other species with low P/$\overline{B}$ ratio (i.e. K-strategist) (Mistri and Ceccherelli, 1994; Cusson and Bourget, 2005), thus resulting in lower total productivity than other stations along the organic enrichments sewage gradient. Moreover, the average P/$\overline{B}$ estimated (1.3 y^{-1}) in our study area was considerably higher than previous estimates for the South-western Barents Sea (0.25 y^{-1}, Denisenko, 2001) the Sørfjord, North Norway (0.29 y^{-1}, Nilsen et al., 2006), the Barents Sea (0.3 y^{-1}, Denisenko and Titov, 2003) and the Baltic Sea (0.32 y^{-1}, Harvey et al., 2003) (Table 4). Due to the relatively high P/$\overline{B}$ ratios observed in our study area often being accompanied by low mean biomass, the macrofauna communities tended to show a high total production, indicating that the effects of organic enrichment derived from the sewage pipelines were not so negative on the nearby impacted study area. This is possibly due to a combination of discharge levels with respect to the environment and capacity of the system to disperse the loads.

In this study, macrofauna invertebrates were sampled in April and the assessments of biotic variables did not reflect seasonal changes. Furthermore, the sampling period was before the settling of juveniles for most macrofauna organisms. However, P/$\overline{B}$ values may differ due to seasonal variations in environmental variables and the relative contribution of juveniles (with small body size and high P/$\overline{B}$ ratios) (Fuhrmann et al., 2015). Hence, estimates given in this study were likely not subject to large seasonal changes. We could therefore infer that P/$\overline{B}$ measured represents the average annual values fairly well and essentially mirrors the impact of the sewage discharge along the sampled gradient.

Regarding station 0M, we observed higher values of biomass, production, and P/$\overline{B}$ than other stations placed nearby the pipes (i.e. 0D and 0P). This was probably due to the lower amount of organic enrichment received here, compared to stations 0D and 0P located in front of the two outfalls. Indeed, these features indicate that probably the environmental context at 0M is fairly similar to that observed at stations located 100 m away from the pipes and it is reasonable to expect the similar environmental patterns in terms of sewage organic loads influence. This is also confirmed by the overall macrofauna composition and richness present at 0M station, which is much more similar to those observed at stations 100 m away rather than those placed in front of the two sewage outfalls, as is clearly shown in the nMDS plot (Auriemma et al., 2016; Vesal et al., 2021).

Our results support the hypothesis that increased organic enrichments due to the sewage discharges could decrease biomass but increase transfer efficiency at the stations located <5 m away from the pipelines (group 0) compared to other groups of stations. The mechanisms accounting for these responses are somewhat difficult to discern as they result from a combination of local factors enhancing productivity and community changes. This approach now ensures that transfer efficiency is important to study macrofauna communities, not only influenced by sewage discharge given in the present study outcomes. Transfer efficiency is shaped by the nature of species involved, the diversity of food web interconnections and energy fluxes of organic material. However, fluctuations in species abundances can control energy pathways through food webs, and systems dominated by a small number of species may have limited resilience (Steneck et al., 2011). In our case, in front of the pipeline outfalls, the sewage discharge causes the occurrence of tolerant species with smaller individual bodies, fast turnover and lowest values of biomass (i.e. the small-sized polychaeta, *Capitella capitata*) that can suggest the locally high TE. Moving away from the outfalls (group 1), TE decreased and biomass increased, with TE and B values shifting to normal at increasing distances (group 2). Therefore, we hypothesized *a priori* that sewage discharges would lead to a decrease in production and productivity rates for macroinvertebrates near the pipelines but nonetheless resulting in a maximum of biomass at an intermediate disturbance level.

A wide range of processes and scales affect transfer efficiency results and its estimation can be challenging (Eddy et al., 2020) since TE results from diverse metabolic aspects, such as life cycle, consumption, excretion, respiration and exploitation. Here, TE was roughly estimated for the benthic community on the basis of the trophic level of organisms within an ecosystem determined by their diets, and production at each trophic level (Ullah et al., 2018; Eddy et al., 2020) and our results indicate that the macrofauna species with low trophic levels, low biomass, at group 0, have higher overall TE compared to the farther stations from sewage discharge.

The estimated average TE equal to 13.9% for macrofauna communities in the whole sampling area (see Table 2) is larger than the global average of 10% estimated for organisms from zooplankton and benthic organisms to fish, but it is consistent with average estimates for trophic levels 1-2 in the temperate Northern hemisphere marine ecosystems (13%) (Harrison et al., 2000).

4.2. Relationship between biological factors and environmental variables

Similarly, the macrofauna P, P/$\overline{B}$, and TE were influenced by the environmental variations due to the sewage effluents. In this study, we attempted to identify the relationships between environmental variables and biological factors of benthic macrofauna.

The RDA analysis showed that all stations grouped by distance (<5, 100, and 200 m) were distinctly different from each other according to their biological factors and environmental variables. The analysis indicated that the influence of Eh and the grain size characteristics were the most important for biological factors of macrofauna communities. However, environmental conditions, in particular the characteristics of sediments, typically structure soft-bottom benthic communities (see e.g., Nilsen et al., 2006; Gray and Elliott, 2009).

