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Identifying the drivers of microbial community changes and interactions in polluted coastal sediments

Ana Ramljak¹, Stephanie Jurburg², Antonis Chatzinotas^{2,3}, Mavro Lučić¹, Marta Žižek¹, Ivana Babić¹,
Nikolina Udiković-Kolić¹ and Ines Petrić^{1*}

Abstract

Despite over three decades of research into the composition and distribution of microbial communities, gaps remain in our mechanistic understanding of microbial community assembly processes, especially in benthic communities in coastal zones continuously exposed to anthropogenic pressures. We analyzed the microbial communities (prokaryotes, fungi, and protists) in sediment samples from ports and bays located along the Adriatic coast chronically exposed to chemical and nutrient pollution, and explored how selective pressures (pollutants, nutrients, and environmental conditions) and dispersal shape these communities. We found that biogeographic factors (i.e. location) play a key role in structuring microbial communities, with benthic fungi also being shaped by the presence of pollutants and nutrients. Strong correlations between nutrient loads and pollutants were observed, along with weakened interactions between microbial communities, particularly between prokaryotes and protists, in the presence of specific pollutants (bismuth, cadmium, copper, zinc, mercury). These results are an important step in disentangling the complex interactions between pollutants and microbial community dynamics in aquatic ecosystems. Further research is needed to assess how these shifts in microbial community dynamics may affect ecosystem services in vulnerable coastal zones.

Keywords Sediment pollution, Anthropogenic pressures, Adriatic Sea, Microbial biogeography, Microbial interactions, Coastal ecosystems

Introduction

Identifying the mechanisms responsible for the assembly and distribution of microorganisms is central for our understanding of microbial ecology. Microbial community assembly is driven by four main ecological processes: diversification, dispersal, selection, and drift, as outlined in Vellend's conceptual synthesis of community ecology [1, 2]. Specifically, dispersal (location, region, depth, sediment grain size) refers to the movement of microorganisms across space by wind, or water, or as mobile microorganisms. In contrast, the selection process is affected by environmental factors including physicochemical parameters (pH, salinity, temperature) and

*Correspondence:

Ines Petrić
ipetric@irb.hr

¹Division for Marine and Environmental Research, Ruder Bošković Institute, Zagreb, Croatia

²Department of Applied Microbial Ecology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany

³Institute of Biology, Leipzig University, Leipzig, Germany



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ecological interactions, which are still not fully understood [2]. Selection pressures may also arise from exposure to pollutants such as heavy metals [3].

The distance-decay relationship (DDR) describes the biogeographical properties and spatial structuring of ecological communities through drift, selection, dispersal, and diversification, resulting in a decrease in similarity between two communities as space between them increases [4–6]. It has been suggested that the small size and high dispersal ability of bacteria make them less susceptible to dispersal limitations than eukaryotes (i.e. protists and fungi) [7]. In these cases, environmental factors have a more significant role in shaping bacterial communities [8]. Additionally, aquatic ecosystems (e.g., seas), have a weaker DDR than structured and heterogeneous environments (e.g. soils and sediments), likely due to the greater connectivity and dispersal ability of aquatic microbes [5].

Marine sediment environments are shaped by a combination of physical, chemical, and biological processes, such as currents, sedimentation rates and interactions with marine organisms. They also serve as sinks for pollutants entering marine ecosystems, which pose risks to both ecosystem health and humans. Pollutants can be resuspended into the water column and bioaccumulated in marine organisms, ultimately influencing all trophic levels within the food web [9]. Benthic microbial communities form a boundary layer between the water column and the seafloor communities [3, 10, 11], where they play important roles in organic matter decomposition, biogeochemical cycling, and contaminant remediation [12, 13]. However, sediment microbial communities remain relatively understudied, and the factors that affect their distribution are an active area of research. Benthic communities are shaped by marine processes including suspension, deposition, erosion and dispersal by currents, as well as the substantial small-scale heterogeneity [5, 11, 14]. Previous studies revealed that the assembly of bacterial and archaeal communities in coastal sediments is random [10, 11], and that, while benthic fungal communities are randomly assembled, they are influenced more strongly by local environmental conditions than geographical distance [7, 15]. In contrast, environmental factors play a significant role in shaping protistan communities at a regional scale, with distance-decay effects particularly observed in surface water layers [16]. Reconciling these contradictory findings is particularly important as benthic microorganisms provide crucial ecosystem services and are affected by climate change and growing anthropogenic pressures [12]. While many studies examine responses of individual microbial communities, such as bacteria, it is equally important to investigate the interactions between microbial communities under combined pressures in coastal ecosystems.

These dynamics can significantly affect essential ecosystem functions, such as nutrient cycling and the microbial food web. To date no study has investigated the drivers of the entire microbial community—bacteria, fungi, and protists—in coastal environments, despite the critical role of interactions between these compartments in driving community assembly.

Coastal sediments, particularly those in ports, are heavily impacted marine environments where microbial communities are exposed to a variety of pollution sources, including industrial discharges, shipping activities, shipyards, urban runoff, and wastewater. In the coastal zones of the Adriatic Sea, the risk of pollution is increasing due to growing pressures from tourism and maritime traffic [17]. Benthic microbial communities are known to respond quickly to multiple pollutants entering the coastal ecosystems, including heavy metals and nutrient loads [13, 18], and their responses can provide insights into the extent and impact of anthropogenic pressures in coastal zones [18, 19].

As outlined in Ramljak et al. [17], we sampled sediments from seven long-term polluted ports and bays along the Croatian coast and investigated the extent of sediment disturbance through detailed chemical analysis. This included measuring specific pollutants (tributyltin, heavy metals), sediment toxicity, and nutrient levels (total phosphorus, total nitrogen, and total organic carbon), as well as identifying the microbial community composition (prokaryotes, fungi, and protists) through metabarcoding. The previous study revealed that Proteobacteria, Dinoflagellata and Ascomycota dominated the microbial communities and that community diversity was shaped by disturbance levels. In particular, beta diversity was strongly impacted by disturbance levels, especially for prokaryotes. Additionally, an initial evidence of geographic clustering of microbial communities was observed. As reported in our previous study, DESeq2 analysis revealed several pollution-tolerant taxa, including the families *Ectothiorhodospiraceae*, *Rhodobacteraceae* and *Thermoanaerobaculaceae*, as well as the genera *Boseongicola*, *B2M28*, *Subgroup 23*, *Sva0485*, *Thiogramnum*. Building on these findings, the present study aims to clarify the drivers of microbial community assembly and the changes of microbial interactions in anthropogenically disturbed coastal zones. Specifically, this study focuses on three main objectives: (1) assessing the effects of individual dispersal and selection factors on microbial communities (prokaryotes, fungi, and protists); (2) examining interactions among specific selection factors; and (3) investigating microbial community interactions in the presence of specific pollutants. This study provides valuable knowledge of the factors shaping these communities in long-term polluted ports and bays along the Croatian coastline.

