Venezia2021

Programma di ricerca scientifica per una laguna “regolata”

Linea 2.1

Qualità del sedimento lagunare a supporto della sua gestione sostenibile

D2.1.3.1

Report sui processi microbici all’interfaccia sedimento-acqua

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Table of contents

Sommario

Nella laguna di Venezia, l'interfaccia acqua-sedimento è un ambiente estremamente attivo dal punto di vista microbico. È caratterizzato da forti gradienti di materia organica, gas (es. ossigeno) e concentrazione di inquinanti (es. metalli, tossine e contaminanti emergenti) e di microorganismi. Questa sottile "pellicola" è il cuore dell'ecosistema lagunare.

Nel 2019 e 2020 sono stati svolti prelievi di sedimento stagionali con carotaggi e benne in cinque stazioni (Chioggia, Marghera, Palude della Rosa, Sacca Sessola e Tresse) con diversi livelli di pressione antropica e distribuite nei quattro sottobacini della laguna di Venezia identificati sulla base delle linee guida per l'analisi del rischio nel sedimento.

Il nostro obiettivo è stato lo studio della diversità, delle dinamiche e dei processi metabolici delle comunità microbiche del primo centimetro del sedimento (Figura 1), andando a definire delle "impronte digitali microbiche" caratteristiche per le 5 stazioni lagunari.

I campioni di sedimento sono stati sottoposti ad analisi routinarie (contenuto idrico, granulometria, carbonio organico totale (TOC)) ed analisi caratteristiche di ecologia microbica per la stima dell'abbondanza, la diversità ed i metabolismi microbici (con 16S rRNA metabarcoding e metagenomica, quest'ultima applicata per la prima volta nella Laguna) ed i loro flussi di carbonio organico disciolto (DOC) e di nutrienti dall'interfaccia acqua-sedimento. I nostri dati hanno contribuito alla comprensione dell'importanza dell'interfaccia acqua-sedimento nei cicli biogeochimici microbici di carbonio, nutrienti, metalli, inquinanti, antibiotici e xenobiotici dal livello di ecosistema a quello di microscala.

Per quanto riguarda la diversità e i processi metabolici dei microorganismi, le comunità microbiche sono diverse nei diversi sottobacini. Presentano un'elevata biodiversità principalmente dovuta ai taxa meno abbondanti. Le caratteristiche chimiche e fisiche come la salinità, la granulometria e la concentrazione di TOC del sedimento determinano la struttura della comunità e le potenziali funzioni metaboliche.

Nella prospettiva di una gestione integrata e sostenibile dei sedimenti, i nostri dati contribuiscono ad una caratterizzazione molto fine dei microrganismi, che sono alla base dell'ecosistema della laguna di Venezia. In tutte le stazioni, nello strato superiore del sedimento, sono presenti microorganismi associati a feci e liquami e potenzialmente patogeni. Questi tipi di inquinamento rappresentano un grave problema nelle aree costiere e di transizione, e una potenziale minaccia per la salute umana. Abbiamo rilevato la maggiore presenza di batteri associati alle feci a Chioggia, il sito con la maggiore contaminazione di origine urbana. Nella gestione dei sedimenti, questo implica che la loro mobilizzazione potrebbe portare queste cellule in sospensione nella colonna d'acqua e rappresentare una fonte secondaria di contaminazione.

Per quanto riguarda le funzioni metaboliche potenziali, le aree cronicamente inquinate (Marghera e Tresse) sono *hot spot* di geni legati alla resistenza a composti tossici e agli antibiotici. Nell'ottica della gestione, la mobilitazione del sedimento da queste aree ad altre meno colpite potrebbe portare alla diffusione e all'accumulo di questi tratti genetici. In quest'ottica, la trasmissione genica tra i microrganismi può intensificare il ruolo dei sedimenti come serbatoio di geni di resistenza, complicando il già difficile controllo delle infezioni batteriche nei pesci, nei crostacei e nell'uomo.

La contaminazione dei sedimenti ha effetti ampi e duraturi sulle comunità microbiche, che possono influenzare anche i livelli trofici più alti e l'intero ecosistema, compreso l'uomo. Per questo motivo, la riduzione della contaminazione dei sedimenti dovrebbe essere di primaria importanza per l'ecosistema lagunare per diminuire i potenziali rischi ambientali e per la salute.

1 Introduction

Globally, marine microbes living in the top 10 - 50 cm account for 1.7 - 5 × 10^{28} cells (Whitman et al., 1998, Danovaro et al., 2015, Flemming et al., 2019). Within transitional shallow-water benthic ecosystems, the top centimeter of the sediment is a highly spatially and temporally dynamic habitat. It is subjected to frequent resuspension and deposition events, mixing, transport, and it is shaped by significant biotic activities of macro- to microorganisms (Daumas 1990, Mayer 1993). The top centimeter is highly heterogeneous and characterized by high water content, steep physical and chemical gradients that structure the ecology, the interactions, and the metabolism of microorganisms (*e.g.,* light, oxygen, and organic matter) (Zinger et al., 2011). The microbes within this layer play an essential role in the decomposition of organic matter, nutrient cycling, benthic food webs, thus governing the ecosystem functioning (Schallenberg and Kalff, 1993, Azam and Malfatti, 2007). In respect to the microbial diversity and functions of the pelagic realm, fewer information is available on benthic microbial communities (Probandt et al., 2017). Surface sediments usually present higher microbial abundance and diversity in terms of taxa, potential functions, and metabolisms (Zinger et al., 2011) in comparison to the deeper ones. This microbial environment is influenced by the water column biogeochemistry, and on the other side, from the deeper sediment biogeochemistry, via porewater fluxes (*e.g.,* anoxic conditions, reduced state of organic matter, and contaminants). How these two "biogeochemistries" interplay at the water-sediment interface is still not fully resolved (Catania et al., 2018). Therefore, the study of the microbial distribution patterns within "sediment skin" is fundamental to understand and predict the responses of the marine ecosystem to environmental changes (Zinger et al., 2011). In contrast to the water environment (with the exception of marine snow particles), microbial abundances reach 10^9 cells for cm^3 of sediment and within these universes there is harsh microscale competition for resources (*e.g.,* energy and nutrients) and space (Petro et al., 2017).

Microbes exert their ecosystem role at the microscale (Azam and Malfatti, 2007, Stocker 2008) displaying a great variety of adaptive strategies from dormancy to exchanging electrons and specialized behaviors. Specifically, motility, two-component systems, antiviral systems, the propensity to exchange genetic material, secretion system, and production of redox active antibiotics may promote overall their persistence and success in such crowded environment.

The Venice Lagoon (northern Adriatic Sea), with a surface of 550 km^2, is one of the largest transitional systems in the Mediterranean Sea (Madricardo et al., 2019). This lagoon includes different environments, such as salt marshes, channels, fish farms, natural and artificial islands. It is subjected to different and extensive natural and anthropogenic stressors (Solidoro et al., 2010) such as sea-level rising and subsidence, elevated touristic pressure on Venice city (Seraphin et al., 2018), and the presence of industrial-chemical areas (Bellucci et al., 2002). The essence to be a lagoon relies on the tight coupling between the water and sediments; the sediment environment provides information on the dynamic history of these transitional areas. In the Venice Lagoon, several contaminants have been detected, coming mainly from industrial processes, incinerators, drainages, and urban wastes (Solidoro et al., 2010). These include heavy metals, toxins, polychlorinated biphenyls (PCBs), and others (Han et al., 2007, Borin et al., 2009, Solidoro et al., 2010, Gieskes et al., 2015). These pollutants were discharged in the water column and then they accumulated in the sediments, by the interaction with the downward fluxes of particulate matter (Depinto et al., 2010). The quality of the Venice Lagoon sediments has been evaluated via different risk assessment screenings: several contaminants exceed sediment quality guidelines thresholds, with the highest screening risk level in the north and central part of the lagoon, near the two hotspots of contamination Porto Marghera and Venice city canals (Apitz et al., 2007).

Our research is composed by two sections, focusing *(i)* on the fine characterization of the microbial communities in the sediments, and *(ii)* on the sediment fluxes.

Even if the Venice Lagoon is one of the most studied coastal ecosystems (Solidoro et al., 2004), more effort needs to be done by employing molecular approaches focusing on the microbial role within this transitional area. So far, a handful of high-profile studies have been carried out, but none of them have employed the

metagenomic approach. In 2007, Danovaro and Pusceddu used automated ribosomal intergenic spacer analysis (ARISA) to study microbial diversity in some Mediterranean coastal lagoons, including Venice. They found no relationships between the environmental features of the lagoon and the bacterial diversity, but, on the other hand, the latter was significantly and positively correlated with the ecosystem functioning and efficiency (Danovaro and Pusceddu, 2007). Borin and coworkers (2009) used 16S rRNA gene clone libraries to assess microbial community structure and diversity in the different sub-basins of the Lagoon. They highlighted that the sediments, mainly anoxic, were colonized by microbial communities that presented a species richness correlated with total elemental sulfur and acid-volatile sulfide (Borin et al., 2009). More recently, Quero and coworkers (2017) applied DNA metabarcoding to Venice Lagoon planktonic and benthic bacterial assemblages to explore diversity patterns and the role of environmental gradients. They found evidence of different temporal dynamics in the pelagic and benthic domains (Quero et al., 2017).

We aimed to contribute to the understanding of the importance of the top centimeter of sediment within the microbial biogeochemical cycles of carbon, nutrients, metals, pollutants, antibiotics, and xenobiotics from the large to the micro-scale using molecular techniques. In 2019 and 2020, we have seasonally sampled five sites broadly covering the Venice Lagoon to finely characterize the microbial communities at the taxonomic and functional level using DNA metabarcoding (16S rRNA gene amplicon sequencing) and metagenomics. These data provided new insights on the spatial and temporal distribution and dynamics of microbes in terms of biodiversity, functions, and metabolisms. The metagenomic approach was employed for the first time in the Venice Lagoon, aiming at enhancing our understanding of the behavior of the top-layer sediment microbial communities.

The energy and nutrients exchange are central components of ecological functions of shallow benthic ecosystems and are directly dependent on *in situ* environmental conditions (Roth et al., 2018). Processes such as primary production, organic matter remineralization, and nutrient cycling are important indicators of ecosystem status (Roth et al., 2018). However, the complexity of interactions underlying these processes requires a holistic assessment with accurate measurements of community metabolism and biogeochemical fluxes (Griffiths et al., 2017). In 2019 and 2021, we have seasonally sampled three sites of the Venice Lagoon to assess microbial and viral abundances, estimate dissolved organic carbon, inorganic nutrients, and diffusive benthic fluxes. The measurements of biogeochemical properties of benthic communities are a prerequisite for ecosystem management, and gave us knowledge on the processes that occur at the water-sediment interface of the Venice Lagoon.

In the perspective of an integrated and sustainable sediment management, the knowledge gained within this study have highlighted the importance of the fine characterization of the microbial communities of the top surface layer. For instance, the detection of fecal/sewage related microorganisms and the presence and spread of genes linked to antibiotic and toxic compounds resistance should be considered when sediments are resuspended or mobilized between the different sub-basins, in order to prevent secondary sources of contaminations and reduce potential environmental and health risks.

2 Materials and Methods

2.1 Sampling

The sampling was carried out in 2019, 2020, and 2021 (Table 1) at different sites of the Venice lagoon, Italy (Figure 1): st. 15 Chioggia (C), Pili 1 (L), st. 2 Marghera (M), st. 5 Palude della Rosa, (P), Sacca Sessola 1 (S), Tresse 3 (T). The sites were chosen to cover the four sub-basins, defined according to risk analyses following the international sediment quality guideline of the Lagoon (Apitz et al., 2007). They are located in the southern (C), in the central (S), in the central-north (M, T, L), and in the northern (P) sub-basins. The Chioggia site is near the city of Chioggia, very close to the southern lagoon inlet and more subjected to marine influence. The Sacca Sessola site, situated in the center of the Lagoon, south of Venice city, is potentially affected by urban contamination. The Marghera site is inside the active industrial area of Porto Marghera and is characterized by high anthropogenic impact. The Tresse site is next to an artificial island created as a dumping site for urban and industrial waste. The Pili site is located near both Porto Marghera and Tresse island and is characterized by anthropogenic impact.. Finally, Palude della Rosa site is less impacted by human activities (Picone et al., 2016).

In Chioggia, Marghera, and Palude della Rosa samples were collected with a Van Veen grab (0.045 m^2) in collaboration with Vezzi (UNIPD), while in Sacca Sessola and Tresse and Pili with manually operated corers (6-10 cm diameter; Figure 2) in collaboration with Zonta and Cassin (CNR-ISMAR). Sediments were collected in three replicates. Water temperature and salinity were recorded with the multiparametric probes HI98128 (HANNA Instruments, Italy) and AP-2000 (Aquasearch, Italy) (Table 2).

In all sites, sediment from the 0-1 cm layer was collected for water content estimation, grain size composition, total organic carbon (TOC), prokaryotic abundance. In samples collected seasonally in 2019 and 2020 from C, M, P, S, and T genetic analyses (16S rRNA gene amplicon sequencing and metagenomics) were performed.

In L, S, and T, overlaying water was sampled for bacterial, picoeukaryotic and virus-like particles (VLP) abundance estimation, dissolved organic carbon (DOC), dissolved inorganic nutrients quantification and diffusive fluxes calculations. Pore water was extracted and collected for DOC, dissolved inorganic nutrients quantification, and diffusive fluxes calculations.