The Eh trend was the only environmental parameter that showed a positively significant correlation with richness, production, productivity, and TE estimates. Indeed, the values of biological factors and Eh ones, increased with the distance from the pipelines to stations less impacted by sewage discharge (e.g. stations located 100 meters' distance), suggesting that the amount of organic matter can directly influence the environmental sedimentary conditions and, therefore, it can affect these biological factors with deeply changes, even on small spatial scales.

However, it is possible that while certain environmental conditions enhance productivity for one species, others may show an increase in productivity under different values of environmental variables (Bolam et al., 2010); indeed, higher P/$\overline{B}$ ratios suggest higher population resilience to environmental perturbations (Tumbiolo and Downing, 1994). We observed that C/N contents along the gradient of sewage effluents discharge showed no correlation with TE, but there was a notable negative correlation with B, P, and P/$\overline{B}$.

In this study, the proportion of TE made up of the macrofauna, increased at stations located near the pipelines with increasing sand fraction and low Eh, similarly to how Kutti et al. (2008) reported that the station close to high loading of organic matter was characterized by low Eh values. In other words, the impact of sediment deposition tends to be more taxon-selective; ultimately it favors the taxa that have an inherent ability to vertically migrate through the disposal of sediments (e.g. Hinchey et al., 2006; Bolam et al., 2011; Last et al., 2011; Burd et al., 2012).

However, the direct correlations between the macrobenthos and sediment characteristics clearly showed that variation in the measured environmental variables was linked with the observed decreases in biomass, production, and P/$\overline{B}$ of the macrofauna. Environmental variables influence community compositions and P/$\overline{B}$ that are largely a function of the intrinsic characteristics of species (Cusson and Bourget, 2005; Bolam et al., 2010). In general, sediment composition and the available organic matter, are known to structure benthic communities and determine the distribution of benthic infauna (e.g. Pearson & Rosenberg 1978, Wieking and Kröncke, 2003; Kröncke et al., 2004; van Hoey et al., 2004).

B, P/$\overline{B}$, and TE, as well as all other parameters investigated here, are known to vary over time, to be influenced by external environmental factors, and also might be deeply affected by unquantified spatial exchanges and flows (because of transport and active movement of species). Therefore, since estimates are based on assumption that sampled areas are closed systems, a limitation of the above might result from the influences of possible lateral energy flows.

5. Conclusion

This study quantifies the potential impacts of the organic matter loading from sewage discharge to the coastal marine environment and its influence on structure, biomass, production, productivity, and transfer efficiency of macrofauna communities. On a local scale, we observed a clear change in the macrofauna community in the stations studied along the sewage gradient. Distance from the source, grain size characteristics and Eh were the most important drivers for the variations of the functional processes of macrofauna. Our results showed minimum B, P, and P/$\overline{B}$ nearby the pipelines and maximum ones for intermediate distance (stations located at 100 m from the pipelines). This study showed a negative relationship between TE and B of benthic macrofauna at sampling stations. TE displayed the highest value at stations close to the pipeline outfalls (group 0), where benthic communities are also characterized by opportunistic smaller species with low biomass values and fast turnover (i.e. Polychaeta, *Capitella capitata*). Therefore, this suggests that stations placed 100 m away (group 1) from the source of organic matter could be considered as an intermediate/transition zone, where species numbers and biomass are usually higher. Close to the pipelines, there is no exclusion of sensitive species but only the presence of some opportunistic species. Overall, our results indicate a direct influence of the sewage discharge on the biological features of macrofauna communities and show surrounding bottoms influenced by moderate organic impact. However, the effects of the sewage loads were not so dramatic. Indeed, compared to other sites, it indicates an average increasing productivity contribution, where the local (proximity) effects were never too negative.

Additionally, to reduce the effects of sewage discharge on macrofauna communities, we suggest planning the sewage discharge pipelines by placing the maximum possible number of diffusion towers to minimize the impact at the end of the pipeline main outfall. Furthermore, due to the lack of information on TE of macrobenthic communities, not only in stressed conditions, we strongly recommend applying this approach in further studies to better understand the behaviour of TE and the related role of the energy fluxes among macrobenthic trophic webs concerning different environmental conditions and macrofauna compositions.

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Table 1. Sample stations, depth range, coordinates, sampling stations distance from the pipelines, and physical-chemical parameters were measured at sampling stations in April 2018. Sediment grain-size, TN (Total Nitrogen); Corg (organic carbon); C/N (carbon and nitrogen ratio); Eh (redox potential).