Materials and methods

Sampling locations

Sediment samples ($n = 67$) were collected in spring 2021 from seven locations in the eastern Adriatic Sea. These locations, Pula port (7 samples), Raša Bay (10 samples), Rijeka port (5 samples), and Bakar Bay (11 samples) in the northern Adriatic, along with Šibenik Bay (7 samples), Vranjic Basin (9 samples), and Split port (10 samples) in the southern Adriatic, have historically been subjected to anthropogenic pressures from various sources. Additionally, 2 samples were collected from each reference

location: Cape Kamenjak, Zlarin Island, and Vis Island (Fig. 1). The selected locations within ports and bays were identified as highly polluted areas, based on monitoring campaigns conducted as part of the Marine Strategy Framework Directive (MSFD) agreement obligations. These areas continue to be affected primarily by industrial, port and tourism activities. During the sampling campaigns physicochemical parameters were also measured: sediment and bottom water temperature, salinity, depth, pH, and redox potential. These data were

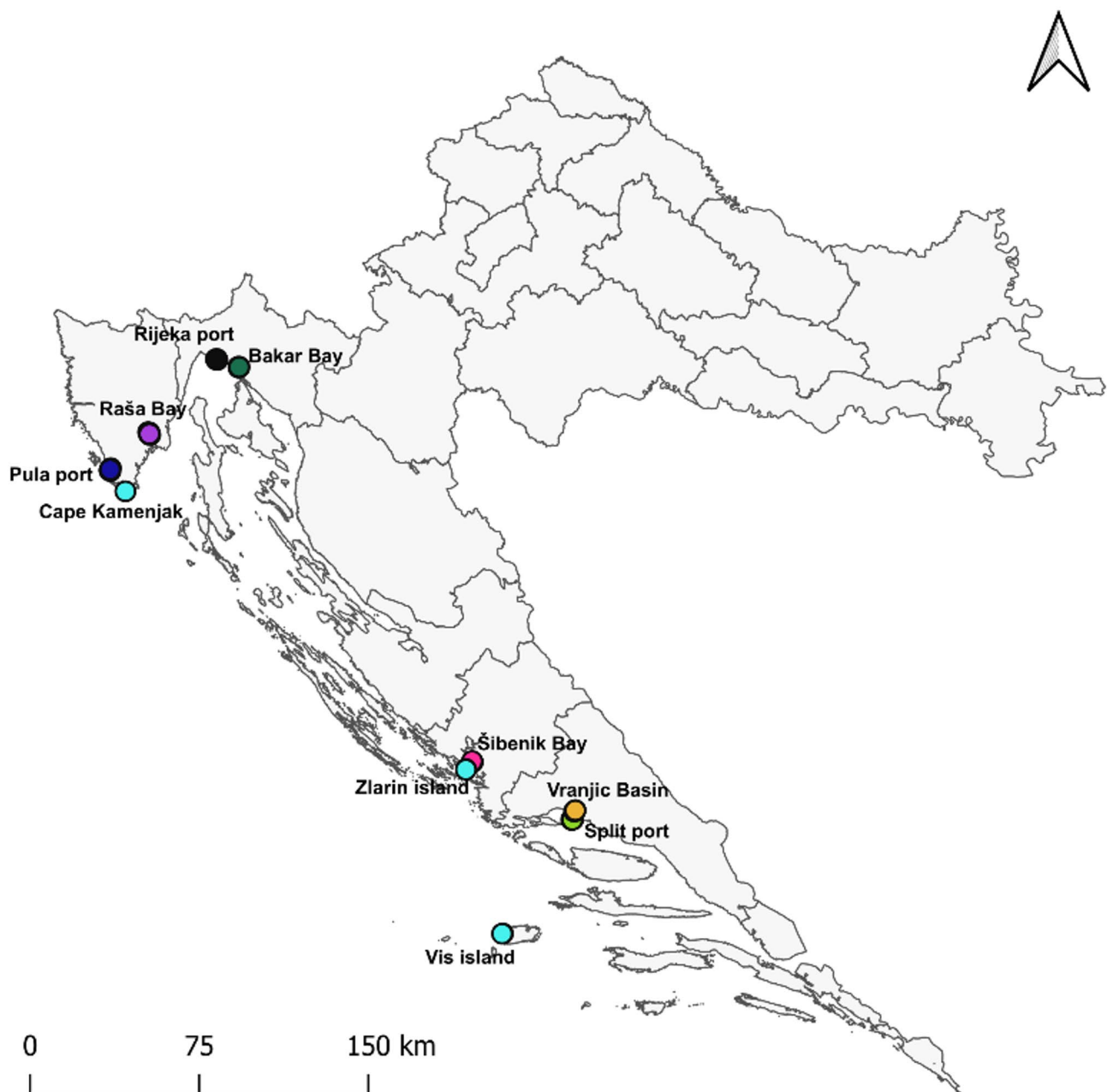


Fig. 1 Map of the eastern Adriatic Sea showing seven sampling locations and three reference locations (marked in cyan blue)

previously published in Ramljak et al. [17] and are available on Mendeley Data [20].

Data sources and statistical analysis

Chemical, toxicity, and metabarcoding data used in this study were previously generated and published in Ramljak et al. [17] and associated datasets [20, 21]. In brief, sediment characterization included grain size, metal(loid)s (total and mercury), tributyltin, nutrients (total N, total P, TOC), and toxicity (Microtox test), following standardized and published methods [22–25]. Sediments were clustered into five disturbance levels (low–extreme) using k-means [17]. For the purpose of this study, the bioavailable fraction of metal(oid)s was newly determined in all sediment samples using the modified BCR (the European Community Bureau of Reference) sequential extraction procedure [26]. This method involved treating 2.0 g of lyophilized sediment with 0.11 M acetic acid, followed by overnight shaking (300 rpm). The samples were then centrifuged for 20 min at 4000 g and filtered using the Milipore 0.45 pore size PES (polyethersulfone) membrane filters. Finally, the filtrates were diluted 100-fold and analyzed using high-resolution inductively coupled plasma mass spectrometry (HR ICP-MS; Element 2, Thermo, Germany) [17].