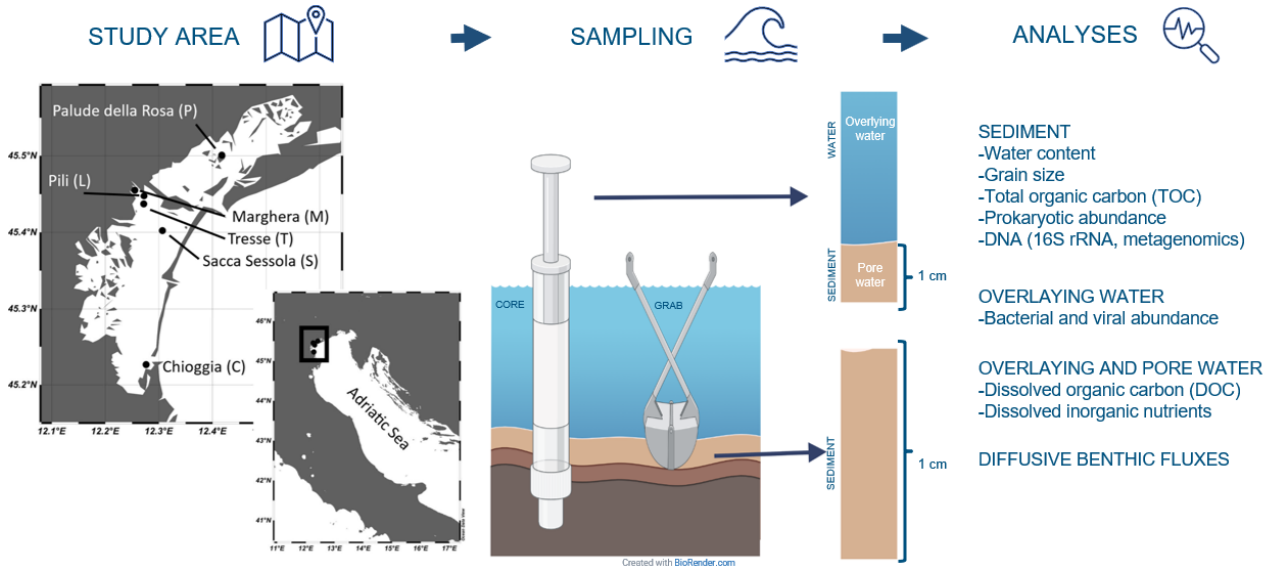

Figure 1. Study area, sampling strategy and microbiological, genetic, physical, and chemical analyses performed. The Venice Lagoon maps were created with the software Ocean Data View (Schlitzer 2018).

Figure 2. Cores and sediments sampled in the Venice Lagoon.

Table 1. Sampling stations, coordinates, and sampling data of the study in the Venice Lagoon. "NS" not sampled due to Covid19 emergency. C: Chioggia; M: Marghera; L: Pili, P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

	Coordinates		**2019**				**2020**				**2021**	
Site	**N**	**E**	**Win**	**Spr**	**Sum**	**Aut**	**Inv**	**Spr**	**Sum**	**Aut**	**Spr**	**Spr**
C	45°13'59.8"	12°16'56.2"	13/2	15/5	22/08	4/11	17/02	29/5	28/8	6/11	--	--
L	45°27'29.268"	12°16'55.1748"	--	--	--	--	--	--	--	--	15/4	14/5
M	45°27'30.3"	12°15'32.5"	13/2	15/5	22/08	4/11	17/02	29/5	28/8	6/11	--	--
P	45°30'06.1"	12°25'03.0"	13/2	15/5	22/08	4/11	17/02	29/5	28/8	6/11	--	--
S	45°24'13.608"	12°18'38.844"	20/3	24/5	23/7-12/9	24/10	NS	NS	NS	NS	15/4	14/5
T	45°26'23.44"	12°16'29.207"	20/3	24/5	23/7-13/9	25/10	NS	NS	NS	NS	--	--

Table 2. Temperature (Temp: °C) and Salinity (Sal) measured during sampling. C: Chioggia; M: Marghera; L: Pili, P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

		Win		**Spr**		**Sum**		**Aut**	
Station	**Year**	**Temp**	**Sal**	**Temp**	**Sal**	**Temp**	**Sal**	**Temp**	**Sal**
C	19	8.03	35.2	14.25	29.2	26.4	33	16.6	35.6
C	20	11.1	34.2	19.7	36.1	26.9	34.1	16.1	34.7
M	19	8.6	33.9	15.38	24.5	27.8	33	16.1	30.8
M	20	11.2	34.3	22.7	31.8	28.1	33.5	16.7	32.5
P	19	8.79	27.4	14.26	22	25.6	30	14	30.2
P	20	10.7	29.2	23.5	32.0	27.8	32.6	15.3	29.5
S	19	10.81	34.1	20.65	26.9	28.6	28	20.1	28
T	19	12.06	33.3	20.28	23.5	28.4	25	20.7	26.7
L	21(Apr)	--	--	13.8	21.5	--	--	--	--
L	21(May)	--	--	22.5	19.6	--	--	--	--
S	21(Apr)	--	--	12.9	21.45	--	--	--	--
S	21(May)	--	--	21.7	19.7	--	--	--	--

2.2 Sediment characterization

The water content was estimated by weighing fresh and dried (105 °C for 24 h) sediment aliquots (2 cm^3).

The grain size composition was determined with a laser Particle Size Analyzer (BECKMAN COULTER LS 13 320) after treating 1 g of wet sediment with H_2O_2. The sediment was described using Shepard's classification (Shepard 1954) and the data were expressed as percentages of sand, silt, and clay. The limit between silt and clay was fixed to 4 µm, following the Udden-Wentworth classification (Wentworth 1922).

2.3 Total organic carbon (TOC) in sediment

Freeze-dried sediment was grounded in a ceramic mortar and sieved on a 250 µm iron steel sieve (Endecotts LTD, UK). Triplicate subsamples of about 8–12 mg were weighed on a microultrabalance with an accuracy of 0.1 µg. Before Total organic carbon (TOC) determination, subsamples were treated directly into capsules with increasing concentrations of HCl (0.1 N and 1 N) to remove the carbonate fraction (Nieuwenhuize et al., 1994). Carbon content was determined using a CHNO-S elemental analyzer ECS 4010, Costech, Italy) according to Pella and Colombo (1973). Standard acetanilide (Costech, purity ≥ 99.5%) was used to calibrate the instrument and empty capsules were also analyzed to correct for blank. Measurement quality control was performed using internal standards and it was also verified for carbon against the certified marine sediment reference material PACS-2 (National Research Council Canada).

Significant differences in TOC were calculated in the R environment (v. 4.0.3, R Core Team 2019) by Kruskal-Wallis and Wilcoxon non-paired tests. False discovery rate (FDR) was used for p-value correction. Results with q-value < 0.05 were considered significant.

2.4 Prokaryotic abundance in sediment

2 cm^3 of sediment were fixed with 5 mL of 0.2 µm-filtered seawater-formaldehyde solution (4 %) and kept at 4°C for 24 h. The samples were washed with 7.5 mL of phosphate buffered saline solution (PBS) 1 ×, resuspended in 5 mL of a solution of 1:1 ethanol: PBS 1 ×, and stored at -20 °C until processing.

The prokaryotic abundance was estimated by flow cytometry following Deng et al. (2019) with modifications, using a FACSCanto II (Becton Dickinson) instrument equipped with an air-cooled laser at 488 nm and standard filter setup. A diluted sediment slurry was mixed with Milli Q water and the Detergent solution [100 mM ethylenediaminetetraacetic acid (EDTA), 100 mM sodium pyrophosphate, 1% (v/v) Tween 80]. The slurry was subjected to mechanical shaking, ultrasonication, and hydrofluoridric acid (1%, final concentration) treatment. Then, an aliquot was mixed with the Stop solution [1 M Tris–HCl, pH 8.0; 0.125 M Calcium chloride and 25% methanol] and filtered through a 10 µm polycarbonate filter. A 1:800 aliquot was diluted in 0.2 µm-filtered Tris-EDTA buffer 1 × and stained with SYBRGreen I (2 ×, final concentration). Negative controls were prepared by autoclaving sediments and running unstained sediments. The acquisition threshold was set to green fluorescence and stained prokaryotic cells were identified in the side scatter versus green fluorescence plot. The flow rate was calibrated daily, by running distilled water and weighing it before and after the run (at least five replicates). Data were acquired and processed with the FACSDiva software (Becton Dickinson).

Prokaryotic cells per gram of dry sediment were calculated using the acquired cell counts, the flow rates and the respective sample water content, and statistical analyses were run as described for TOC data.

2.5 DNA extraction from sediment and sequencing

DNA was extracted with DNeasy PowerSoil Pro Kit (Qiagen) following the manufacturer's instructions. Quality and quantity of the extracted DNA was assessed with Nanodrop spectrometer (Thermo Fisher Scientific) and with Qubit Fluorimeter (Thermo Fisher Scientific).

For the DNA metabarcoding analysis, the V4-V5 region of 16S rRNA gene was amplified using 515-Y (5'-GTGYCAGCMGCCGCGGTAA-3') and 926R (5'-CGYCAATTYMTTTRAGTTT-3') primers (Parada et al., 2016). Libraries were prepared following the 16S Metagenomic Sequencing Library Preparation protocol and run on an Illumina MiSeq System for a read length of 2 × 250 bp at the genetic and epigenetic ARGO Open Lab Platform, Area Science Park, Trieste, Italy.

For the metagenomic analysis, libraries were prepared following the Illumina Nextera DNA Flex Library Prep protocol and run on an Illumina NovaSeq 6000 System for a read length of 2 × 300 bp at the genetic and epigenetic ARGO Open Lab Platform, Area Science Park, Trieste, Italy.

2.6 Sediment 16S bioinformatic pipeline

Bioinformatic analyses were performed with QIIME2 2020.6 (Bolyen et al., 2019). Raw sequences were quality filtered and denoised with DADA2 (Callahan et al., 2016). Alpha-diversity metrics were estimated after samples were rarefied. To assess the reliability and robustness of the replicates, a correlation analysis (Spearman's ρ) on the amplicon sequence variants (ASVs) table was performed with the R package *ggplot2* (Wickham et al., 2016). Taxonomy was assigned to ASVs using the sklearn naïve Bayes taxonomy classifier (Bokulich et al., 2018) against the Silva 138 99% reference database with 7-level taxonomy (Quast et al., 2013). Reads belonging to Eukarya, mitochondria, chloroplast, and with frequency < 2 (singletons) were removed.

Statistical analyses were conducted in the R environment (v. 4.0.3, R Core Team 2019). Rarefaction curves on ASVs table were plotted with the R package *vegan* (Oksanen et al., 2019). Significant differences in alpha diversity metrics were calculated by Kruskal-Wallis H test. FDR was used for p-value correction. Results with q-value < 0.05 were considered significant.

To visualize similarity patterns of prokaryotic communities, a Principal Coordinates Analysis (PcoA) using Bray-Curtis dissimilarity matrices was constructed using normalized read abundances in the R package *vegan* (Oksanen et al., 2019). A permutational multivariate analysis of variance (PERMANOVA) with 4999 permutations was computed on normalized ASVs table to investigate the effect of site and season using the function *adonis* in the R package *vegan* (Oksanen et al., 2019).

To further explore the environmental drivers of the community, we used a distance-based redundancy analysis (dbRDA) using the *capscale* function in the R package *vegan* (Oksanen et al., 2019). The environmental variables (temperature, salinity, grain size, and TOC) were normalized using a z-score transformation. The significance of the variables was tested with ANOVA and 4999 permutations after building a reduced model. As the grain size components (sand, silt, and clay) present multicollinearity, only one component (sand) was included in the analysis.

An indicator species analysis was performed to verify the taxa fidelity at site level with the R package *indicspecies* (De Cáceres and Legendre, 2009). The results were visualized as networks by mean of the R package *igraph* (Csárd and Nepusz, 2006).

Microbial diversity visualization at different taxonomic level was done using the average normalized abundance of the three replicates of each sample, with package *phyloseq* (McMurdie and Holmes, 2012) and *ggplot2* (Wickham et al., 2016). Microbial signatures of fecal- and sewage-associated bacteria (McLellan et al., 2010, Newton et al., 2013, Paliaga et al., 2017) were also evaluated.

Statistical Analyses of Metagenomic Profiles 2.1.3 (STAMP; Parks and Beiko, 2010) was used to assess differences in the relative proportion of sequences belonging to the different taxa considering the whole dataset. Significant differences were calculated by ANOVA and Tukey-Kramer post-hoc test. FDR was used for p-value correction. Results with q-value < 0.001 were considered significant.

2.7 Sediment metagenomic analysis

The number and quality of reads were checked with FastQC (Andrews 2010). Reads were assembled into contigs using MEGAHIT-1.2.9 (Li et al., 2015) combining the three replicates of each sample after testing the significance of their correlations. Gene prediction was performed with Prodigal 2.6.3 (Hyatt et al., 2010) on contigs longer than 1000 bp (Van der Walt et al., 2017).

The contigs > 1000 bp were submitted to Metagenome Analysis Rapid Annotation using Subsystem Technology (MG-RAST; Keegan et al., 2016) for a similarity search using the SEED Subsystem (Overbeek et al., 2005) and the Kyoto Encyclopedia of Genes and Genomes (KEGG; Kanehisa and Goto, 2000) databases, using a cut-off E-value of 1e-5, minimum identity of 60% and a minimum alignment length of 15 bp. SEED and KEGG annotations were normalized by the number of reads assigned to the prokaryotic single copy gene recA (Acinas et al., 2021) prior to calculating the relative abundances.

Key oceanic marker genes (Sunagawa et al., 2015) and key sediment metabolic genes (Dombrowski et al., 2018) were evaluated to provide an overview of the main biochemical cycling metabolisms present. We have created a novel ad-hoc specific microscale marker function list to explore the microbial potential at the small-scale in the sediment environment.