Station	Depth	Latitude	Longitude	Distance from the pipeline	Sand	Silt	Clay	TN	Corg	C/N	Eh
	(m)			(m)	(%)	(%)	(%)	(mg N g^{-1})	(mg C g^{-1})		mV
0D	22.6	45°38'605"	13°40'862"	5	39.6	38	22.4	3.8	60.5	18.6	-290
0M	20.1	45°38'601"	13°41'788"	5	39.4	37.3	23.3	1.7	13.5	9.4	-25
0P	22	45°38’435’’	13°41’600"	5	25.7	46.6	22.7	2.5	27.8	13	-204
1FD	22.7	45°38'601"	13°40'788"	100	9	54.3	36.7	1.9	16.7	10.1	-150
1D	23	45°38'662"	13°40'849"	100	13.6	53.3	33.1	1.8	16.8	10.8	-210
–1D	22.3	45°38'561"	13°40'872"	100	10.5	54.2	35.3	2.5	21.9	10.2	-101
1M	20.6	45°38'599"	13°41'233"	100	11.4	54.6	34	1.9	15.9	10	-117
–1M	18.3	45°38'493"	13°41'258"	100	34.9	40.8	24.3	1.3	12.3	11.3	70
1P	21.9	45°38’483"	13°41’588"	100	12.1	54.3	33.6	2.5	17.6	8.2	-57
–1P	22	45°38’989"	13°41’612"	100	17.2	50.1	32.7	2.3	16.8	8.5	-117
2FD	23.1	45°38'592"	13°40'718"	200	5.9	52.7	41.4	1.8	15.2	10	-130
2D	23.5	45°38'716"	13°40'834"	200	7	54.1	38.9	1.9	16.1	10	-150
–2D	21.9	45°38'506"	13°40'886"	200	7	54.6	38.4	1.8	16.2	10.3	-210
2M	22,00	45°38'649"	13°41'215"	200	5.7	55	39.3	1.9	16	9.7	35
–2M	18.8	45°38'441"	13°41'271"	200	21.3	48.9	30	1.6	13.3	9.9	8
2P	22	45°38’531"	13°41’576"	200	8	54.5	37.5	2.1	16.7	9.2	-15
–2P	21.4	45°38’344"	13°41’623"	200	7.8	53.2	39	1.8	14.1	9.3	-115
RS	22.5	45°37'540"	13°41'118"	2000	7	50.5	42.5	1.2	10.7	10.5	54

Table 2. Total Biomass-B, Production-P, Productivity-P/$\bar{B}$ and Transfer efficiency-TE, number of Species-Sp. and Abundance-Abu from different stations. RS was excluded to calculate the mean of each variable.

Station	**B** (kJ m^{-2})	**P** (kJ m^{-2} yr^{-1})	**P/$\bar{B}$** (yr^{-1})	**TE** (%)	**Sp.** N°	**Abu** (ind. m^{-2})
0D	5.8	3.9	0.6	17	35	1556
0M	16.3	27.4	1.6	28	76	1406
0P	8.9	5.7	0.6	8	36	343
1FD	6	7.7	1.2	13	53	916
1D	6.4	6	0.9	10	46	616
–1D	6.9	10.2	1.4	14	69	3436
1M	27.1	61.7	2.2	11	91	1679
–1M	16	27.4	1.7	14	74	1316
1P	27	54	2	11	73	1459
–1P	28.1	40	1.4	10	61	1023
2FD	12.3	11.9	0.9	17	45	426
2D	8.6	9.1	1	7	47	833
–2D	14.1	16.8	1.1	9	50	636
2M	19	28.4	1.4	18	62	1299
–2M	6.8	9.3	1.3	15	57	723
2P	24.8	40.9	1.6	17	74	1013
–2P	7.7	14.2	1.8	14	68	1513
RS	14.1	17.7	1.2	18	46	740
Means	14.2±7.9	21.7±17.3	1.3±0.4	13.9±4.9	59.0±15.4	1162.9±697.6

Table 3. Spearman's rank correlation coefficient (rs) for the relationship between biological factors and environmental variables. **p<0.01; *p<0.05

	Eh	C/N	Corg	TN	Clay	Silt	Sand	Sp.	Abu.	TE	P/$\overline{B}$	P
B	0.448	-.0565*	-0.186	0.017	0.089	0.262	-0.115	0.596**	0.189	0.044	0.617**	0.923**
P	0.593**	-0.656**	-0.315	-0.090	0.176	0.380	-0.180	0.771**	0.386	0.207	0.923**	
P/$\overline{B}$	0.632**	-0.630**	-0.343	-0.112	0.003	0.265	0.029	0.938**	0.658**	0.187		
TE	0.582*	-0.144	-0.439	-0.307	0.105	-0.209	-0.044	0.190	0.255			
Abu.	0.246	-0.251	0.090	0.320	-0.212	0.079	0.235	0.625**				
Sp.	0.589*	-0.566*	-0.294	-0.098	-0.119	0.257	0.110					

Table 4. Total biomass (g ww m^{-2}), production (kJ m^{-2} yr^{-1}), and average production/ biomass (P/$\bar{B}$) ratio (yr^{-1}) of benthic macrofauna from different study areas.