Metabarcoding data were obtained in the previous study [17] from DNA extracted from sediment, with 16S and 18S rRNA amplicons sequenced on Illumina NovaSeq and processed in QIIME2 using standard pipelines (DADA2, MAFFT, FastTree2, SILVA v138) [27–33]. Raw sequences are available in the European Nucleotide Archive (ENA) underproject accession number PRJEB72621. Full details are provided in Ramljak et al. [17]. In this study, we extended these datasets by performing additional analyses. Amplicon sequences were rarefied in RStudio (version 4.3.2) [34] (prokaryotes: 37,149; protists: 4,228; fungi: 2,481 reads/sample), with low-read samples removed (CV1 from reference site, ST5 from Split port and BA3 from Bakar Bay).

The above-mentioned datasets were further analyzed in the study using a range of statistical tools to explore how dispersal and selection factors shape microbial communities. Dispersal factors included: (i) *sampling location* (Pula port, Raša Bay, Rijeka port, Bakar Bay, Šibenik Bay, Vranjic Basin, Split port, reference sites), (ii) *sampling region* (northern vs. southern Adriatic), (iii) *grain type* (silt, silty sand, sandy silt, clayey silt), (iv) *depth*. Selection factors included: (i) *sediment temperature*, (ii) *bottom water layer temperature*, (iii) *sediment pH*, (iv) *sediment redox potential*, (v) *sediment toxicity*, (vi) *contamination* (contaminated vs. non-contaminated sites), (vi) *disturbance level* (low, mild, medium, high and extreme), (vii) *pollutants* - TBT, metals (total and bioavailable

fractions), and (viii) *nutrients* - total nitrogen, total phosphorus, total organic carbon.

Data manipulation and visualization were done using RStudio (version 4.3.2) and the *phyloseq* (version 1.46.0) [35], *vegan* (version 2.6.4) [36], *ggplot2* (version 3.4.4) [37], *data.table* (version 1.14.10) [38], *reshape2* (version 1.4.4) [39], *dplyr* (version 1.1.4) [40], *stringr* (version 1.5.1) [41], *cowplot* (version 1.1.3) [42], and *patchwork* (version 1.2.0) [43] packages. A Principal Coordinate Analysis (PCoA) was used to visualize the similarity between microbial communities. The DDR was assessed through a nonlinear regression of Bray-Curtis dissimilarities over geographical distance (in kilometers) between communities. The function *distHaversine* from the *geosphere* package (version 1.5.19) [44] was used to calculate the shortest distance between two points while assuming a spherical Earth, and the function *decay.model* from *betapart* package (version 1.6) [45] was used to fit a nonlinear model describing the increase of assemblage dissimilarity with distance. To identify significant factors influencing each microbial community, PERMANOVAs (adonis) were performed on the abundance (Bray-Curtis) and incidence-based (Sorensen) similarity matrices, and *p* values < 0.01 were considered significant. Incidence, defined as the presence or absence of specific taxa, is commonly used to analyze rare taxa [46]. To further assess the impact of each factor on microbial abundance and incidence, forward selection based on distance-based redundancy analysis was performed (with 10,000 permutations), and Pearson's correlations between the significant selection factors were calculated and visualized using the package *corrplot* (version 0.92) [47]. To examine patterns in alpha diversity for each community, we calculated effective Shannon's diversity using the *hillR* package (version 0.5.2) [48, 49]. Finally, to assess the impact of pollution on interactions between microbial communities, we first divided the samples into high and low pollutant groups according to the median concentration of each pollutant. For each pollutant, samples with low and mild disturbance levels were grouped as low pollution and those with medium, high and extreme disturbance levels were grouped as high pollution (as described in Ramljak et al. [17]). Then, Mantel tests (with a Pearson correlation) were applied between prokaryotes-fungi, prokaryotes-protists, protists-fungi within each pollutant grouping.

Results

Location shapes microbial communities

The effects of dispersal and selection factors on prokaryotic, fungal, and protistan communities were explored through PERMANOVAs of Bray-Curtis dissimilarities. Among all tested factors (Tables S1 and S2), location explained the highest proportion of variation in

prokaryotic ($p=0.001$, $R^2=0.46919$), fungal ($p=0.001$, $R^2=0.49092$), and protistan communities ($p=0.001$, $R^2=0.51253$), (Fig. 2). Pairwise PERMANOVAs confirmed significant differences between all locations ($p<0.01$), except for prokaryotes between the Reference site–Vranjic Basin ($p=0.025$) and Split port–Vranjic Basin ($p=0.036$), fungi between Split port–Vranjic Basin ($p=0.017$), and protists between the Reference site–Raša Bay ($p=0.011$). A regional differentiation (northern vs. southern Adriatic) was observed in the PCoA, where communities from southern sites (Split port, Vranjic Basin, and Šibenik Bay) separated from northern sites (Bakar Bay, Rijeka port, Pula port). This was confirmed by PERMANOVAs for all three communities, prokaryotes ($p=0.001$, $R^2=10.393$), protists ($p=0.001$, $R^2=15.59$) and fungi ($p=0.001$, $R^2=15.378$). Notably, unique prokaryotic ($p=0.001$, $R^2=15.693$), fungal ($p=0.001$, $R^2=8.391$), and protistan ($p=0.001$, $R^2=16.895$) communities were observed in the estuarine sediment of Raša Bay (Fig. 2). Community dissimilarity (Bray-Curtis) increased with geographical distance. This relationship was strongest for fungi ($p<0.01$, $R^2=0.22$) (Fig. S1B), followed by protists ($p<0.01$, $R^2=0.12$) and prokaryotes ($p<0.01$, $R^2=0.05$) (Fig. S1A and S1C).