Annotation results were visualized using Statistical Analyses of Metagenomic Profiles 2.1.3 (STAMP; Parks and Beiko, 2010) to assess differences in their relative proportion considering the whole dataset. Significant differences among samples were calculated by ANOVA and Tukey-Kramer post-hoc test. FDR was used for p-value correction. Results with q-value < 0.001 were considered significant.

2.8 Bacterial and viral abundance in overlaying water

1.5 mL of the overlaying water of cores was fixed with of glutaraldehyde (0.5% f.c.). The samples were kept 10 min at 4°C, then frozen and kept at –80 °C until processing, within one week.

The abundance of Synechococcus (SYN), Picoeukaryotes (PE), heterotrophic bacteria (HB), and virus-like particles (VLP) was estimated by flow cytometry following Gasol et al. (1999) and Brussaard (2004). A FACSCanto II (Becton Dickinson) instrument was used, equipped with an air-cooled laser at 488 nm and standard filter setup. Prior to enumeration, samples were thawed at room temperature and diluted 1:10 (HB) and 1:50 (VLP) with 0.2 µm-filtered Tris-EDTA buffer 1 ×. Then samples were stained with SYBR Green I nucleic acid dye (Life Technologies), according to Marie et al. (1999) and Brussaard (2004) for HB and VLP, respectively. HB were stained (1 ×) and incubated for 10 min in the dark at room temperature. VLP were stained (0.5 ×) and incubated for 15 min in the dark at 80 °C. Total VLP abundance was obtained by correcting the total count for noise, with 0.2 µm-filtered Tris-EDTA buffer 1 × as blank. Data were acquired and processed with the FACSDiva software (Becton Dickinson). Abundances were calculated using the acquired cell counts and the respective flow rates.

2.9 Dissolved organic carbon (DOC) in overlaying and pore water

Samples for DOC were filtered onto pre-combusted (450 °C for 4 h) Whatman GF/F filters in acid-washed glass vials and kept frozen (−20 °C) until laboratory analysis.

DOC was determined using the Shimadzu TOC 5000 Analyzer. Water samples were previously acidified with HCl and after CO_2 elimination the DOC concentration was determined with a 1.2% Pt on silica as catalyst at 680 °C.

2.10 Dissolved inorganic nutrients in overlaying and pore water

Samples for dissolved inorganic nitrogen (DIN = nitrites N-NO_2 + nitrates N-NO_3 + ammonium N-NH_4), phosphate P-PO_4, and silicates Si -Si$(OH)_4$ were filtered onto pre-combusted (450 °C for 4 h) Whatman GF/F filters in acid-washed polyethylene vials and kept frozen (−20 °C) until laboratory analysis. Inorganic nutrient concentrations were determined colourimetrically with a QuAAtro Seal Analytical autoanalyzer according to Hansen and Koroleff (1999). The detection limits of nutrient concentrations reported by the analytical methods are 0.02, 0.02, 0.04, 0.02 and 0.02 µM, respectively, for N-NO_2, N-NO_3, N-NH_4, P-PO_4 and Si-Si$(OH)_4$.

The accuracy and precision of the analytical procedures at low concentrations are checked annually through the quality assurance programme QUASIMEME and the relative coefficient of variation for five replicates was less than 5 %. Internal quality control samples were used during each analysis.

2.11 Diffusive benthic fluxes

Diffusive fluxes are instantaneous measure, along a gradient of concentration, of the movement of solutes diffusing from sediment porewater to the overlying water column (De Vittor et al., 2012). Diffusive fluxes of the dissolved inorganic nutrients were calculated with Fick's first law as described in Covelli et al. (2008) and De Vittor et al. (2012).

The diffusive fluxes of solutes in porewaters were determined using Fick's first law:

$$F = -(\Phi D_{\mathrm{w}}/\theta^2)\partial C/\partial x$$

where F (µmol $m^{-2}d^{-1}$) is the flux of a solute with concentration C at depth x, Φ the sediment porosity, ϑ the tortuosity (dimensionless) and D_{w} is the diffusion coefficient of the solute in water in the absence of the sediment matrix. For the calculations, nutrients concentration in overlaying and pore water (extracted by 20 min of centrifugation at 3000 × g) at 1 cm depth were used. Porosity, considering the dry weight, and tortuosity were estimated following Serpetti et al. (2016) and Boudreau (1996) respectively. Diffusion coefficients (D_w) of the solutes in water were calculated following Schulz (2000) and corrected for temperature.

3 Sediment microbial and molecular analyses

3.1 Sediment characterization

The five stations were characterized by different percentages of the grain size classes of sand, silt, and clay (Table 3), in agreement with previous studies (Borin et al., 2009, Picone et al., 2016, Zonta et al., 2018). Based on Shepard's classification, Chioggia was categorized as sand, Marghera and Pili as clayey silt, Palude della Rosa as silty-loam, Sacca Sessola as loam, and Tresse as silty sand.

Table 3. Grain size composition and Shepard's classification of the sediment samples. C: Chioggia; L: Pili; M: Marghera, P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

Site	Season	Year	%SAND	%SILT	%CLAY	Shepard's classification
C	Win	2019	97.6	1.7	0.7	Sand
	Win	2019	93.6	4.3	2.1	Sand
	Win	2019	97.3	1.9	0.8	Sand
	Spr	2019	96.5	2.4	1.1	Sand
	Spr	2019	95.4	3.2	1.4	Sand
	Spr	2019	90.3	6.6	3.1	Sand
	Sum	2019	90.8	6.5	2.7	Sand
	Aut	2019	97	2	1	Sand
	Win	2020	97.9	1.5	0.6	Sand
	Win	2020	78.4	14.6	7	Sand
	Spr	2020	89.2	7.8	3	Sand
	Spr	2020	98	1.5	0.5	Sand
	Sum	2020	94.5	3.9	1.6	Sand
	Aut	2020	98	1.5	0.5	Sand
	Aut	2020	84.4	10.6	5	Sand
L	Spr	2021	12.2	61.8	26.0	Clayey silt
	Spr	2021	17.7	59.5	22.8	Clayey silt
M	Win	2019	9.1	62.3	28.6	Clayey silt
	Spr	2019	3.1	51.2	45.7	Clayey silt
	Spr	2019	13.1	57.3	29.6	Clayey silt
	Spr	2019	13.8	59.8	26.4	Clayey silt
	Sum	2019	11.5	60.1	28.4	Clayey silt
	Sum	2019	9.1	60.2	30.7	Clayey silt
	Sum	2019	11.8	59.8	28.4	Clayey silt
	Aut	2019	15.4	55.4	29.2	Clayey silt
	Win	2020	19.8	54.6	25.6	Clayey silt
	Spr	2020	6.2	56	37.8	Clayey silt
	Sum	2020	7.5	56.1	36.4	Clayey silt
	Sum	2020	10.3	61.3	28.4	Clayey silt
	Aut	2020	9.2	60.3	30.5	Clayey silt
P	Win	2019	18.1	57.5	24.4	Clayey silt
	Spr	2019	17.1	57.7	25.2	Clayey silt
	Spr	2019	16.5	60	23.5	Clayey silt
	Spr	2019	38.8	43.4	17.8	Sandy silt
	Sum	2019	11.6	62.9	25.5	Clayey silt
	Sum	2019	12.1	63	24.9	Clayey silt
	Sum	2019	35	45.8	19.2	Sandy silt
	Aut	2019	34.1	45	20.9	Sand silt clay
	Win	2020	50.6	35.3	14.1	Silty sand
	Spr	2020	21.6	56	22.4	Sand silt clay
	Sum	2020	29.9	48.3	21.8	Sand silt clay
	Aut	2020	24	52.5	23.5	Sand silt clay
S	Win	2019	17.3	60.6	22.1	Clayey silt

Site	Season	Year	%SAND	%SILT	%CLAY	Shepard's classification
	Win	2019	18.9	60.1	21	Clayey silt
	Spr	2019	50.1	37.9	12	Silty sand
	Spr	2019	43.5	41.9	14.6	Silty sand
	Sum	2019	40.5	44.8	14.7	Sandy silt
	Aut	2019	40.9	44.6	14.5	Sandy silt
	Spr	2021	42.6	43.7	13.7	Sandy silt
	Spr	2021	47.9	41.4	10.7	Silty sand
T	Win	2019	63.5	27.3	9.2	Silty sand
	Win	2019	71.1	21.1	7.8	Silty sand
	Spr	2019	73.8	19.2	7	Silty sand
	Spr	2019	67.9	23.3	8.8	Silty sand
	Sum	2019	62.6	27.8	9.6	Silty sand
	Aut	2019	68.5	23.4	8.1	Silty sand

3.2 TOC in sediment

Total organic carbon (TOC) ranged from 0.87 to 32.8 mg g_d^{-1} (Table 4). Marghera (16.4 ± 7.5) and Palude della Rosa (14.8 ± 7.5) showed significantly higher abundances than Sacca Sessola (8.2 ± 2.1), Tresse (5.5 ± 1), and Chioggia (3.3 ± 3.2). No significant differences were detected among seasons.

Table 4. Total organic carbon (TOC; mg g_d^{-1}) quantified in the sediment samples. C: Chioggia; L: Pili; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

Site	Season	Year	TOC
C	Win	2019	1.99 ± 0.04
	Spr	2019	1.23 ± 0.02
	Sum	2019	1.72 ± 0.03
	Aut	2019	1.04 ± 0.02
	Win	2020	0.87 ± 0.04
	Spr	2020	2.82 ± 0.02
	Sum	2020	3.06 ± 0.02
	Aut	2020	10.7 ± 0.14
L	Spr	2021	13.96 ± 0.21
	Spr	2021	11.81 ± 0.04
M	Win	2019	16.42 ± 0.08
	Spr	2019	14.91 ± 0.08
	Sum	2019	16.63 ± 0.21
	Aut	2019	15.83 ± 0.03
	Win	2020	14.42 ± 0.05
	Spr	2020	32.81 ± 0.05
	Sum	2020	15.32 ± 0.04
	Aut	2020	16.13 ± 0.25
P	Win	2019	16.49 ± 0.07
	Spr	2019	17.33 ± 0.07
	Sum	2019	17.06 ± 0.06
	Aut	2019	13.6 ± 0.02
	Win	2020	13.13 ± 0.05
	Spr	2020	9.77 ± 0.05
	Sum	2020	15.37 ± 0.21
	Aut	2020	18.26 ± 0.10
S	Win	2019	7.86 ± 0.15
	Spr	2019	7.68 ± 0.13

Site	Season	Year	TOC
	Sum	2019	5.92 ± 0.03
	Aut	2019	6.2 ± 0.08
	Spr	2021	10.57 ± 0.29
	Spr	2021	10.92 ± 0.04
T	Win	2019	5.24 ± 0.01
	Spr	2019	4.68 ± 0.01
	Sum	2019	7.13 ± 0.07
	Aut	2019	4.76 ± 0.02

3.3 Prokaryotic abundance in sediment

The average sediment prokaryotic abundance was 25.7 ± 21.5 × 10^9 Cells g_d^{-1} (Figure 4). Pili (60.9 ± 13 × 10^9 Cells g_d^{-1}) and Palude della Rosa (45.4 ± 32.5 × 10^9 Cells g_d^{-1}) showed significantly higher abundances than Marghera (27.7 ± 24.6 × 10^9 Cells g_d^{-1}), Chioggia (8.3 ± 7.5 × 10^9 Cells g_d^{-1}), Sacca Sessola (7.8 ± 4.3 × 10^9 Cells g_d^{-1}), and Tresse (4.5 ± 4.1 × 10^9 Cells g_d^{-1}). Overall, Autumn data (30.2 ± 26.9 ×10^9 Cells g_d^{-1}) were significantly higher than Spring (10.1 ± 8.5 × 10^9 Cells g_d^{-1}) and Summer ones (16.0 ± 12.5 × 10^9 Cells g_d^{-1}).

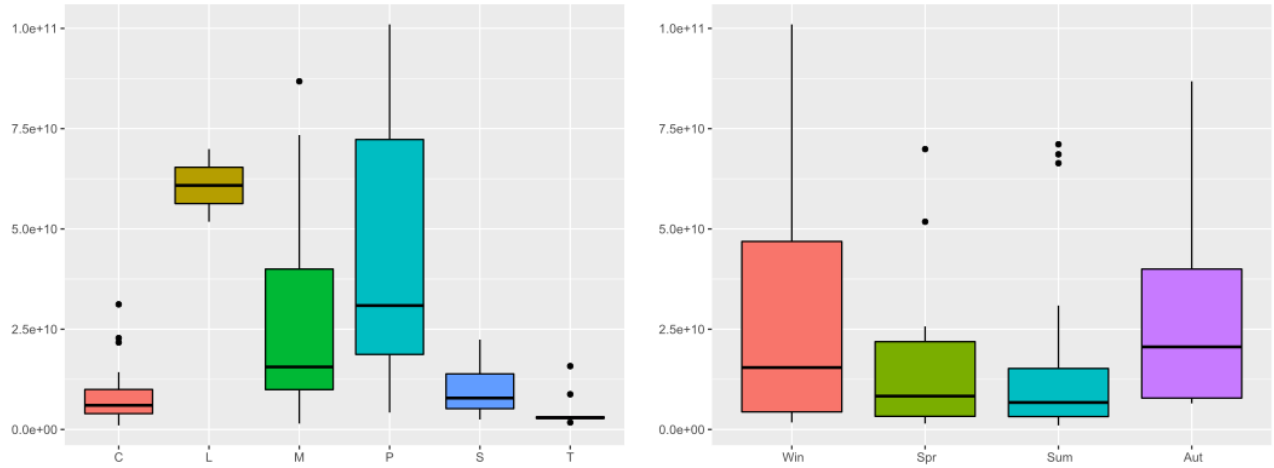

Figure 4. Prokaryotic abundance (Cells g_d^{-1}) quantified by flow cytometry on the samples grouped by site, season, and site-season for 2019 and 2020 combined. C: Chioggia; L: Pili; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

3.4 Sediment prokaryotic diversity and structure (16S rRNA dataset)

A total of 31,189,504 raw sequences were generated. After the denoising procedure, 20,191,333 were retained with an average of 219,471 ± 92,086 sequences per sample. The average number of ASVs was 5,230 ± 1,536. The rarefaction curves indicated that the sequencing effort was enough to assess the sample biodiversity (Figure 5). The replicates significantly correlated ($p < 0.05$) with an average ρ of 0.86 ± 0.09.