Study area	**Biomass** (g ww m^{-2})	**Production** (kJ m^{-2} yr^{-1})	**P/$\bar{B}$ ratio** (yr^{-1})	**References**
Gulf of Trieste	344.2	392.3	1.3	Present study
Sørfjord, North Norway	307	nd	0.29	Nilsen et al., (2006)
Tyne/Tees	nd	19.8	1.2	Bolam et al., (2010)
Anglia	nd	99.6	1.6	Bolam et al., (2010)
Barents Sea	59.5	nd	0.3	Denisenko & Titov, (2003)
Humber/Wash	nd	47.1	1.9	Bolam et al., (2010)
Southwestern Barents Sea	nd	nd	0.25	Denisenko, (2001)
Eastern Channel	nd	180.4	1.4	Bolam et al., (2010)
Baltic Sea	53.8	nd	0.32	Harvey et al., (2003)
Western Channel	nd	94.3	1.3	Bolam et al., (2010)
Cardigan Bay	nd	196.6	1.7	Bolam et al., (2010)
North Sea, 57°N	20–90	nd	0.1–5.0	McLusky & McIntyre (1988)
North Sea, 51–57°N	76	nd	1.9	Duineveld et al., (1991)
Severn	nd	86.5	1.3	Bolam et al., (2010)
Irish Sea	nd	157	1.2	Bolam et al., (2010)
Cape Hatteras, USA	540	nd	1.3	Aller et al., (2002)
Minches and Malin Sea	nd	66.2	1.4	Bolam et al., (2010)
Chukchi Sea	nd	0.5–1603.1	0.2–1.1	Lin et al., (2016)
North Scotland Coast	nd	67.5	1.3	Bolam et al., (2010)
Beaufort Sea	nd	0.5–278.7	0.4–0.9	Lin et al., (2016)
English Channel	nd	75.0–350.0	nd	Cooper et al., (2008)
Porsangerfjord, North Norway	65	1744	1.02	Fuhrmann et al., (2015)

Figure captions

Figure 1: Study area and location of sampling sites in the Gulf of Trieste, Italy.

Figure 2: nMDS of the 18 stations from square-root transformed abundances of 270 species and Bray-Curtis similarities with the three distance groups and the reference station from the sewage discharge area indicated by different symbols and colors.

Figure 3: Bar plots showing the macrobenthic abundance (ind. m^{-2}) (a), number of species (N°) (b), biomass (kJ m^{-2}) (c), production (kJ m^{-2} y^{-1}) (d), P/$\overline{B}$ (yr^{-1}) (e) and transfer efficiency (TE, %) (f), along distance gradient from the pipelines (5, 100 and 200 meters). The data are presented as means (±SD) for each of the 3 groups.

Figure 4: Relationship between the values (±SE) for transfer efficiency (TE) and biomass (B) along the sewage gradient with increasing distance from the pipelines (5, 100, and 200 meters).

Figure 5: Two-dimensional PCA plot of the sampling stations include stations placed at <5 m from the Pipelines- group 0 of stations (0D, 0M and 0P), stations placed at 100 m from the Pipelines-group 1 of stations (1FD, 1D, –1D, 1M, –1M, 1P and –1P), stations placed at 200 m from the Pipelines- group 2 of stations (2FD, 2D, –2D, 2M, –2M, 2P and –2P) and RS, according to biological factors. The different groups are indicated with different symbols and colors.

Figure 6: RDA ordination diagram of biological factors (B, P, P/$\overline{B}$, and TE), abundance, and environmental variables (Sand, Silt, Clay, TN, Corg, C/N, and Eh) among the stations. The different groups are indicated with different symbols and colors.

Figure 1.