Differences in alpha diversity between sampling locations

Prokaryotes showed the highest average diversity (799 ± 165), followed by protists (97 ± 31) and fungi (59 ± 34). Prokaryotic alpha diversity ranged from 410 (Raša Bay) to 1,462 (reference site); protistan diversity ranged from 29 (Rijeka port) to 156 (Bakar Bay); and fungal diversity ranged from 11 (Bakar Bay) to 177 (Raša Bay) (Fig. 3, Table S4).

Despite this, the Kruskal-Wallis test revealed no significant differences in overall diversity between locations for prokaryotes and protists. However, fungal diversity significantly differed ($p<0.01$) between specific locations, particularly between Vranjic Basin and Bakar Bay ($p=0.0098$), and Vranjic Basin and Šibenik Bay ($p=0.0098$).

Impact of dispersal and selective factors in shaping benthic microbial communities

We further investigated the impact of dispersal and selection factors on the abundance and incidence (presence/absence) of benthic prokaryotic, protistan, and fungal communities. The PERMANOVA analyses revealed that the abundance of all three communities was strongly influenced by dispersal factors, with location being a key driver in community assembly (Fig. 4A, Table S1). Microbial abundance was significantly impacted by other dispersal factors (region, depth, sediment grain size) ($p<0.01$). Among the selection factors (Fig. 4B, Table S1), disturbance level, sediment and bottom water

temperature significantly influenced the abundance of all microbial communities ($p<0.01$). Additionally, salinity was proved to significantly affect prokaryotic and fungal abundance ($p<0.01$). Fungal and protistan abundance were significantly affected by Bi, distance from shore, bioavailable fraction of As and total organic carbon ($p<0.01$) (Fig. 4B, Table S1, Table S5). Only fungal abundance was additionally significantly impacted by contamination, sediment redox potential, Hg, Cu, Zn, Cd, total nitrogen, bioavailable fraction of Cu and Sb (Fig. 4B, Table S1, Table S5). Selective factors which were also analyzed, but did not prove significant for the abundance of any community included toxicity level, Cu, As, Pb, Sb, Sn, total phosphorus, bioavailable fractions of Pb, Zn, Cd.

When considering microbial incidence, all dispersal factors were also significantly affecting the three communities, though with the smaller R^2 value in comparison to abundance (Fig. 4B, Table S2). The incidence of all three microbial communities ($p<0.01$) was significantly influenced by selection factors including disturbance level, sediment and bottom water temperature (Fig. 4B, Table S2). In contrast to prokaryotic abundance, contamination, Bi and the bioavailable fraction of As were shown to significantly affect prokaryotic incidence ($p<0.01$). Fungal incidence was significantly affected by fewer selective factors than fungal abundance which included salinity, distance from shore and Bi ($p<0.01$). Similarly, protistan incidence was significantly impacted by Bi, total organic carbon and the bioavailable fraction of As ($p<0.01$) (Fig. 4B, Table S2).

Relationships between selection factors and microbe-microbe relationships

No significant correlations (Pearson's correlation) were observed between environmental factors (depth, sediment and bottom water layer temperature, sediment redox potential, salinity, distance from shore) and pollutants mercury (Hg), bismuth (Bi), copper (Cu), cadmium (Cd), zinc (Zn), bioavailable fraction of copper (Cu), arsenic (As) and antimony (Sn), except for the environmental factors correlating with each other. Most metal(oid)s were strongly positively correlated with each other, and with the bioavailable fraction of Cu, As and Sn. Nutrients (total organic carbon, total nitrogen) exhibited a strong positive correlation with each other, along with the sediment redox potential. A strong positive correlation was also found between Cd and Zn and nutrients (Fig. S2).

To further explore the effect of pollution on microbial community interactions, we performed Mantel's tests comparing Bray-Curtis dissimilarity matrices of pairs of communities, differentiating sediment samples classified as having low and high pollution levels (Fig. 5, Table S6). Pollutants tested were heavy metals Bi, Cd, Cu, Zn, Hg (selection factors) which showed significance for at least