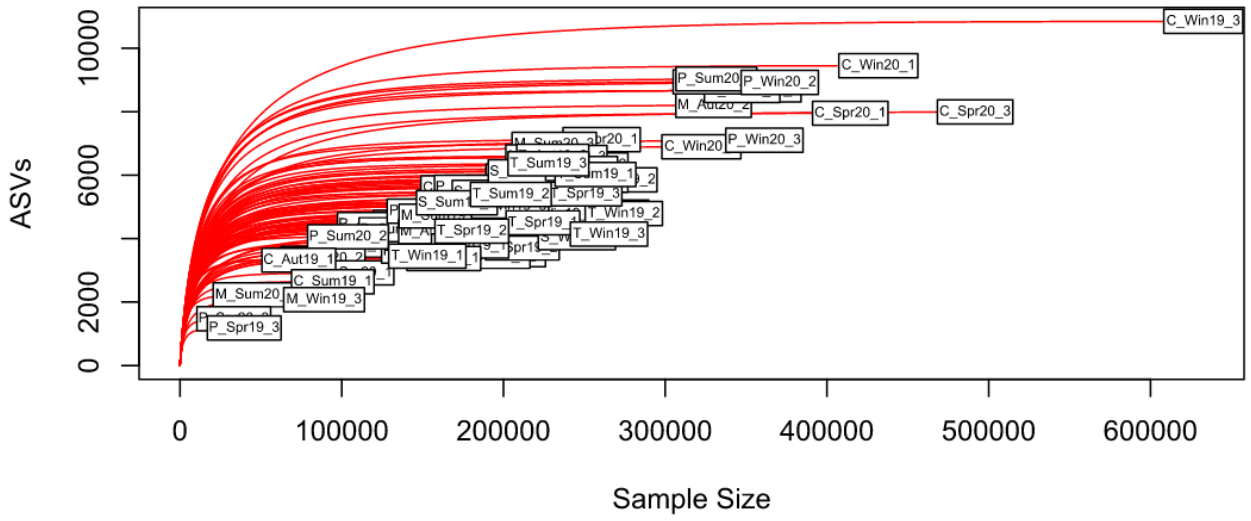

Figure 5. Rarefaction curves constructed on the number of reads (sample size) and the number of ASVs in each sample.

Shannon's diversity index showed an average of 10.7 ± 0.4 and Pielou's evenness showed an average of 0.89 ± 0.02 (Figure 6). Overall, autumn had a significantly higher index than the other seasons. No significant differences in biodiversity indices were detected among sites and site-season combinations.

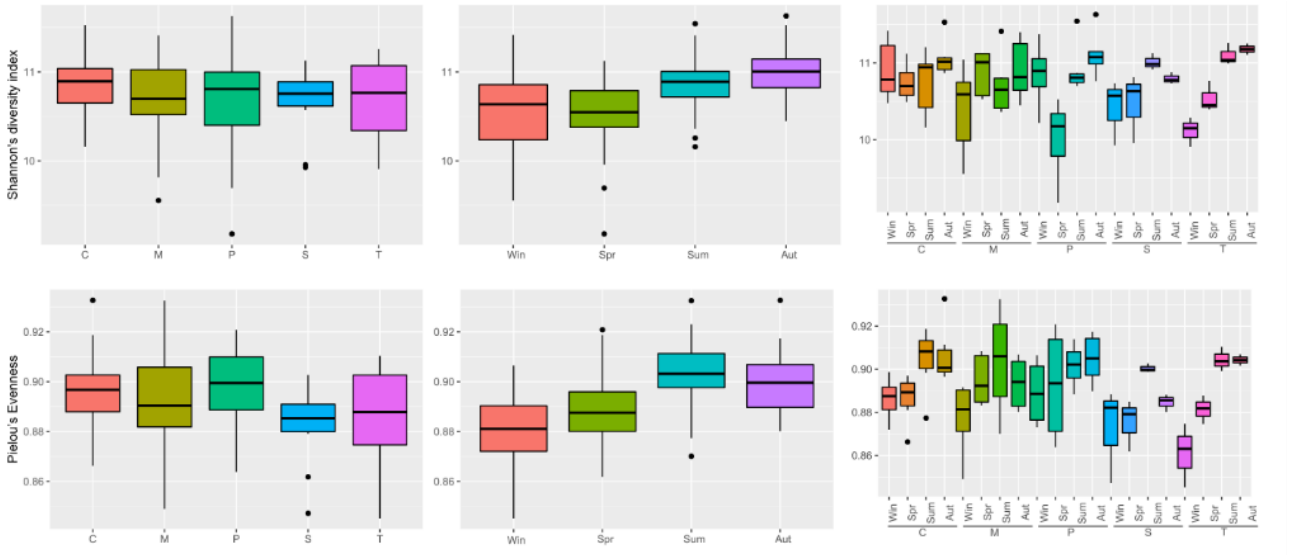

Figure 6. Alpha diversity metrics calculated on the samples grouped by site, season, and site-season for 2019 and 2020 combined. C: Chioggia; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

The PERMANOVA highlighted that site, season, and their interaction had a significant effect ($p < 0.001$) on the prokaryotic community structure (Table 5). The highest amount of variance was explained by the site (37%), followed by season-site interaction (11%) and season (6 %).

Table 5. Results of permutational multivariant analysis of variance (PERMANOVA) of the prokaryotic communities based on Bray-Curtis dissimilarities of reads relative abundance table.

	PERMANOVA				
Factor	**d.f.**	**SS**	**MeanSqs**	**F.Model**	**R^2**
Site	4	7.2376	1.8094	15.0385	0.37521*
Season	3	1.2415	0.41382	3.4393	0.06436*
Site*Season	12	2.1475	0.17896	1.4874	0.11133*
Residuals	72	8.6629	0.12032	0.4491	
Total	91	19.2895	1		

d.f., degrees of freedom; SS, sum of squares. * p < 0.001, calculated after 4999 permutations.

The PCoA (Figure 7), in agreement with the PERMANOVA, grouped the samples by site. The dbRDA (Figure 7) revealed that grain size, TOC, and salinity had a significant role (p < 0.001) in shaping the communities, while temperature was not a significant variable (p > 0.05).

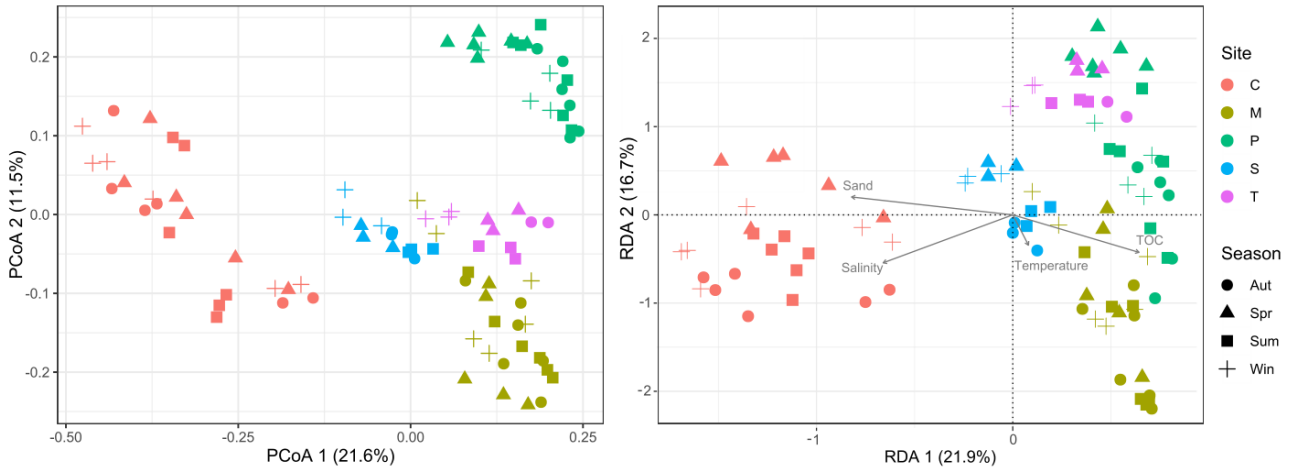

Figure 7. Principal Coordinate Analysis (PCoa, left panel) and distance-based redundancy analysis (dbRDA, right panel) based on Bray-Curtis dissimilarity in community composition. C: Chioggia; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse. TOC= total organic carbon.

Microbial communities in marine sediment are extremely diverse due to the combination of different factors such as habitat temporal stability, high niche diversity, and resource partitioning due to the complex physical-chemical gradients (Zinger et al., 2011, Acosta-Gonzales and Marques, 2016). As such, they represent a major reservoir of genetic variability in the marine environment (Polymenakou et al., 2005).

Our analysis detected a pronounced diversity in the communities, in agreement with 16S studies in the Venice Lagoon (Borin et al., 2009, Quero et al., 2017) and in other transitional and coastal benthic systems (Bianchelli et al., 2000, Miksch et al., 2021).

The sampling site specific features were the main drivers in shaping the prokaryotic assemblages, while seasonality had a significant but minor role (Figure 7). Depending on the area, seasonality in the sediment microbial communities is not as regularly and clearly found as in the water column (Gobet et al., 2012, Tšertova et al., 2011, Miksch et al., 2021), even in the Venice Lagoon (Quero et al., 2017). Quero and collaborators (2017), in a study including pelagic and benthic communities in the central part of the Venice Lagoon, found seasonality in both compartments but less pronounced in surface sediments than in the overlaying water, where this feature is well documented (Celussi et al., 2009).

In marine sediments, it is now known that physical-chemical characteristics including salinity, chlorophyll a, TOC content, grain size, and benthic fauna can influence the microbial communities (Sapp et al., 2010, Pala et al., 2018, Hoshino et al., 2020). Generally, the composition of the sediment bacterial communities can present high dissimilarities between sites, reflecting the heterogeneity of these environments, mostly due to limited physical mixing and the geographical distance (Acosta-Gonzales and Marques, 2016).

We found that salinity, grain size, and TOC significantly contributed to the structure of the microbial communities in the different sites of the Venice Lagoon.

Salinity is recognized as a major environmental determinant of microbial composition in benthic communities (Lozupone and Knight, 2007, Bolhuis et al., 2013) in coastal (Bolhuis and Stal, 2011, Severin et al., 2012, Bolhuis et al., 2013) and lagoon systems (Tsuboi et al., 2013, Pavloudi et al., 2016, Behera et al., 2017). In our study, the more marine-influenced site (Chioggia, located at the southern inlet of the Venice Lagoon) was confirmed as the most diverse one, forming a separated group in the multivariate analyses (Figure 7).

It is known that grain size can affect microbial abundance and activity by providing different surface properties (e.g., fine sediments provide greater areas that can be colonized) and influencing permeability, water chemistry, hydraulic conductivity, and pore space between sediment particles (Dale 1974, Zhang et al., 1998). A positive correlation between bacterial abundance, sediment grain size, and organic carbon content is well established (Dale 1974) and reported in different studies (Santmire and Leff, 2007, Legg et al., 2012, Tsuboi et al., 2013, Fazi et al., 2020). In another Italian Adriatic lagoon, it was found that lowest salinity, higher percentage of fine grain sediment and organic matter corresponded to the highest microbial abundances (Cibic et al., 2019). This was confirmed also in our study, in which the sites enriched in silt, Marghera and Palude della Rosa, presented the highest prokaryotic abundance and TOC concentration.

Sediment grain type influences indirectly the microbial assemblage at the microscale, due to the variable water fluxes (Ahmerkamp et al., 2020) that changes the redox conditions thus driving more diverse and able-to-adapt communities in sandy sites compared to the clay and silty ones.

Being and important source of energy for heterotrophs the organic carbon (TOC) pool is an important structuring agent for benthic microbial communities (Oni et al., 2015). As such, in our study the content of TOC was important in structuring the differences among the communities (*e.g.*, Chioggia vs other sites), even though we did not analyze its quality (biopolymers' concentration, labile vs recalcitrant matrices, etc.), which is also known to affect the composition of bacterial communities (e.g., Fazi et al., 2020). Likewise, ecological factors that were not investigated in this study can contribute to the differentiation of the sites: interspecific bacteria competition, grazing pressure, viral lysis, vegetation, contaminants, and also stochastic events (Böer et al., 2009, Luna et al., 2013, Acosta-Gonzales and Marques, 2016, Bianchelli et al., 2020).

3.5 Sediment prokaryotic community composition

The microbial communities were characterized by few dominant taxonomic groups, the core microbiome, and a high number of low abundant taxa, the so-called rare biosphere (Sogin et al., 2006), that accounted for most of the phylogenetic diversity.