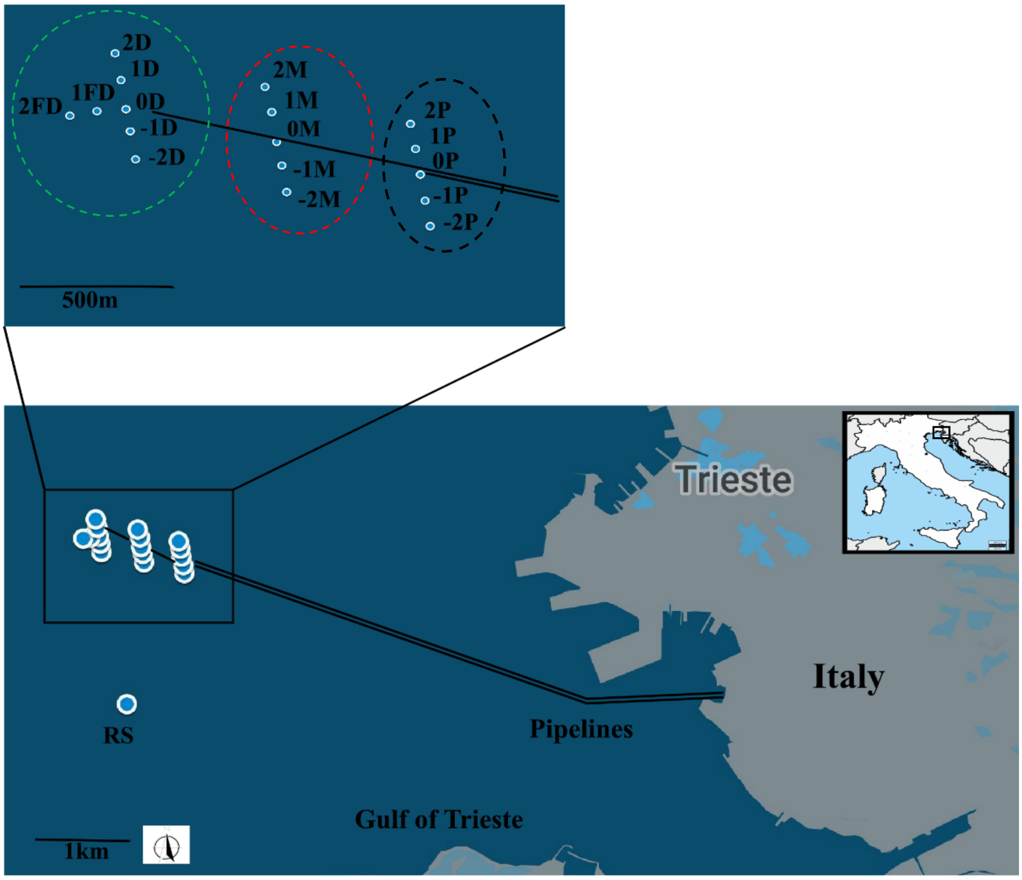

2D
1D
2FD
1FD
0D
-1D
-2D
2M
1M
0M
-1M
-2M
2P
1P
0P
-1P
-2P
500m
Trieste
Italy
RS
Pipelines
Gulf of Trieste
1km

Figure 2.

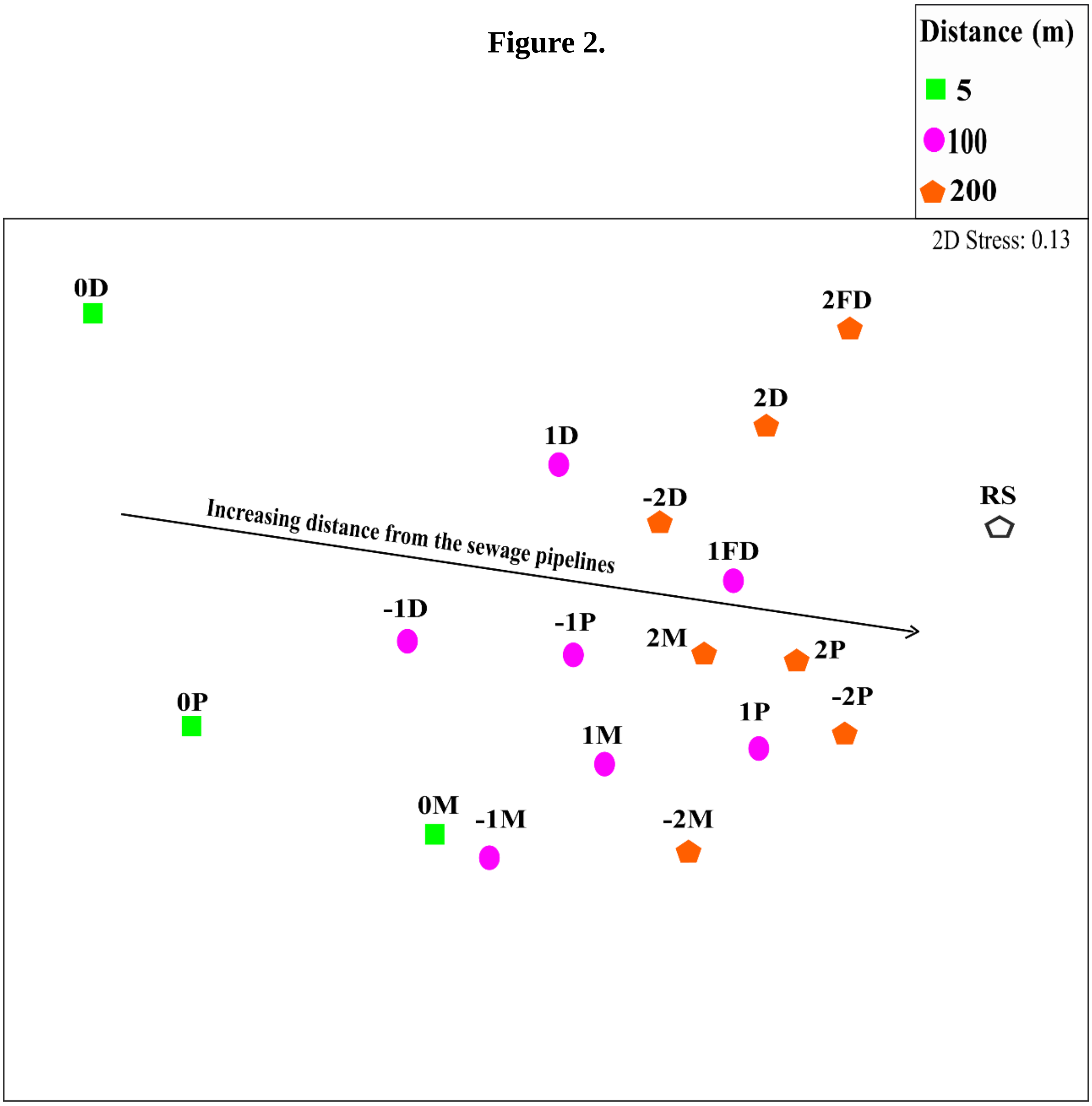

Distance (m)
5
100
200
2D Stress: 0.13
0D
2FD
2D
1D
-2D
RS
Increasing distance from the sewage pipelines
1FD
-1D
-1P
2M
2P
0P
-2P
1P
1M
0M
-1M
-2M

Figure 3.