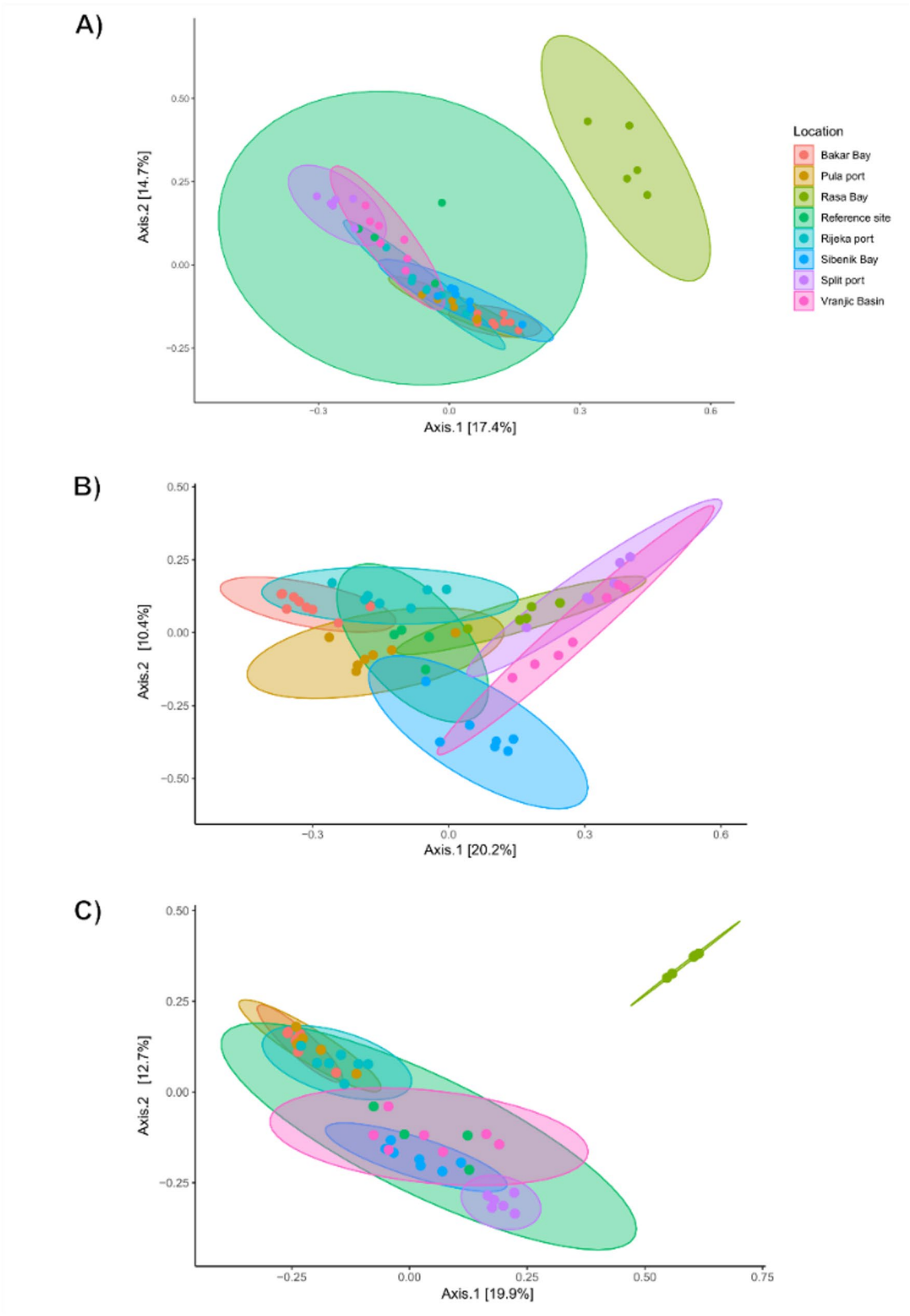


Fig. 2 Principal coordinate analysis (PCoA) plots based on Bray-Curtis dissimilarity matrices across sampling locations, for (A) prokaryotes, (B) fungi, and (C) protists. Each point represents one sediment sample and colors indicate sampling locations

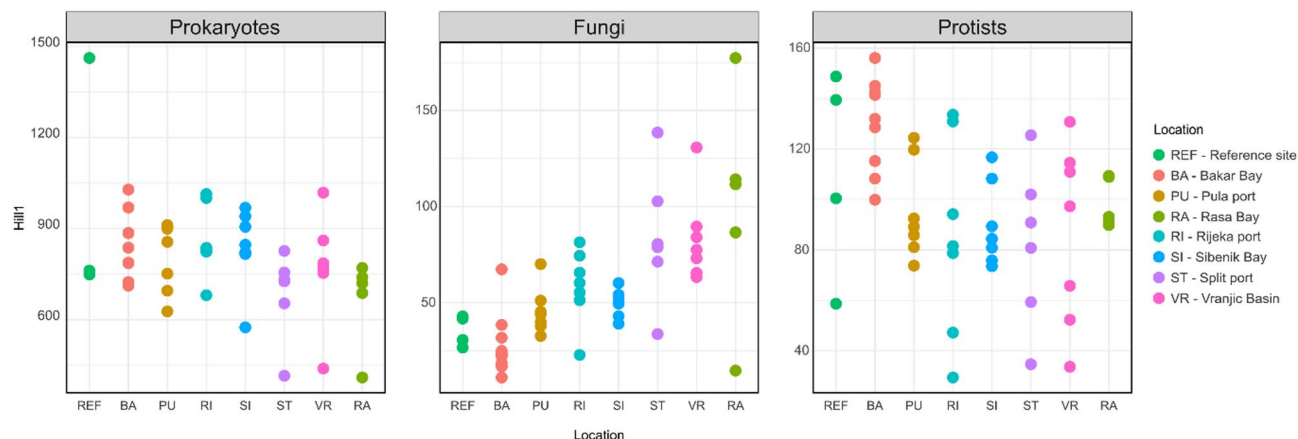


Fig. 3 Effective Shannon's diversity (Hill number with $q=1$) for each microbial community based on location. The locations are ordered on x-axis as follows: REF – Reference site, BA – Bakar Bay, PU – Pula port, RA – Raša Bay, RI – Rijeka port, SI – Sibenik Bay, ST – Split port, VR – Vranjic Basin

one community (abundance/incidence) by above-mentioned PERMANOVA analysis ($p < 0.01$). Results showed that, under low pollution level, interactions among all three pairs of communities (prokaryotes-fungi, fungi-protists, prokaryotes-protists) were similar, with Mantel r values around 0.80. Prokaryotes-protists interaction was the strongest under low pollution levels of all heavy metals (from $r = 0.824$ to $r = 0.849$), except in the presence of Cu, where fungi-protists interaction showed the strongest correlation ($r = 0.787$) (Table S7).

Conversely, a decline in Mantel r values was recorded under heavy metal pollution (sediments under high pollution level). This was most pronounced for the interaction between prokaryotes and protists, with the largest decline in correlation in samples under high levels of pollution with Bi ($r = 0.226$), followed by Cd, Hg, Zn and Cu. The prokaryotes-fungi interaction also strongly declined, especially for Cd ($r = 0.172$) and Hg ($r = 0.116$), followed by Bi, Zn and Cu. The interaction between fungi and protists declined under high levels of pollution with Cd ($r = 0.091$), followed by Bi, Zn and Cu. In contrast, fungi-protists interaction was not impacted by high levels of pollution with Hg, as the correlation increased from $r = 0.809$ to $r = 0.813$ (Table S7). The smallest negative effect on the interactions among all the studied communities was observed for high pollution level with Cu.

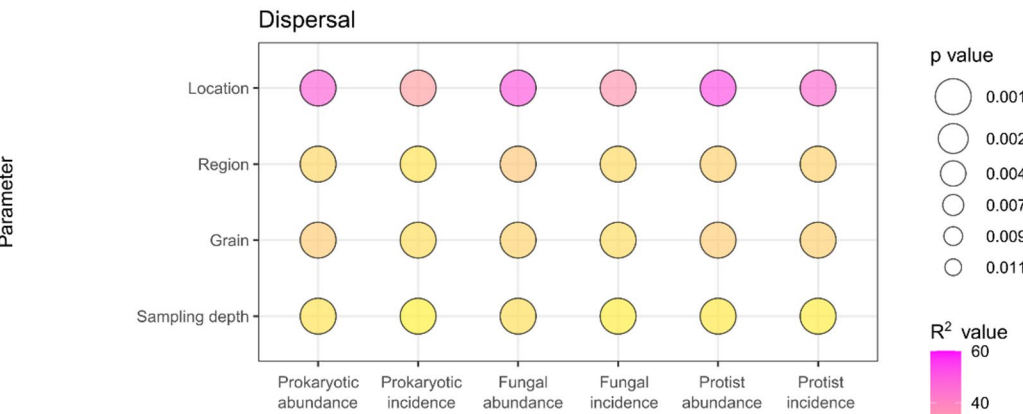
Discussion

Knowledge of the ecology of benthic microbiomes (prokaryotes, protists, and fungi) is limited by a lack of understanding of the relative contributions of dispersal and environmental factors in their assembly. Benthic microbiota play essential roles in nutrient cycling, organic matter decomposition, and overall ecosystem health [12, 50, 51]. While many studies report significant correlations between microbial composition and environmental or habitat characteristics [52–55], comprehensive