For instance, among the 84 phyla detected in the whole dataset, 49 were considered rare, as represented by an average relative abundance < 0.01 % (Galand et al., 2009, Gobet et al., 2012), as well as for 1181 out of the 1587 detected genera. The presence of such rare biosphere is of ecological importance as these taxa, often overlooked, can have an over-proportional role in biogeochemical cycles and can represent a hidden driver of ecosystem functions (Jousset et al., 2017).

Even if we detected significant differences among sites in high abundant taxa, from the phylum to the genus level, the numerous low-abundant taxonomic units were those that more contributed to their site-specific fingerprint. These taxa were mostly uncharacterized (*e.g.* belong to uncultured bacteria typically detected only with molecular techniques and are still not defined in terms of physiology and metabolism), and more studies are needed to define their ecological roles.

In accordance with Jousset and colleagues (2017) we suggest that the rare biosphere in the Venice Lagoon can be considered as an ensemble of keystone species that provide stability and seeds for ecosystem functions, specifically in terms of water purification, nutrients, and global climate regulations (da Mosto et al., 2020).

Regarding the taxonomic composition, at the domain level most reads were assigned to Bacteria (average on the overall dataset 96.1 ± 2.6 %), and a smaller percentage (3.8 ± 2.6 %) to Archaea.

The most represented phyla were Proteobacteria (29.3 ± 4.4 %), followed by Bacteroidota (15.5 ± 4.4 %) (Figure 8). Desulfobacterota were significantly less abundant in Chioggia than the other sites (9.2 ± 2.4 % *vs* 12.8 ± 0.8 %), while Actinobacteriota presented the opposite distribution pattern (4.8 ± 1.2 % *vs* 2.2 ± 0.5 %).

The most represented classes were Gammaproteobacteria (23.9 ± 3.7 %), followed by Bacteroida (14.7 ± 4.3 %). Gammaproteobacteria were confirmed to be prevalent at all sites, as generally detected in sediment in both the costal and the deep seafloor (Fry et al., 2008, Zinger et al., 2011, Acosta-Gonzales and Marques, 2016), including the Venice Lagoon one (Borin et al., 2009, Quero et al., 2017).

The most represented orders were the bacterial Flavobacteriales (6.8 ± 3.8 %), Desulfobacterales (6.1 ± 2.2 %), and Pirellulales (5.2 ± 2.3 %). The most represented families were Flavobacteriaceae (6.2 ± 3.6 %), Desulfosarcinaceae (5.2 ± 1.8 %), and Pirellulaceae (4.5 ± 0.9 %).

The ubiquitous genus *Woeseia* (4.4 ± 0.9 %) was the most abundant (Figure 8). Recent molecular characterization suggested that it is likely to grow on proteinaceous matter, potentially derived from detrital cell membranes, cell walls, and other organic remnants (Mußmann et al., 2017, Hoffmann et al., 2020).). The ammonia-oxidizing archaeon *Candidatus Nitrosopomilus* was significantly higher in Chioggia (1.2 ± 0.7 % *vs* 0.1 ± 0.1 %)(Figure 8). *Blastopirellula* and *Rubripirellula* were significantly higher in Chioggia and Sacca Sessola in respect to the other sites (1.4 ± 0.3 % *vs* 0.5 ± 0.1 % for the former and 1.2 ± 0.1 % *vs* 0.6 ± 0.1 % for the latter respectively) (Figure 3). Among the genera related to microscale processes, we detected the cable bacteria *Candidatus Electrothrix* at all sites (0.1 ± 0.2 %); these bacteria have long, multicellular filaments that can conduct electric currents over centimeter-scale distances (Trojan et al., 2016). Likewise, lignin-degrader genera (*Bacillus*, *Novosphingobium*, *Pseudomonas*, *Rhodococcus*, and *Streptomyces*; Lee et al., 2019) were found at all sites (0.006 ± 0.008 %).

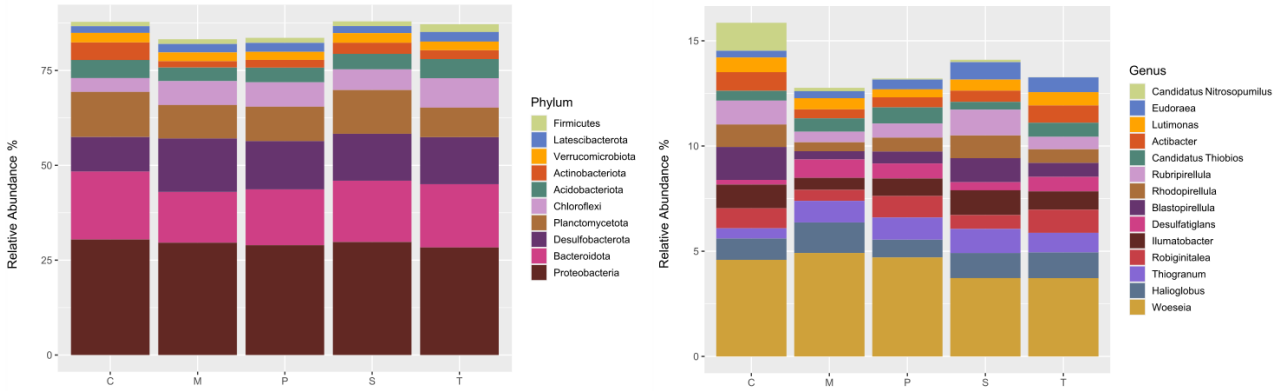

Figure 8. Taxonomic composition (average relative abundance) at the phylum (left panel) and the genus (right panel) levels in the five sampling sites for 2019 and 2020 combined. Taxa representing > 1% and 0.5% are shown, respectively. C: Chioggia; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

The Indicator Species analysis showed that each site hosted a set of high-fidelity bacterial genera (Figure 9), with Chioggia showing the highest number (n = 21). On the contrary, excluding Chioggia, the highest number of genera (n = 27) were shared among Marghera, Palude della Rosa, Sacca Sessola and Tresse. Chioggia was characterized by the presence of aerobic (or strictly aerobic), halophilic bacteria such as *Aquimarina, Aureisphaera,* and *Pseudoalteromonas* and by the absence of genera of sulfate-reducing Desulfobacteraceae that were shared among the other sites, like *Desulfoconvexum*, *Desulfonema,* and *Desulfobacterium*.

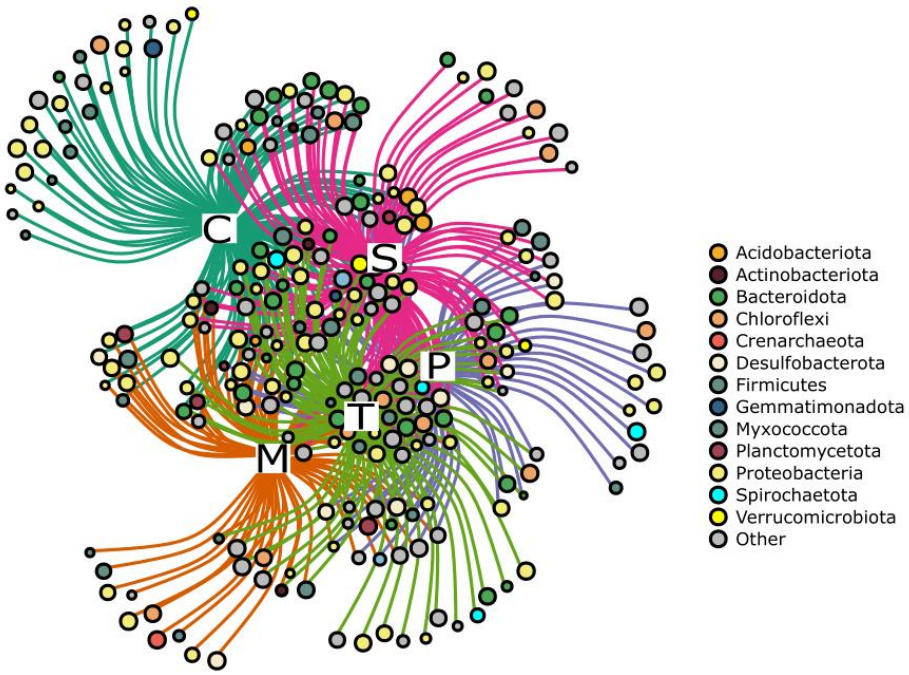

Figure 9. Network representation of the Indicator Species analysis at the genus level. Each node represents a genus, and the node size is scaled based on the fidelity to the sampling site for 2019 and 2020 combined. Colours refer to the phylum to which each genus belongs. Grey nodes represent low abundant phyla (< 1%). C: Chioggia; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

Several significant differences in taxonomic composition were found in sulphur-related bacteria (Desulfobacteria, Dedulfobacterales) involved in sulphate reduction. In our study, these taxa were less represented in Chioggia where, differently form the inner area of the Lagoon where the sediment is mostly anoxic, hypoxic conditions are commonly found (Borin et al., 2009). Chioggia sandy sediment is in fact more exposed to the effect of winds, tidal currents, and ship movements, that favor partial oxygenation (Borin et al., 2009). Permeable sandy sediment pore space is constantly flushed by the overlying water and could trap detritus and living cells from the water column (Boudreau et al., 2001, Gobet et al., 2012). In the other sites, the higher amount of organic matter contributes to the oxygen depletion and to the activity of sulphate-reducing bacteria in sulphide production (Zaggia et al., 2007). This is further supported by the metagenomics analysis, which also highlighted a significantly lower number of genes related to sulphur metabolism in Chioggia.

3.6 Sediment functional characterization (metagenome dataset)

A total ~120 million of raw reads (30 Gbp) were generated, specifically 14.8 ± 3.7 millions of paired-end reads with quality score of 35 ± 2 for each metagenome. On average, contigs derived from the three co-assembled replicates for each sample were 5,208,341 ± 376,210 contigs > 1.000 bp, and 652,755 ± 174,565 coding sequences.In the KEGG functional characterization, the most represented level 1 categories were Metabolism (63.2 ± 0.4 % on the overall dataset), Genetic Information Processing (17.0 ± 0.2 %), and Environmental Information Processing (14.2 ± 0.5 %); at level 2, Amino acid metabolism (21.5 ± 0.6 %), Carbohydrate metabolism (12.9 ± 0.3 %), and Translation (7.9 ± 0.1 %); at level 3, ATP-binding cassette (ABC) transporters (6.3 ± 0.4 %), Two-component system (4.7 ± 0.2 %), and Aminoacyl-tRNA biosynthesis (4.2 ± 0.1 %).

In the SEED functional characterization, the most represented level 1 categories were Clustering-based subsystems (14.7 ± 0.1 %; functional coupling evidence with unknown function), Carbohydrates (11.0 ± 0.3 %), Amino Acids and Derivatives (8.8 ± 0.1 %), and Protein metabolism (8.6 ± 0.1 %), whereas at the level 2, Plant-Prokaryote DOE project (6.8 ± 0.1 %), Protein biosynthesis (5.3 ± 0.1 %), and Central carbohydrate metabolism (3.7 ± 0.1 %).

3.7 Anthropogenic-related pressures influence benthic microbial communities

DNA metabarcoding and metagenomics allowed the exploration of microbial features related to different anthropogenic pressures, of particular importance in the monitoring of fecal and sewage bacteria, antibiotic and metal resistant microbes.

The use of DNA-based approach is increasingly applied to microbial profiling in wastewater plants, coastal areas, and in tracking and identifying microbial signatures in the environment; such studies contributed to the identification of bacterial taxa associated with sewage- and fecal- contamination (Newton et al., 2013, Tan et al., 2015). Marine sediments are a reservoir of fecal bacteria (Luna et al., 2010) and have the potential to favor the persistence of fecal microbes and to contaminate the overlying water through resuspension, which likely poses important public health and environmental threats (Luna et al., 2016).

Microbial signatures of selected anthropogenic-related taxa (Figure 10) presented overall 0.1 - 0.3 % relative abundance for fecal-associated bacteria and 0.01 - 0.04 % for sewage-associated bacteria. Sacca Sessola and Chioggia had a higher percentage of fecal-associated taxa, being mostly affected by urban contamination (Picone et al., 2017). For the sewage-associated bacteria, Marghera showed on average the highest proportion of reads due to *Acinetobacter* and *Trichococcus* while Palude della Rosa, considered less impacted by human activities, showed the lowest proportion.

Overall, we detected several indicators of fecal and sewage contamination (Figure10), and their relative abundance and taxonomic assignment are in line with a previous study in central part of the Venice Lagoon (Luna et al., 2016), confirming a diffuse contamination and accumulation of these bacteria in the Lagoon sediment.

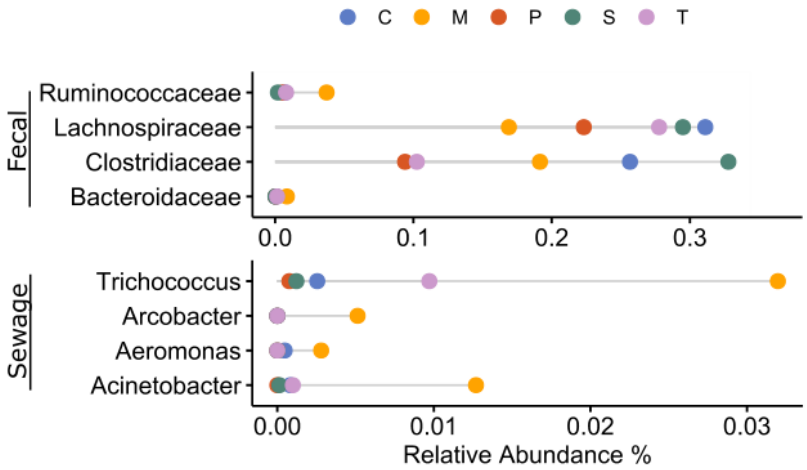

Figure 10. Average relative abundance of fecal- and sewage-associated taxa in the sampling sites. C: Chioggia; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

Within metagenomics analysis, in SEED database the "Virulence, Disease and Defense" category was significantly higher in the two more impacted sites of Marghera and Tresse in respect to the other sites (3.4 ± 0.02 % *vs* 3.0 ± 0.03 %), mainly due to a significantly higher presence of genes in the "Resistance to antibiotics and toxic compounds" group (3.1 ± 0.04 % *vs* 2.7 ± 0.03 %).