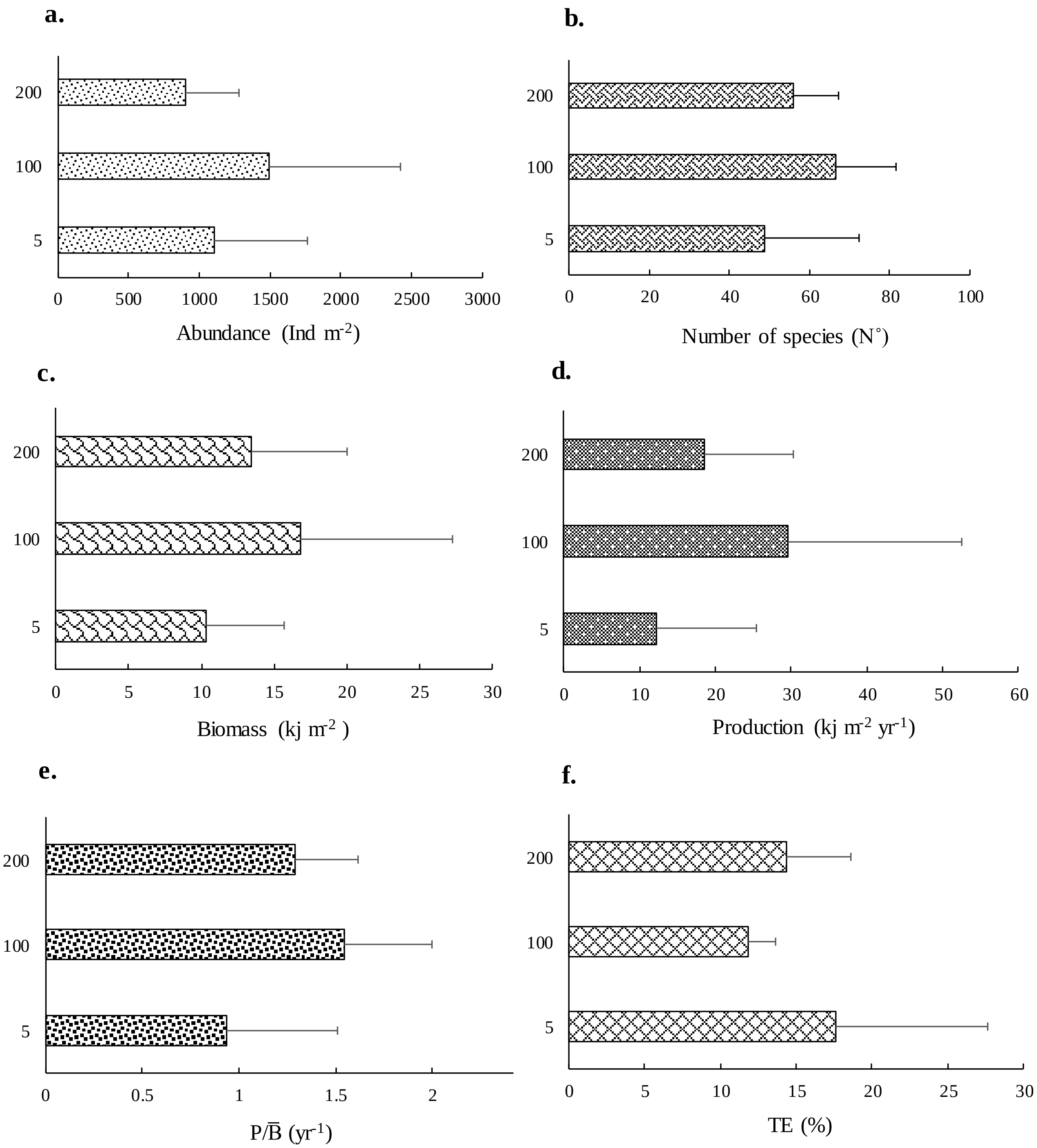

a.
200
100
5
0
500
1000
1500
2000
2500
3000
Abundance (Ind m^{-2})
b.
200
100
5
0
20
40
60
80
100
Number of species (N°)
c.
200
100
5
0
5
10
15
20
25
30
Biomass (kj m^{-2})
d.
200
100
5
0
10
20
30
40
50
60
Production (kj m^{-2} yr^{-1})
e.
200
100
5
0
0.5
1
1.5
2
P/$\overline{B}$ (yr^{-1})
f.
200
100
5
0
5
10
15
20
25
30
TE (%)

Figure 4.