investigations addressing the multiple drivers, including both environmental parameters and pollutants, shaping these communities are scarce [56]. To address this research gap, we conducted a series of statistical analyses to investigate the influence of 12 dispersal and selection factors on sediment microbial communities and explore the effect of pollution on interactions among microbial compartments in the northern and southern Adriatic Sea. Notably, this study represents the first of its kind to focus on the benthic coastal zones of Croatia.

Location emerged as the strongest driver of microbial communities, with β -diversity patterns revealing distinct location-specific and regional clustering patterns (northern and southern Adriatic). Interestingly, samples from estuarine sediment in Raša Bay formed separate clusters, suggesting adaptation to local estuarine conditions [55, 57, 58]. Considering the interlocation differences, the similarity of prokaryotic and protistan communities between Split port and Vranjic Basin may reflect geographical proximity. Dispersal-related factors (location, region, depth and grain size) were the primary drivers of both microbial abundance and incidence, highlighting the strong influence of biogeography on microbial community assembly. This supports previous findings that geographic isolation and habitat-specific conditions drive microbial diversity and adaptation across marine ecosystems [2, 59, 60]. Additionally, the results suggest that specific microbial assemblages evolve within the particular port or bay, with biogeography playing a more dominant role than anthropogenic influences or environmental conditions. This was further supported by the observed distance-decay patterns across all microbial groups, consistent with previous studies for benthic prokaryotes, fungi, and protists across various spatial scales in the Mediterranean [5, 7, 11]. For instance, Trouche et al. [11] concluded that environmental conditions and historical processes strongly influence community assembly at

Panel A



Panel B

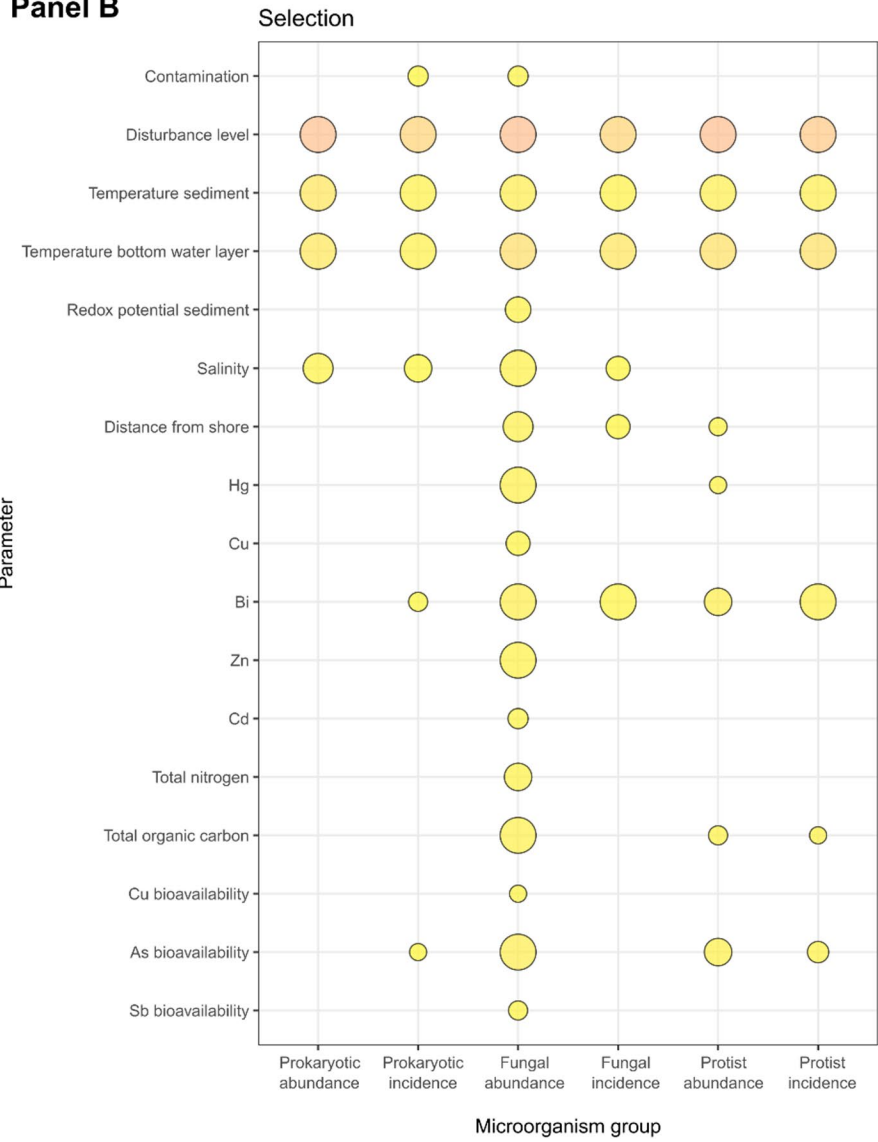


Fig. 4 Bubble plot illustrating significant dispersal (Panel A) and selection factors (Panel B) (PERMANOVA, $p < 0.01$), for each of the three microbial communities (abundance and incidence)