Antibiotic resistance spread is one of the main threats to global human, fish, shellfish, and in overall ecosystem health (Yang et al., 2012, Ventola 2015, Mackenzie and Jeggo, 2019, Helsens et al., 2020). For this reason, the detection, tracking, and assessment of the increasing antibiotic resistant microbes and genes, is necessary in terms of risk management (Heß et al., 2018). The marine environment is considered a global reservoir of antibiotic resistance genes, but we still have little understanding of their presence and diversity in both pelagic and benthic environments (Chen et al., 2013, Hatosy and Martiny, 2015). Several antibiotic resistance genes were detected in our dataset (Figure 11), with a significantly higher presence in the two more impacted sites of Marghera and Tresse. The Multi-drugs and Beta-lactamase resistance genes were found as the most abundant at all sites (0.9 ± 0.01 % and 0.6 ± 0.03 % respectively)(Figure 11), as previously detected in seawater (Hatosy and Martiny, 2015) and sediment (Huang et al., 2019).

Several toxic compound resistance genes were also detected (Figure 11). Cobalt-zinc-cadmium was the most present at all sites (0.06 ± 0.002 %). Marghera and Tresse sites presented the highest number of genes related to Mercury resistance Tresse (0.009 ± 0.00009 % *vs* 0.004 ± 0.002), due to the release of such metals from industrial plants. In particular, the mercury contamination mainly originates from the chloralkali discharge in Porto Marghera, which occurred until the 1980s (Bloom et al., 2004). Mercury is one of the most severe pollutants, and its flux to Venice Lagoon water is mostly due to the resuspension of contaminated sediment (Bloom et al., 2004), underlying the importance of its regulated management. Chioggia and Palude della Rosa presented the highest number of genes coding for Arsenic resistance (0.22 ± 0.002 % *vs* 0.20 ± 0.004). Even if the presence of this contaminant in the lagoon is mainly due to the geochemical characteristics of the sediments, human activities can contribute to its accumulation (Zonta et al., 2018).

Surface sediment metal contamination is another consequence of anthropogenic pressure in transitional environments, representing a potential threat to both public health and marine ecosystems (Han et al., 2011). Metals not only accumulate in the sediment but can be subjected to complex cycling that can make toxic compounds available to benthic organisms; sediment mobilization and resuspension under particular pH and redox conditions may release them in the water column becoming a secondary source of pollution (Eggleton and Thomas, 2004, Turner and Millward, 2002, Zonta et al., 2018). Furthermore, microbes that are antibiotic resistant can carry information for metal resistance and *vice versa*, thus exacerbating the problem of resistance spreading (Chen et al., 2019).

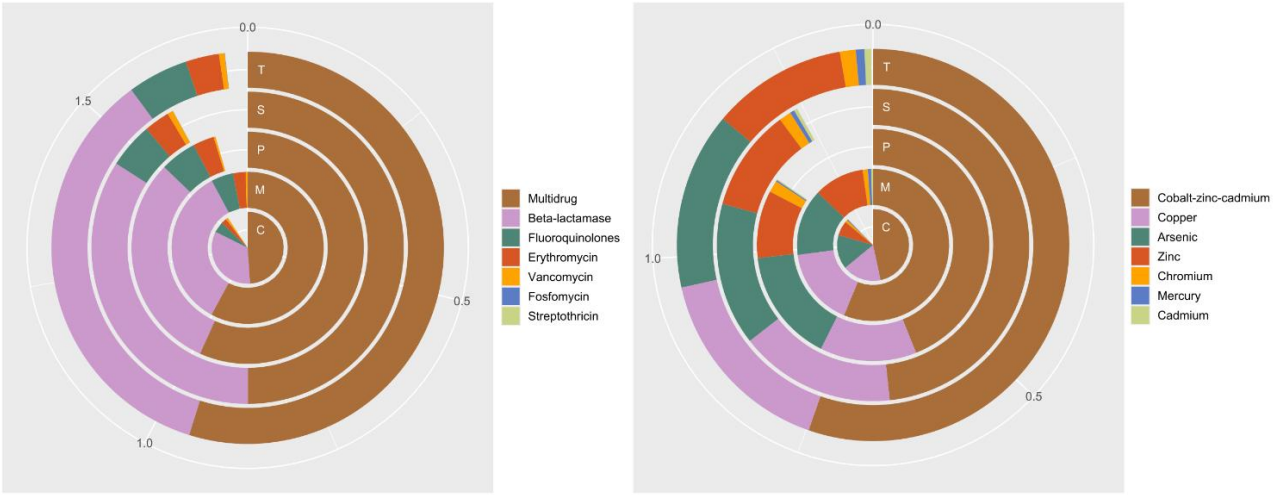

Figure 11. Average relative abundance of genes coding for resistance to antibiotics (left panel) and toxic compounds (right panel) in the sampling sites. C: Chioggia; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse.

3.8 Ecosystem-level functions

Sediment bacteria play a key ecological and biogeochemical role in marine ecosystems. They present high abundance and genetic variability, critical role in the transformation and speciation of major bioactive

elements (*e.g.,* carbon, nitrogen, phosphorus, oxygen, and sulphur), and in the degradation of organic pollutants (Polymenakou et al., 2005, Wu et al., 2008). The metagenomics approach adopted in this study allowed the identification of the genes involved in biogeochemical cycles providing insights into the potential metabolic functioning of the microbial communities (Doney et al., 2004, Dombrowski et al., 2018, Acinas et al., 2021). Following the "carbon currency" in the microbial sediment compartment we were able to map the carbon fixation processes, the energy and metabolism processes, the carbon-nutrient coupling, the carbon reworking processes, which includes the degradative processes, and the behavior and anthropogenic-related metabolisms (Figure 12).

To do so, key oceanic marker genes (Sunagawa et al., 2015) and key sediment metabolic genes (Dombrowski et al., 2018) have been selected and clustered as follows: Carbon fixation, Energy and metabolism, and Carbon-nutrient coupling.

The category of Carbon fixation included: oxygenic photosynthesis, anoxygenic photosynthesis, and other autotrophic pathways (Wood-Ljungdahl, Hydroxypropionate bicycle, hydroxypropionate/hydroxybutyrate, C1-metabolism). The category of Energy and metabolism included: aerobic respiration, fermentation, manganese-related metabolism, nitrogen metabolism, iron-related metabolism, sulfur metabolism, methanogenesis, butane metabolism, and hydrogenase. The category of Carbon-nutrient coupling included: phosphorus metabolism, peptide degradation, motility and chemotaxis, transport, and fatty acids degradation. We also assessed Anthropogenic-related metabolisms regarding hydrocarbons and xenobiotics (Figure 12).

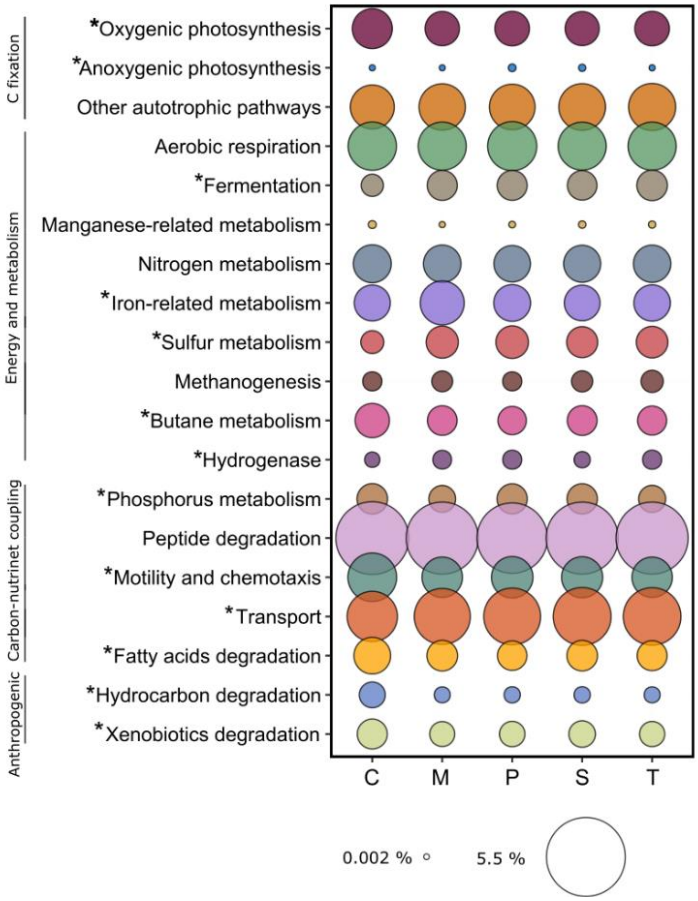

Figure 12. Average relative abundance of functions related to ecosystem-scale processes identified in the sampling sites. C: Chioggia; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse. Asterisks indicates significant differences among sites.

Overall, in the sediment microbial environment, Peptide degradation was the most represented category (4.5 ± 0.1 %), followed by Transport (2.7 ± 0.1 %), Aerobic respiration (1.8 ± 0.03), Other autotrophic pathways (1.7 ± 0.09 %), and Motility and chemotaxis (1.3 ± 0.09 %). Here we present the significant differences detected in the above-mentioned categories across the sites (Figure 12). Within Carbon fixation, Oxygenic photosynthesis was higher in Chioggia (0.9± 0.02 % *vs* 0.8 ± 0.01 %), while Anoxygenic photosynthesis was higher in Palude della Rosa (0.008 ± 0.001 % *vs* 0.003 ± 0.002 %).

Within Energy and metabolism, Chioggia presented a lower number of genes coding for functions related to Fermentation (0.5 ± 0.03 % *vs* 0.7 ± 0.03 %), Sulphur metabolism (0.6 ± 0.01 % *vs* 0.7 ± 0.03 %), and Butane metabolism (0.7 ± 0.01 % *vs* 0.6 ± 0.03 %) in respect to the other sites. Marghera was higher in Iron-related metabolism (1.1 ± 0.03 % *vs* 0.9 ± 0.03 %) whereas Palude della Rosa, Tresse and Marghera harboured the highest number of Hydrogenase genes (0.2 ± 0.005 % *vs* 0.1 ± 0.01 %). Within the Carbon-nutrient coupling category, Phosphorus metabolism functions were less present in Marghera and Tresse (0.6 ± 0.007 % and 0.7 ± 0.008 % respectively). Regarding Nitrogen metabolism, we detected significant amounts of genes coding for ammonia monooxygenase subunits (amoA, amoB, and amoC) in Chioggia in respect to the other sites (0.0007 ± 0.0003 % and 0.00008 ± 0.00002 % respectively), in accordance with the presence of *Candidatus Nitrosopumilus* (Walker at al., 2010, Bayer et al., 2019). This sandy site was significantly different from the others for Motility and chemotaxis (1.5 ± 0.2 % *vs* 1.3 ± 0.3 %, flagellum-related genes), and Transport (2.5 ± 0.1 % *vs* 2.7 ± 0.08 %, lipid and vitamin). Chioggia presented the highest number of genes related to Fatty acid degradation (0.7 ± 0.001 % *vs* 0.6 ± 0.002 %).

In our sites, we found that oxygenic photosynthesis (by Prokaryotes) was the main carbon fixation pathway. Anoxygenic photosynthesis was also detected together with five additional carbon fixation pathways which differ in reducing compounds, energy source, and oxygen sensitivity of enzymes alternative pathways (Wood-Ljungdahl, Hydroxypropionate bicycle, hydroxypropionate/hydroxybutyrate, C1-metabolism). These findings highlighted the metabolic flexibility of the community in fixing CO_2 from different sources thus suggesting high diversity in C fixation modes in the sediment. Once the carbon is turned into biomass and organic carbon, it can be metabolized via aerobic and anaerobic respiration or fermentation. The top sediment is affected by the light diurnal cycle and by the presence of oxygen (as a by-product of photosynthesis and from the oxygenated seawater column) (Petersen et al., 1994, Nealson 1997). Aerobic respiration was the main pathway to respire the organic matter and secondly, anaerobic respiration (N, Mg, Fe, S; *i.e.* changing the terminal electron acceptor according to their availably, Nealson 1997) was detected. Furthermore, often understudied metabolisms, such as methanogenesis, butane, proton degradation (hydrogenase, with a great diversity of enzymes), and fermentation were also important in enriching the metabolic repertoire of the microbial communities across sites (Falkowski et al., 2008). Within the concept of shared metabolisms, cable bacteria (family Desulfobulbaceae, Kjeldsenet al., 2019) were present in our samples. These microbes are formed by centimeter long filaments that span from the oxic water-sediment interface to the sulphidic deeper sediment layer. Within their filament, they couple sulfide oxidation with oxygen or nitrate reduction via long-distance electron transport.