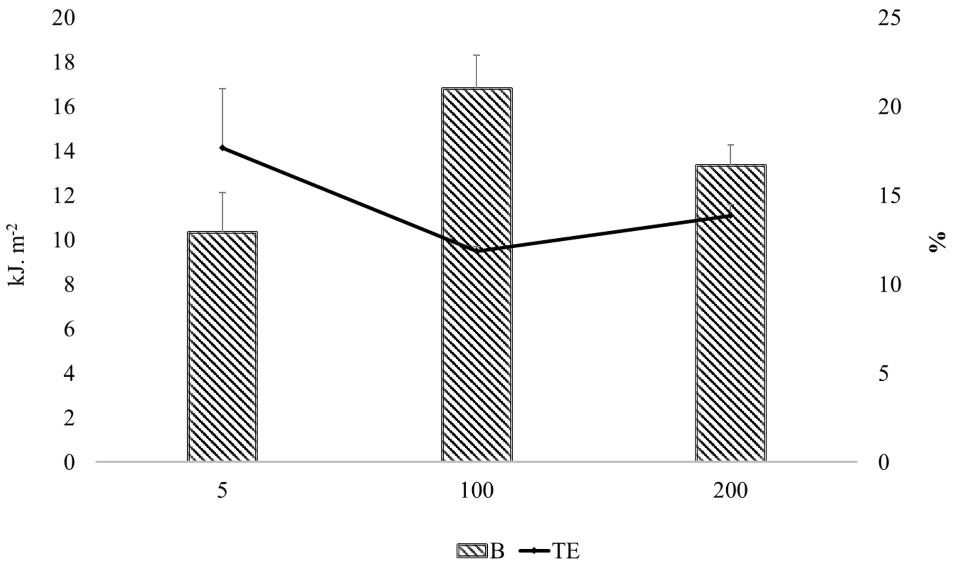


Figure 5.