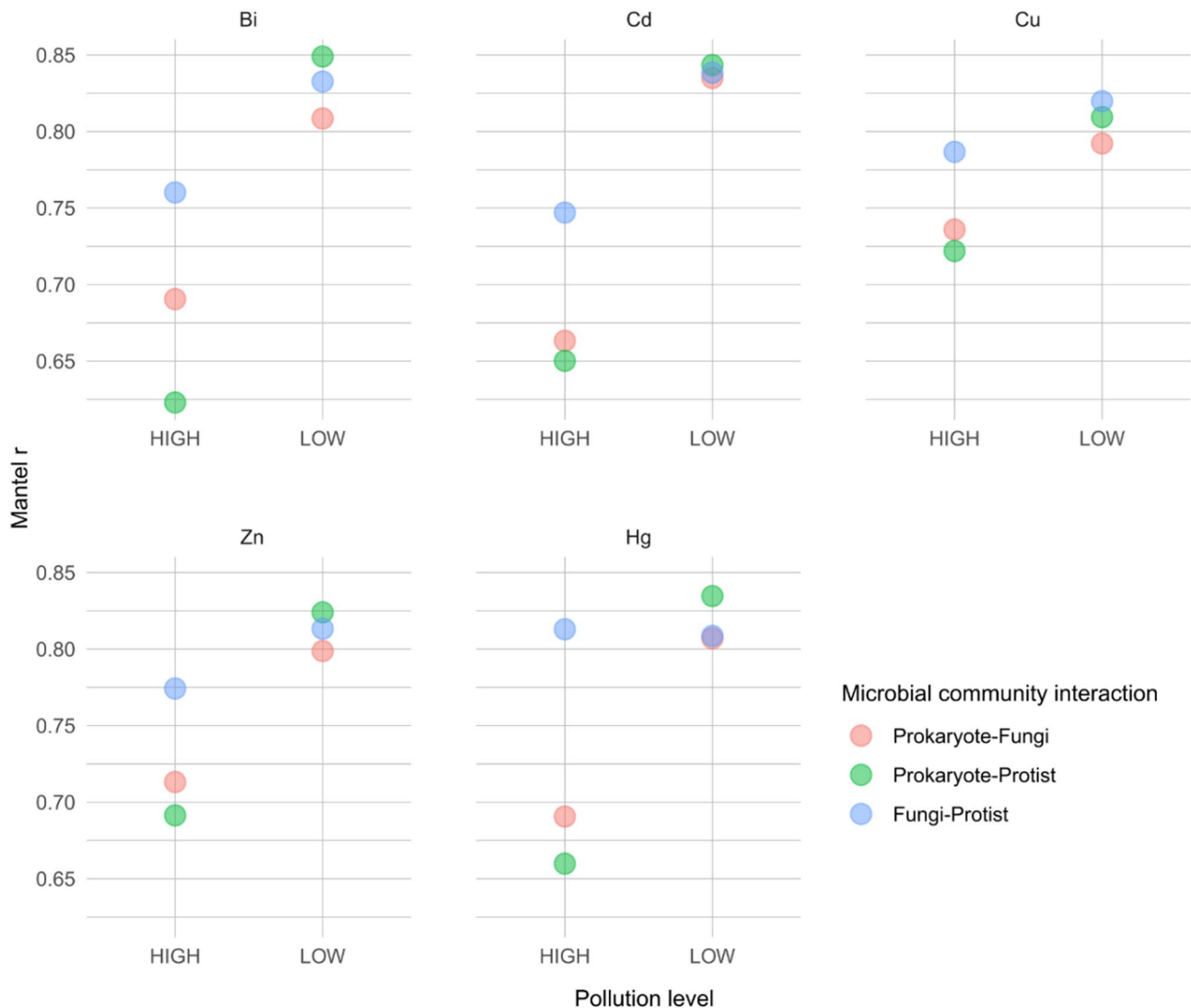


Fig. 5 Changes in interactions among microbial communities (prokaryotes, fungi, and protists) under low and high pollution levels, assessed using Mantel's test. Each point represents the interaction between two microbial communities (e.g. prokaryotes-protists) based on Bray-Curtis dissimilarity matrices. The y-axis shows Mantel r values, indicating the strength of correlation between microbial communities. Pollutants included (Bi, Cd, Cu, Zn, Hg) identified as significant selection factors (PERMANOVA, $p < 0.01$) are included

local and regional scales, while historical processes tend to dominate at inter-regional scales.

Among the selection factors, temperature (sediment and bottom water) and anthropogenic disturbance level significantly influence microbial communities, with temperature emerging as the only consistent driver across all groups. This highlights the vulnerability of benthic microbiome to both climate-related and human-induced stressors [61]. To date, temperature shifts have been shown to alter microbial structure and growth, community composition, function and metabolism in seawater, plankton and benthic ecosystems [62–66], with potential consequences on nutrient regeneration and essential ecosystem services [12, 67]. These responses are likely to vary across regions and microbial communities, with a

shift in diversity towards more resilient species, though further research is needed to fully understand these dynamics [12].

Prokaryotic abundance was primarily influenced by broad-scale environmental gradients, notably temperature, anthropogenic disturbance level and salinity, suggesting a potential tolerance to localized stressors such as nutrients and heavy metals or dormancy mechanisms [58]. The significant impact of general anthropogenic disturbance levels indicates the need for inclusion of additional pollutants in the analysis, which could explain the observed effect. In contrast, protistan abundance responded to a wider range of selection factors, including specific pollutants (Hg, TOC, Bi, As), indicating greater sensitivity to environmental pressures. However,

their abundance could also be shaped by other untested factors, such as trophic interactions, primarily by grazing of bacteria which can be affected by local pressures [68]. Additionally, dormant protistan taxa may mask the influence of selection factors as they react weakly to local environmental conditions [68]. While previous studies emphasize stronger selection pressures on protists, especially with increasing depth [69], these findings support a more dominant role of dispersal for both prokaryotes and protists, consistent with recent evidence [58].

Fungal abundance showed a distinct sensitivity to selection factors, particularly sediment redox potential and specific pollutants (Cu, Zn, Cd, total nitrogen), unlike prokaryotic and protistan communities. This suggests that fungi are more responsive to local disturbances and may occupy narrower ecological niches, making them more vulnerable to anthropogenic impacts. These patterns support previous findings that fungal communities are shaped more strongly by dispersal factors than bacterial ones [7]. Similarly, both environmental factors and geographical location were shown to influence pelagic fungal communities, though environmental factors were more significant [15].

Microbial incidence, which reflects the presence or absence of taxa and is associated with rare community members, was affected by fewer selection factors than microbial abundance. This pattern suggests that the abundant taxa are more sensitive to changes in benthic environments, whereas rare taxa may exhibit ecological stability, potentially contributing to functional redundancy and community turnover [70, 71]. Notably, among the tested selection factors, Bi exerted a stronger influence on incidence across all three communities than on abundance. The impact of selection on rare taxa was previously mentioned by Ramond et al. [72], who suggested that these taxa thrive only under specific conditions that support their growth. Nevertheless, these interpretations should be considered within the methodological limitations of the study. Specifically, the use of 18S rRNA gene markers for targeting protistan and fungal communities may introduce biases due to incomplete or inconsistent reference databases [28, 73].