Sulphur-related genes were found in high abundance in our sites with the exception of the sandy site of Chioggia. This is possibly due to the sediment texture that is more permeable to water and oxygen thus causing the community metabolism to switch more often from anoxic to oxic functionalities. Sulphur is essential for building amino acids (*e.g.*, methionine and cysteine), cellular components (sulpholipids) and metabolites (DMSP) (Wasmund et al., 2017). It has a paramount role as electron acceptor (e.g., sulphate and thiosulphate) and electron donor (sulphite and elemental sulphur) (Jørgensen et al., 2019). We found genes related to sulphate-reduction and sulphur-oxidation processes that are coupled in the top centimeter of sediment. Overall, sulphur cycling is interconnected with the other elements, the availability of organic matter and redox species present in the lagoon system. In a high-temperature and low-oxygen lagoon scenario, sulphur-related metabolisms might become dominant in the ecosystem functioning.

Phosphorus-cycle-related genes were also highly abundant in our samples, being especially linked to the involvement of nucleic acids in biofilm structures (Karygianni et al., 2020) and organic P degradation. Furthermore, approximately 4.5 % of genes (KEGG annotation) in the metagenomes were addressed to

peptide degradation. Proteolysis is in fact the fastest hydrolytic activity within surface brackish and coastal sediments, aiming to provide prokaryotes with both organic C and N via amino acids, as highlighted by direct measurements (*e.g.*, Cibic et al., 2012, Franzo et al., 2019). Together with carbohydrates, proteins and lipids are indeed the major constituents of the labile organic matter in the sediments (Pusceddu et al., 1999) and microbes are adapted to utilize these compounds heterotrophically (Oni et al., 2015), thus supporting the concept of sediments as hot spots for organic matter degradation (Lipka et al., 2018).

In the Anthropogenic-related metabolisms category, Hydrocarbon degradation was significantly higher in Chioggia (0.2 ± 0.009 % *vs* 0.1 ± 0.003 %), mostly due to hydrolases involved in the degradation of chlorocyclohexane, chlorobenzene fluorobenzoate, including toluene. The Xenobiotic metabolism and degradation in the same site was also significantly higher (0.5± 0.05 % *vs* 0.4 ± 0.002 %), mostly due to haloalkane dehalogenase (dhaA). Interestingly, in this site, both surface and sub-surface sediment were recently found to harbor the highest concentrations of PAH and PBC in the whole Venice Lagoon (Cassin et al., 2018), due to the important urban waste discharge, road/boat traffic, and being located close to the harbor of the second Italian largest fishing fleet. It was worth noting that among the identified hydrocarbon degraders some taxa were also able to utilize lignin. Lignin is a very complex and hard-to-degrade polymer (Hedges et al., 1979) and Bacteria, Archaea and Eukaryotes have evolved adaptive strategies to gain energy from it (Cragg et al., 2015). In the Venice Lagoon, wood has been historically and extensively used for maritime needs, like mooring piles and navigation channel delimitation (named “briccole”, approximately 7,000, formed by two or three poles) (Ghirardini et al., 2010). Briccole are subjected to physical and biotic degradation, the latter mainly due to marine borers (Ghirardini et al., 2010). Since our data highlighted that the sediment represents a reservoir of potential lignin-degrader bacteria, we hypothesize a role of these taxa (alone or in consortia with other microorganisms) in the turnover/recycling of human-introduced wood structures in the Lagoon.

3.9 Microscale-level functions

Within this framework of reference, we have mined our data set to test hypotheses on the putative functional microscale behavior of the microbes in the top centimeter of lagoon sediments (Figure 13). Starting from DNA, we have asked questions about whether the community was able to display adaptive strategies such as competence and gene exchange to improve their persistence in the environment. Among the mobile genetic elements, what were the most abundant, and was it connected with the site features?

To do so, we have selected the key specific microscale marker functions, identified with the SEED database, and clustered as follows: DNA metabolism (CRISPs and DNA uptake and competence), Mobilome (Gene Transfer Agents, integrons, pathogenicity islands, phages and prophages, plasmids, and transposable elements), Regulation and Cell Signaling (biofilm formation, phenazine biosynthesis, osmotic stress, sporulation, siderophores, toxic-antitoxic systems, and two-component systems) and Nanomachines (protein secretion system Type IV and Type VI) (Figure 13).

Overall, in the sediment microbial environment, Phages and prophages was the most represented category (0.6 ± 0.07 %), followed by Osmotic stress (0.27 ± 0.01 %), Pathogenicity islands (0.26 ± 0.01), Toxic-antitoxic systems (0.13 ± 0.02 %), Transposable elements (0.12 ± 0.09 %), and Two-component systems (0.01 ± 0.09 %) (Figure 13).

Here we present the significant differences detected in the above-mentioned categories across the sites. Within Mobilome, Phages and prophages and Transposable elements were lower in Chioggia (0.47 ± 0.02 % *vs* 0.62 ± 0.04 % and 0.1± 0.006 % *vs* 0.12 ± 0.007 % respectively). Within Regulation and Cell Signaling, Biofilm formation was higher in Marghera, Sacca Sessola, and Tresse (0.04 ± 0.005 % *vs* 0.02 ± 0.006), Osmotic stress was higher in Chioggia, Palude della Rosa, and Sacca Sessola (0.28 ± 0.01 % *vs* 0.25 ± 0.01), and Toxic-antitoxic systems was lower in Chioggia (0.08 ± 0.01 % *vs* 0.14 ± 0.01). Within Nanomachines, Protein secretion system type VI was lower in Chioggia (0.03 ± 0.006 % *vs* 0.06 ± 0.008 %).

DNA is an integral part of the sediment, carrying genetic information and providing 3D structure by binding to other molecules (Dell'Anno and Corinaldesi, 2004, Torti et al., 2015, Corinaldesi et al., 2008). It is known that viruses are abundant in the sediment and are major structuring agents, keeping the microbial community under check (Danovaro et al., 2001, Breitbart et al., 2004, Breitbart and Rohwer, 2005). Among the microbial defense systems (Makarova et al., 2013), we detected the CRISPR-Cas (Doudna et al., 2014) associated genes at all the stations, despite the sediment grain size. This suggests that there is a persistent strategy of Bacteria and Archaea to fight off the viral invasions using this adaptive immunity against foreign genetic elements while creating a viral data bank for further viral encounters. Recent studies showed that in Global Ocean Sampling Expedition (GOS) and Tara Ocean data set, CRISPR-Cas were highly diverse and specific to the viral architecture (Cai et al., 2013, Nasko et al., 2019). In addition to CRISPR-Cas, Competence and Sporulation related functions were found throughout the Lagoon. DNA can be taken up during the competence window (Lennon 2007, Dubnau and Blokesch, 2019), while a population of microbes is undergoing major environmental stress such as oxygen depletion/enrichment or nutrient scarcity. Among that population, a few cells can promote the lysis of the other individuals exploiting the released DNA for ensuring success in the future. Temporally subsequent to competence is the sporulation, an important unique microbial mechanism in marine sediment for surviving burial (Wörmer et al., 2019, Morono et al., 2020).

An ability that microbes use to move information relies on the highly diverse architecture of the mobile genetic elements, the Mobilome (Gillings 2013, Carr et al., 2021). Prophages and Transposons were lower in Chioggia, possibly due to the site features such as higher water flow and lower TOC that might select against these mobilome strategies. Overall, mapping the sediment mobilome could give insights on rapid adaptation processes to the presence of pollutants in marine habitat due to anthropogenic activities.

Within the Regulation and cell signaling and Nanomachines categories, we have mined and detected genes related to physiology, adaptations and interactions of the microbial communities and consortia in the sediment. Biofilm in the marine environment is a pervasive structuring feature (de Carvalho 2018). Biofilm formation is essential in keeping the microenvironment stable for the establishing of the microbial communities thus providing protection from grazers and phages, toxic substances (*e.g.,* antibiotics), and accumulating nutrients (Flemming et al., 2016). Toxin-antitoxin (TA) systems regulate dormancy and persistence in microbes (Spoering and Lewis, 2001, Keren et al., 2004, Shah et al., 2006). TA genes are abundant on plasmids, phages, and chromosomes. They are formed by a toxin, that arrests growth by interfering with a vital cellular process, and a cognate antitoxin, that neutralizes the toxin activity to resume normal growth (Page and Peti, 2016). TA plays a major role in supporting microbial resistance to antibiotics by developing a subpopulation of persistent cells. It is interesting to note that in Chioggia, biofilm and TA were lower than the other sites, suggesting that these strategies were not favored in a sandy sediment habitat and or were not so successful as in the other sites. Despite it is known that in sandy sediments, microbes colonize grains via biofilms (Probandt et al., 2018**)**, the biofilm might be more widespread in muddy sediment to create an organic bridge among clay and silt particles.

In terms of competition for space, nutrient, energy sources and ability to resist osmotic stress, phenazine (Price-Whelan et al., 2007, Schiessl et al., 2019), siderophores (D'Onofrio et al., 2010, Sandy and Butler, 2010), two-component systems (Held et al.,2019), osmotic stress genes, and nanomachines for cell-cell warfare were identified in the data set. Microbes constantly under siege could express phenazine, a redox-active pigment produced by *Pseudomonas aeruginosa* that affect gene expression, metabolic flux, and redox balancing to promote their success. *Pseudomonas* despite being a multi-drug resistant, biofilm former, quorum sensing human pathogenic microbe is highly versatile and has been found in the marine environment (Kimata et al., 2004). Siderophores are structures produced by microbes that chelate ions, such as Fe to facilitate the acquisition of the insoluble species thus harshening the competition for this element. We identified, within the two-component signal transduction systems (Yamamoto et al., 2005) single transduction for anaerobic respiration, nitrate and nitrite utilization, nitrogen assimilation metals efflux system, fumarate respiration, outer membrane proteins, phosphate assimilation, magnesium transport, and osmotic and envelop stress response, chemotaxis modulation, and flagellar motor switch. This great diversity found in the sediment microbial communities highlighted the adaptive strategies that microbes need to

exploit while sensing the external environment at the microscale in order to activate genes that promote their success in the microniche. In the water column, a high abundance of these regulative network systems was found to be linked to copiotrophy and diazotrophy (Held et al.,2019).

The last microscale features we have identified in our dataset were the Type IV (Craig et al., 2019) and Type VI (Cianfanelli et al., 2016) secretion nanomachines, that are involved in cell-cell interactions by allowing nucleic information being shuttle from one individual to the other and attaching with effectors the other organisms. Type IV was found in microbes from acetate-stimulated aquifer sediments (Kantor et al., 2013), thus suggesting conjugation being potentially important to exchange genes and associated metabolic potentials. In the ocean, Type VI secretion systems were found to be globally distributed and associated with particle-attached microbes (Kempnich et al., 2020).

Finally, motility and chemotaxis patterns, highly abundant in Chioggia, suggested a specific strategy in sandy sediments, that are more permeable to water in comparison to clayey and silty one and where a more diverse microenvironment can develop within the grains.

In sum, microscale-related genes highlight a very elaborated fine structure of interactions and behavior in the top sediment microbial communities that then results in the large-scale biogeochemical processes on the lagoon system.

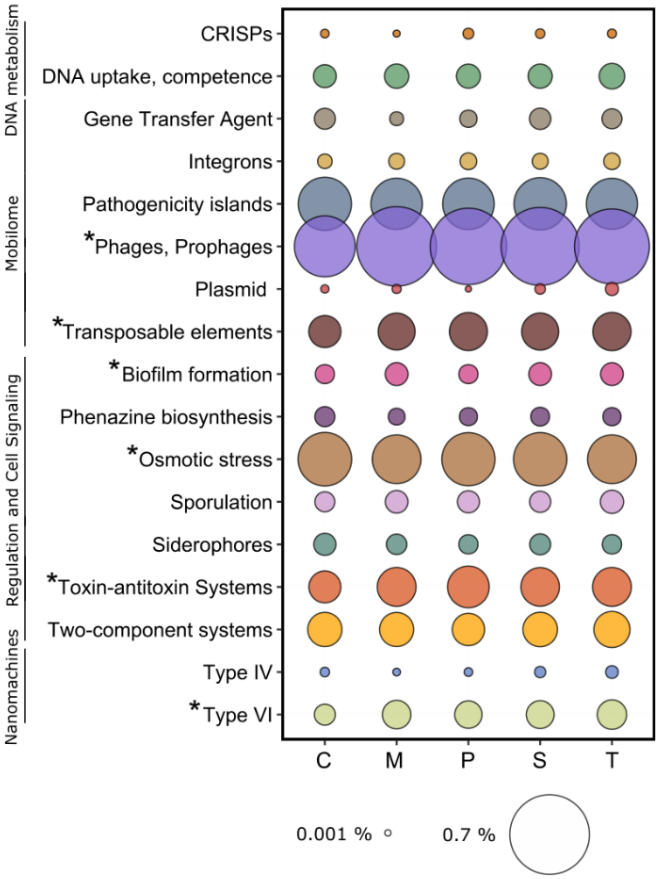

Figure 13. Average relative abundance of functions related to microscale processes identified in the sampling sites. C: Chioggia; M: Marghera; P: Palude della Rosa; S: Sacca Sessola; T: Tresse. Asterisks indicates significant differences among sites.

4 Sediment fluxes

4.1 Bacterial and viral abundance in overlaying water

The presence of the cyanobacterium *Synechococcus* (Figure 14) is indicator of the influence of marine water origin. The abundance data agreed with those found in the literature for the northern Adriatic and the Venice Lagoon (Lasserre and Marzollo 2000, Schmid 2012). *Synechococcus* abundances showed a seasonal trend during 2019 in Sacca Sessola and Tresse. Minima were recorded in Spring at both Sacca Sessola and Tresse, equal to $4.24 \pm 0.42 \times 10^5$ Cells L^{-1} and $5.80 \pm 0.77 \times 10^5$ Cells L^{-1} respectively. While Sacca Sessola showed a progressive increase in Summer and Autumn, Tresse had a peak in Summer and then a decrease in Autumn.