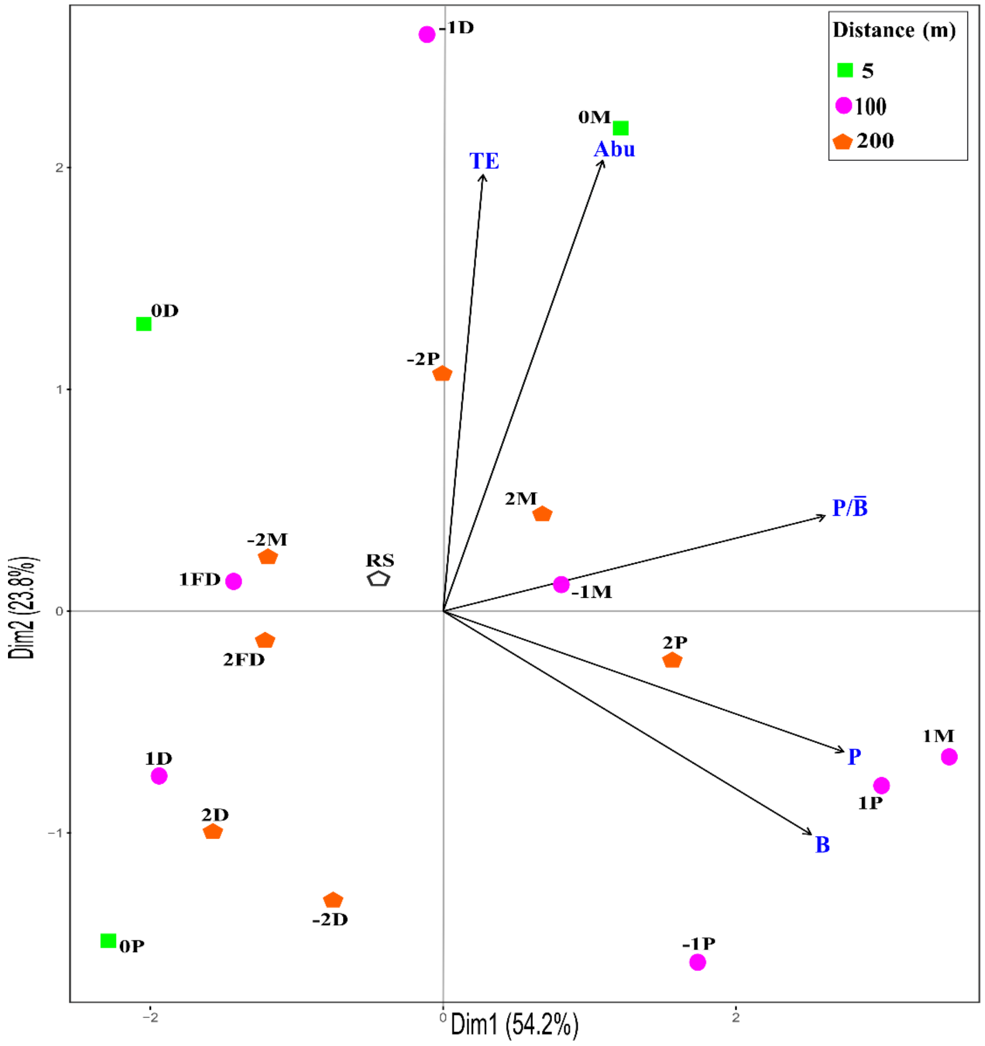
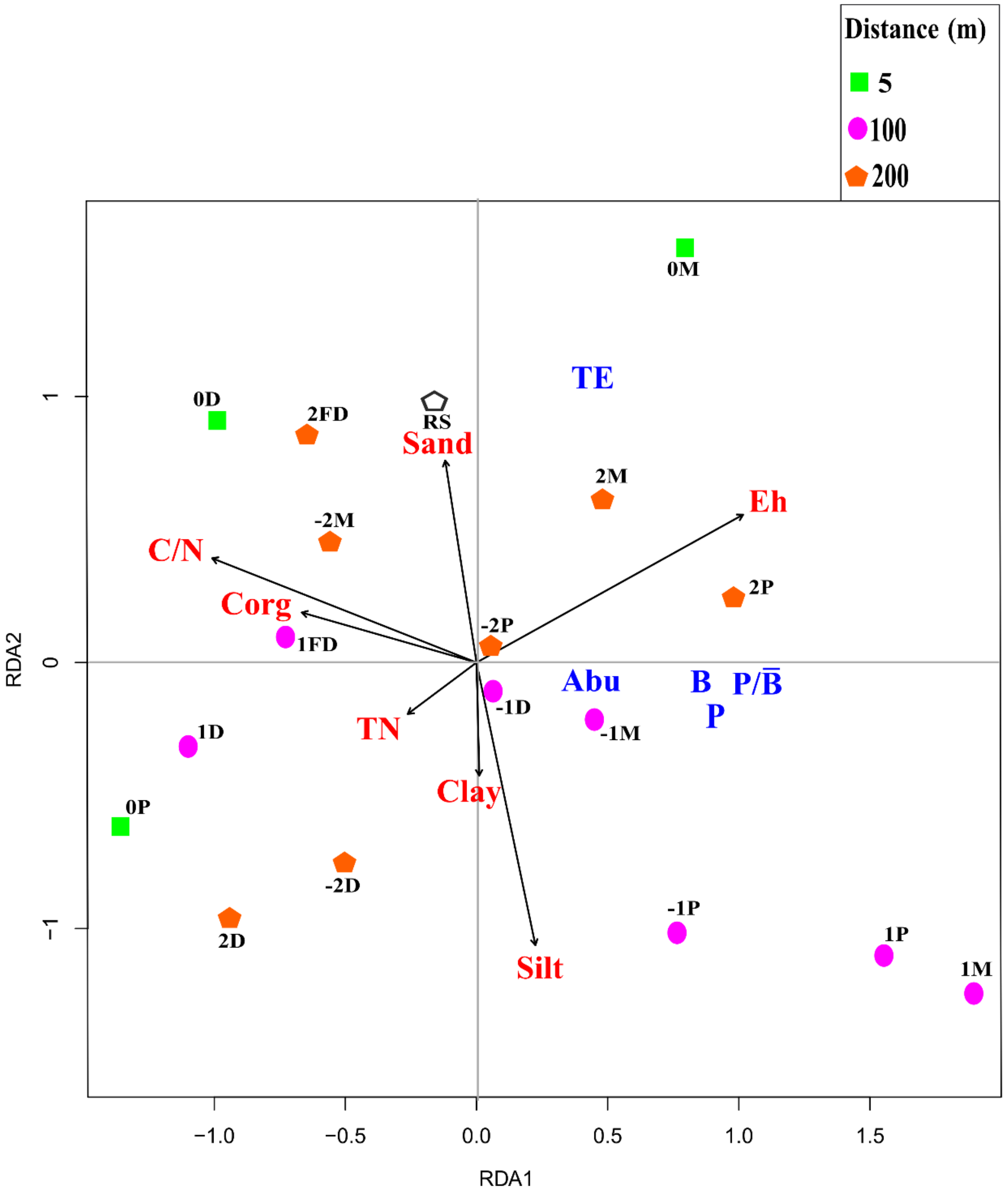

Distance (m)
🟩 5
● 100
⬟ 200
-1D
0M
TE
Abu
0D
-2P
2M
P/B̄
-2M
RS
1FD
-1M
2FD
2P
1M
P
1D
1P
B
2D
-2D
-1P
0P
Dim2 (23.8%)
Dim1 (54.2%)
2
1
0
−1
−2
0
2

Figure 6.


Distance (m)
🟩 5
● 100
⬟ 200
0M
TE
0D
2FD
RS
Sand
2M
Eh
-2M
C/N
2P
Corg
-2P
1FD
Abu
B
P/B̄
-1D
-1M
P
1D
TN
Clay
0P
-2D
-1P
1P
2D
Silt
1M
RDA2
1
0
−1
−1.0
−0.5
0.0
0.5
1.0
1.5
RDA1

Dear MPB editorial manager,

there is no response to reviewers since there are no previous reviewers comments, only for Language Editing Changes, that we addressed.

Best regards,

Seyed Ehsan Vesal

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

CRediT author statement:

SEV: Methodology, Formal analysis, Investigation, Writing-Original Draft; RA: Conceptualization Methodology, Formal analysis, Investigation, Writing-Original Draft; SL: Methodology, Formal analysis, Investigation; FN: Methodology, Investigation, Conceptualization; PDN: Supervision, Funding acquisition.