To fully understand the effects of selection factors on microbial communities, it is essential to consider how interactions between different microorganisms may change. Microbial communities interact through competition for resources or cooperation, reflecting ecological principles such as mutualism, parasitism, predation, and commensalism [74]. While previous studies primarily explored selection factors affecting individual microbial communities, recent research has shifted focus toward examining interactions between different microbial communities, though these efforts have mostly concentrated on soil environments [75–77]. Additionally, microbial

community interactions and the use of co-occurrence networks have been highlighted as a potential metric for monitoring aquatic health [78]. However, this study is among the few that investigate the interactions among three distinct microbial communities and their dynamics in chronically impacted benthic environments, primarily influenced by anthropogenic activities.

In general, heavy metal pollution (Bi, Cd, Cu, Zn and Hg) in sediments led to a decoupling of microbial communities, weakening the interactions in polluted environments compared to less polluted environments. Such disruptions may ultimately affect ecosystem stability and nutrient cycling [74, 79]. These findings align with previous research demonstrating that microbial co-occurrence networks are highly responsive to stressors, including heavy metal contamination, with such disturbances found to reduce network complexity, as observed in soil ecosystems [80, 81]. Furthermore, microbial network analysis from coastal estuarine and river sediments has shown that, under pollution stress, distinct microbial subgroups may respond differently, with stress-tolerant taxa forming densely connected clusters associated with functions such as nitrogen, sulfur, or pollutant transformation, while increased associations among resilient taxa suggest enhanced cooperation [82, 83].

In the studied sediments with low levels of pollution, the strongest correlations were found between prokaryotes and protists, highlighting the ecological importance of protistan grazing on prokaryotes. The grazing process influences the composition and diversity of prokaryotes in aquatic ecosystems [84, 85]. Furthermore, a weakening of prokaryotes-fungi interaction was also observed in metal-polluted sites. Previous research has emphasized the significance of bacteria-fungi interactions in marine sediments, particularly in the transformation of complex pollutants like polycyclic aromatic hydrocarbons (PAHs) [86]. Protistan predation of bacteria through grazing has also been reported to hinder hydrocarbon degradation [87]. However, other studies suggest that protists may also enhance pollutant degradation through grazing in sediments and soil [88, 89]. Moreover, protists have been reported to be more sensitive than bacteria to toxic compounds, such as PAHs, potentially causing cascading effects to prey control [90]. It is important to note that tolerance to pollutants varies among protist species, which may be linked to differences in surface-to-volume ratios [90]. Interestingly, Cu had the smallest negative effect on the interactions among all the studied communities, implying a potential tolerance to this metal. Additionally, the strong correlations observed between different heavy metals indicate that the combined effect of coexisting metals may have a more pronounced effect on microbial community interactions, highlighting the need for further investigation.

Fungi-protist interactions exhibited the lowest sensitivity to heavy metal pollution, with even a positive effect observed in highly mercury-polluted sediments. One possible explanation for this is that the extreme heavy metal disturbances in benthic environments may lead to significant reductions in microbial diversity, as previously observed at high mercury concentrations in soil [91]. Such reductions in diversity could make microbial communities more likely to display stronger positive correlations at sites with high pollution levels. In line with this, a previous study reported that in the Hg polluted soils the interactions between protists and other microbial communities (bacteria and fungi) were intensified, potentially enhancing the resistance of microbial communities to environmental changes [92]. Furthermore, recent research has shown that protists in soil act as predators of fungi, influencing the interactions and food web dynamics [93]. Another study by Wang et al. [77] investigated the co-occurrence networks among bacteria, protists, fungi, and nematodes in soil and found that increased heavy metal pollution destabilized trophic interactions. They found a shift from the typical top-down regulation—where predators control prey populations—to a bottom-up regulation under heavy metal stress, meaning that protists at the higher trophic level, were influenced by changes at the lower levels (bacteria and fungi).

While these findings suggest that heavy metal pollution in marine sediments may destabilize essential ecosystem processes and microbial food webs, the interpretation is limited by its reliance on taxonomic data alone. Integrating functional perspectives through metagenomic approaches would provide a more comprehensive understanding of the mechanisms driving these responses. Such an approach would strengthen the interpretations of ecosystem-level consequences, particularly regarding the observed weakening of microbial interactions. Future research should build on these findings to help guide effective management strategies of impacted coastal areas in the Mediterranean region.

Conclusions

Although the sediments in this study were collected from coastal zones heavily impacted by human activity, the results showed that dispersal mechanisms—particularly location—have a dominant influence on the shaping of benthic microbial communities (prokaryotes, fungi, and protists) in these environments, compared to selection factors. Benthic fungi emerged as particularly responsive among the studied communities, displaying a sensitivity to a range of local environmental conditions, including temperature and the presence of pollutants and nutrients. By examining the changes in microbial interactions in the presence of high levels of heavy metals, we

obtained a deeper understanding of the challenges associated with identifying a single factor responsible for microbial community dynamics. Finally, the observed weakening of prokaryote-protist interactions at polluted sites underlines the need for future research into these dynamics, as they are crucial for the overall functioning of marine ecosystems.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40793-025-00785-4>.

Supplementary Material 1

Author contributions

A.R.: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. S.J.: Formal analysis, Methodology, Visualization, Writing – review & editing. A.C.: Methodology, Writing – review & editing. M.L.: Methodology, Writing – review & editing. M.Ž.: Investigation, Methodology, I.B.: Writing – review & editing. N.U.K.: Writing – review & editing. I.P.: Conceptualization, Investigation, Methodology, Funding acquisition, Resources, Writing – original draft, Writing – review & editing. All authors reviewed the manuscript.

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Data availability

Chemical, toxicity, and metabarcoding data used in this study were previously generated and published in Ramljak et al. [17] and associated datasets [20, 21]. Raw sequences are available in ENA (PRJEB72621). Full details are provided in Ramljak et al. [17].

Declarations

Competing interests

The authors declare no competing interests.

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