The abundance of picoeukaryotes, small eukaryotic microalgae (Figure 14), showed a general seasonal trend during 2019. Maxima were recorded in Summer for Tresse and during Autumn for Sacca Sessola. In Winter and Spring, these two sites had comparable trend. Sacca Sessola progressively increased until reaching $4.29 \pm 0.39 \times 10^7$ Cells L^{-1} in Autumn, while Tresse had a peak in Summer with $6.03 \pm 1.36 \times 10^7$ Cells L^{-1} and then decrease in Autumn.

Heterotrophic bacterial abundances (Figure 14) showed similar trends at Tresse and Sacca Sessola during 2019, with maxima in Summer ($8.48 \pm 0.38 \times 10^9$ Cells L^{-1} in Sacca Sessola and $5.59 \pm 0.16 \times 10^9$ Cells L^{-1} in Tresse) and minima in Winter ($1.92 \pm 0.17 \times 10^9$ cell L^{-1} in Sacca Sessola and $1.54 \pm 0.25 \times 10^9$ cell L^{-1} in Tresse). The high seasonal signature of prokaryotic communities was in agreement with other studies in this area (Celussi et al., 2009, Quero et al., 2017).

Viral abundances (virus-like particles VLPs, Figure 14) data showed seasonality and similar trends could be observed in Sacca Sessola and Tresse during 2019. Maxima were recorded in Summer ($8.6 \pm 0.52 \times 10^{10}$ Cells L^{-1} at Sacca Sessola and $6.36 \pm 0.382 \times 10^{10}$ Cells L^{-1} in Tresse) and minima in Autumn ($3.26 \pm 0.38 \times 10^{10}$ Cells L^{-1} in Sacca Sessola and $2.42 \pm 0.31 \times 10^{10}$ Cells L^{-1} in Tresse). VLPs data were in agreement with the literature for both abundances and seasonal pattern (Weinbauer et al., 1995, Breitbart and Rohwer 2005, Suttle 2005, Gainer et al., 2017). Generally speaking, in marine waters, VLPs are 10 times more abundant than heterotrophic prokaryotes.

In Spring 2021, Sacca Sessola (S) and Pili (L) sites were sampled. They showed comparable values for *Synechococcus*, picoeukaryotes, and VLPs in comparison with Tresse and Sacca Sessola in 2019 for the same Season, while they presented a lower abundance of heterotrophic bacteria (Figure 14).

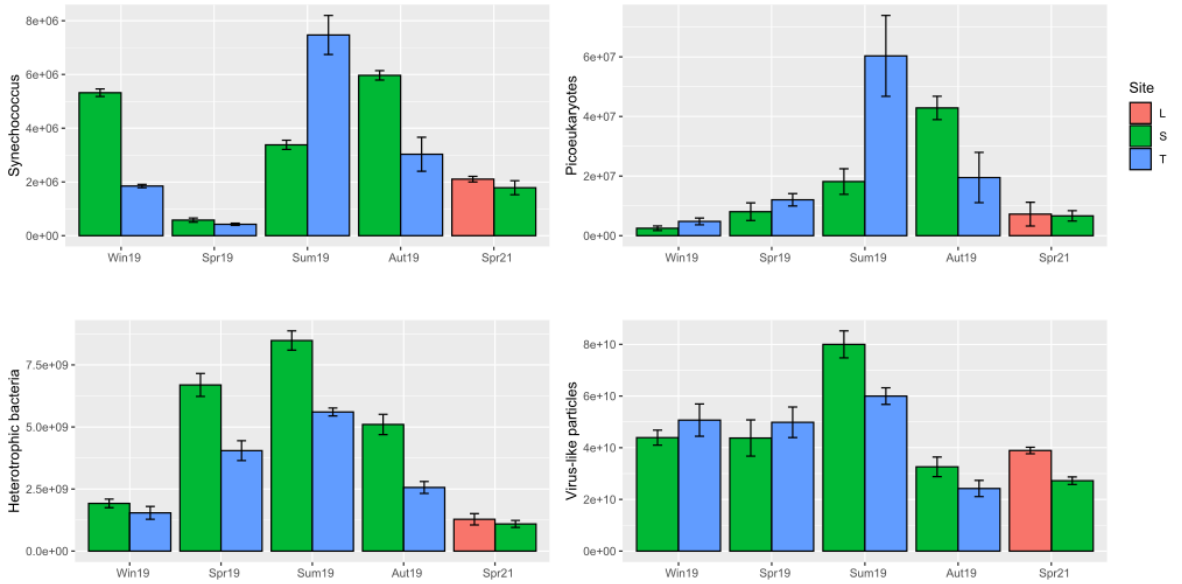

Figure 14. Prokaryotic (Cells L^{-1}), picoeukaryiotic (Cells L^{-1}), and Virus-like (particles L^{-1}) abundance quantified by flow cytometry in overlaying water. L: Pili, S: Sacca Sessola; T: Tresse. The error bars represent the standard deviation of the three replicates.

4.2 DOC in overlaying and pore water

For overlaying water, the dissolved organic carbon (DOC) content (Table 6) had higher values than those commonly measured in the seawater (~ 1.0 mg L^{-1}), but in the range measured in the Venice Lagoon by Martin et al. (1995) and Giani et al. (2008). Pore water values (Table 6) were more variable, and higher than those presented in the work of Sommerfreund et al. (2010) in the Venice lagoon.

Table 6. Dissolved organic carbon (DOC; mg L^{-1}) in overlaying water (OW) and pore water (PW). L: Pili; S: Sacca Sessola; T: Tresse.

Site	Season	Year	OW	PW
L	Spr	2021	2.85 ± 0.04	5.011 ± 0.02
	Spr	2021	3.5 ± 0.02	5.78 ± 0.01
S	Win	2019	2.13 ± 0.01	109.1 ± 0.98
	Spr	2019	2.41 ± 0.01	36.65 ± 0.22
	Sum	2019	3.33 ± 0.02	14.28 ± 0.45
	Aut	2019	3.27 ± 0.03	5.52 ± 0.01
	Spr	2021	3.39 ± 0.04	7.315 ± 0.03
	Spr	2021	3.4 ± 0.04	5.973 ± 0.02
T	Win	2019	1.82 ± 0.01	37.29 ± 0.63
	Spr	2019	2.47 ± 0.01	59.8 ± 0.40
	Sum	2019	3.09 ± 0.05	8.38 ± 0.10
	Aut	2019	3.23 ± 0.06	20.25 ± 0.10

4.3 Dissolved inorganic nutrients in overlaying and pore water

The dissolved inorganic nutrients concentrations (Table 7 and Table 8) were in the range measured in the Venice Lagoon by Acri et al. (2004), Zirino et al. (2006), Celussi et al. (2009).

The concentration of P-PO_4^{3-} and N-NH_4^+ presented positive correlation both in overlaying (0.76, $p < 0.05$) and pore water (0.85, $p < 0.001$), respectively. This was previously detected in other shallow water ecosystems (De Vittor at al., 2016). The linear relationship indicates that porewater P-PO_4^{3-} is released in relation to ammonia by organic matter decomposition (Ogrinc and Faganeli 2006).

Table 7. Dissolved inorganic nutrients (µmol L^{-1}) quantified in overlaying water. L: Pili; S: Sacca Sessola; T: Tresse.

Site	Season	Year	NO_2^-	NO_3^-	PO_4^{3-}	NH_4^+	$Si(OH)_4^{4-}$
L	Spr	2021	2.72	15.60	0.28	34.56	41.75
	Spr	2021	2.74	15.54	0.27	34.71	41.20
	Spr	2021	1.33	6.83	0.25	31.84	38.70
	Spr	2021	1.35	6.85	0.26	32.03	38.11
S	Win	2019	0.41	7.61	0.15	3.9	10.83
	Spr	2019	1.87	24.84	0.07	3.94	17.99
	Sum	2019	0.16	1.06	0.11	1.29	17.24
	Aut	2019	1.21	7.02	0.17	7.11	16.55
	Spr	2021	0.87	8.45	0.20	6.23	20.16
	Spr	2021	0.88	8.38	0.21	6.19	20.21
	Spr	2021	0.85	6.38	0.38	4.62	14.29
	Spr	2021	0.71	5.93	0.16	4.43	14.63
T	Win	2019	0.69	11.92	0.35	5.47	8.35
	Spr	2019	2.79	38.92	0.81	9.83	46.69
	Sum	2019	0.78	4.08	2.47	38.51	42.80
	Aut	2019	1.21	7.02	0.17	7.11	16.55

Table 8. Dissolved inorganic nutrients (µmol L^{-1}) quantified in pore water. L: Pili; S: Sacca Sessola; T: Tresse.

Site	Season	Year	NO_2^-	NO_3^-	PO_4^{3-}	NH_4^+	$Si(OH)_4^{4-}$
L	Spr	2021	0.18	0.21	1.36	68.92	75.69
	Spr	2021	0.20	1.12	2.90	213.88	191.27
S	Win	2019	0.38	2.53	0.53	31.70	70.47
	Spr	2019	0.43	6.78	0.82	70.452	83.98
	Sum	2019	0.26	5.58	0.54	48.6	61.13
	Aut	2019	0.24	4.62	0.36	52.39	88.06
	Spr	2021	0.14	0.87	1.04	56.54	56.98
	Spr	2021	0.19	2.91	0.36	84.16	70.19
T	Win	2019	0.37	1.64	1.56	64.57	49.70
	Spr	2019	0.42	4.24	2.44	89.89	117.9
	Sum	2019	0.16	2.76	5.1	290	177.04
	Aut	2019	0.44	0.93	5.08	152.25	170.35

4.4 Diffusive benthic fluxes

Within the sediment-water exchange, it is of paramount importance to estimate of diffusive fluxes of inorganic nutrients from sediment (pore water) to overlying water. (Covelli et al., 2008). Sediment cores were collected and two horizons were sampled and processed to extract the pore water (Bertuzzi et al., 1996, Bertuzzi et al., 1997). The concentration gradients for the solutes thus obtained were used for the theoretical calculation of diffusive fluxes through the water-sediment interface (Covelli et al., 2008, De Vittor et al., 2012).

The calculated diffusive fluxes in our samples are reported in Table 9.

Table 9. Diffusive fluxes (µmol m^{-2} d^{-1}) of dissolved inorganic nutrients.

Site	Season	Year	NO_2^-	NO_3^-	PO_4^{3-}	NH_4^+
L	Spr	2021	-11.41	-71.09	1.50	158.72
	Spr	2021	-7.26	-39.34	5.33	1254.27
S	Win	2019	-0.23	-39.79	0.91	217.77
	Spr	2019	-6.60	-88.18	1.07	324.77
	Sum	2019	0.40	-20.00	0.55	209.29
	Aut	2019	-5.49	-14.44	0.34	272.36
	Spr	2021	-2.31	-24.14	0.80	161.88
	Spr	2021	-2.13	-12.02	0.02	275.62
T	Win	2019	-1.05	-34.08	1.22	195.94
	Spr	2019	-10.78	-167.89	2.32	387.57
	Sum	2019	-2.71	-6.34	3.62	1207.43
	Aut	2019	-3.42	-28.82	6.82	686.83

Following the concentration gradient, in all samples the flux of $P\text{-}PO_4^{3-}$ and $N\text{-}NH_4^+$ occurred from the sediment pore water to the overlying water. On the other hand, the flux of $N\text{-}NO_2^-$ and $N\text{-}NO_3^-$ occurred from the overlying to the pore water. $N\text{-}NO_3^-$ influxes have already been reported (Al-Rousan et al. 2004, De Vittor et al., 2012) and related to the fact that $N\text{-}NH_4^+$ is the first inorganic product in the regeneration of nitrogenous organic material by microorganisms (De Vittor et al., 2012). The high fluxes of $N\text{-}NH_4^+$ and $P\text{-}PO_4^{3-}$ could be related to the sediment anoxic conditions, that increase the release rate of these species from sediments to the overlaying water (Al-Rousan et al., 2004), and to the dependence of phosphate and ammonium regeneration on the quality of organic matter in the sediment (Clavero et al., 2000).

5 Conclusions

Overall, our data showed a clear spatial distribution of the highly biodiverse top sediment centimeter microbial communities in the different sub-basins of the Venice Lagoon, mainly due to low abundant taxa. Chemical and physical features such as salinity, grain size, and TOC concentration were important drivers in shaping community structure, diversity, potential functions, and metabolisms.

In the perspective of an integrated and sustainable sediment management, our data contributed to a very fine characterisation of the microorganisms living in this environment, which is at the base of the Venice Lagoon ecosystem.

We were able to identify and track a widespread presence of microbes that are fecal- and sewage-associated and potential pathogens in the sediment upper layer. This kind of pollution represents a major problem in coastal and transitional areas and a potential threat to human health. We found the highest presence of fecal-associated bacteria in Chioggia, the site most affected by urban contamination. Sediment management should consider that mobilization could resuspend these cells in the water column and represent a secondary source of contamination.

Our analysis highlighted that chronically polluted areas (Marghera and Tresse) represent hotspots of resistance genes related to toxic compounds and antibiotics. In the view of sediment management, it should be considered that the mobilization of the sediment from these areas to other less impacted could lead to the spreading and accumulation of these traits. Such gene transmission can intensify the role of sediment as reservoir of resistance genes, negatively contributing to the already difficult control of bacterial infections in fish, shellfish, and humans.

In conclusion, this study showed that sediment contamination has vast and long-lasting effects on the microbial communities, that can affect also the higher trophic levels and the whole ecosystem including humans. For this reason, the reduction of sediment contamination should be of primary importance for the Lagoon ecosystem to reduce potential environmental and health risks.

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