


**ORIGINAL ARTICLE**

# Seasonal rather than spatial variability drives planktonic and benthic bacterial diversity in a microtidal lagoon and the adjacent open sea

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**Abstract**

Coastal lagoons are highly productive ecosystems, which are experiencing a variety of human disturbances at increasing frequency. Bacteria are key ecological players within lagoons, yet little is known about the magnitude, patterns and drivers of diversity in these transitional environments. We carried out a seasonal study in the Venice Lagoon (Italy) and the adjacent sea, to simultaneously explore diversity patterns in different domains (pelagic, benthic) and their spatio-temporal variability, and test the role of environmental gradients in structuring assemblages. Community composition differed between lagoon and open sea, and between domains. The dominant phyla varied temporally, with varying trends for the two domains, suggesting different environmental constraints on the assemblages. The percentage of freshwater taxa within the lagoon increased during higher river run-off, pointing at the lagoon as a dynamic mosaic of microbial taxa that generate the metacommunity across the whole hydrological continuum. Seasonality was more important than spatial variability in shaping assemblages. Network analyses indicated more interactions between several genera and environmental variables in the open sea than the lagoon. Our study provides evidences for a temporally dynamic nature of bacterial assemblages in lagoons and suggests that an interplay of seasonally influenced environmental drivers shape assemblages in these vulnerable ecosystems.

**KEYWORDS**

bacterial diversity, benthic, lagoon, planktonic, seasonality

**1 | INTRODUCTION**

Prokaryotes are key components within lagoons, due to their roles as primary producers (e.g., photoautotrophic bacteria) and agents of organic matter remineralization and particles degradation, cycling of biogeochemically relevant elements, pollutants degradation, and transfer of matter and energy to higher trophic levels. A number of studies investigated the relevance of functional prokaryotic processes in lagoons (Abreu, Biddanda, & Odebrecht, 1992; Manini et al., 2003; Pugnetti et al., 2010) while, surprisingly, their

biodiversity has been less studied, with the majority of studies published after the advent of ribosomal gene sequencing (Mohit, Archambault, & Lovejoy, 2015; Mohit, Archambault, Toupoint, & Lovejoy, 2014). Pioneer studies, carried out by cloning and sequencing of rRNA genes, revealed a high diversity (Benlloch, Rodríguez-Valera, & Martínez-Murcia, 1995). Other studies addressed the temporal and spatial patterns of lagoon planktonic prokaryotes (Celussi, Pugnetti, & Del Negro, 2009; LaMontagne & Holden, 2003; Piccini et al., 2006; Simonato, Gómez-Pereira, Fuchs, & Amann, 2010), while less studies addressed diversity in lagoon sediments (Mohit et al.,

2015; Tsuboi et al., 2013). To our knowledge, no studies have simultaneously investigated the diversity of planktonic and benthic assemblages within lagoons over spatial and temporal scales, nor have investigated the pelagic-benthic coupling, or compared microbial diversity in lagoons with the adjacent sea. Consequently, knowledge on the importance of variability in structuring lagoon microbial assemblages, necessary for the holistic understanding of the functioning of these fundamental ecosystems, remains limited.

The diversity of aquatic prokaryotes is shaped by an array of biotic and abiotic drivers. Advances in studying microbial dynamics are showing that their composition follows predictable patterns, which involves complex network interactions (Fuhrman, Cram, & Needham, 2015). The importance of seasonality as a structuring factor for coastal bacterioplankton has recently emerged (Alonso-Sáez et al., 2007; García, Alonso-Sáez, Morán, & López-Urrutia, 2015; Gilbert et al., 2012), while only few studies have investigated the temporal patterns in coastal and lagoon sediments (Böer et al., 2009; Gobet et al., 2012; Mohit et al., 2015), reporting strong seasonal dynamics of microbial communities in these ecosystems. Studies that compared the temporal dynamics in the pelagic and benthic domain at the same time are rare. Only Feng et al. (2009) explored simultaneously bacterial dynamics in water and sediments in an estuary of the China Sea, reporting contrasting patterns for the two domains. Understanding whether planktonic and benthic bacterial assemblages experience similar or different patterns, especially in transitional environments experiencing seasonal fluctuations in the hydrological conditions, is crucial to comprehend how these ecosystems function and to predict how microbes may help in buffering the increasing anthropogenic impact.

Here, we studied, over a one-year period that covered the four seasons, the diversity of planktonic and benthic bacterial assemblages in the Venice lagoon, the largest in Italy and one of the largest of the Mediterranean Sea. We used high-throughput sequencing (HTS) of 16S rRNA gene amplicons to test the hypotheses that (i) assemblages differ between environments (lagoon and open sea) and domains (pelagic and benthic); (ii) bacterial taxa exhibit spatial and/or temporal variability; (iii) different environmental variables drive the diversity patterns in the different environments and domains; and (iv) freshwater inputs from land play a role in structuring assemblages within the lagoon.

## 2 | MATERIALS AND METHODS

### 2.1 | Site description and sampling strategy

Surface water and sediments were collected in nine stations inside (hereafter defined "lagoon": INS, PORT1, WAT2, WAT3 and 7M) and outside ("open sea": WAT4, SEA1, SEA2 and PTF) the Lagoon of Venice, along the northeastern coast of Italy (Figure 1; details in Table S1). The lagoon covers a surface of 500 km<sup>2</sup> (length 50 km, mean width 15 km) and is connected with the adjacent sea by three inlets, ensuring water exchanges with the sea mainly governed by a microtidal regime (Solidoro et al., 2010), with mean ranges of 0.40 m

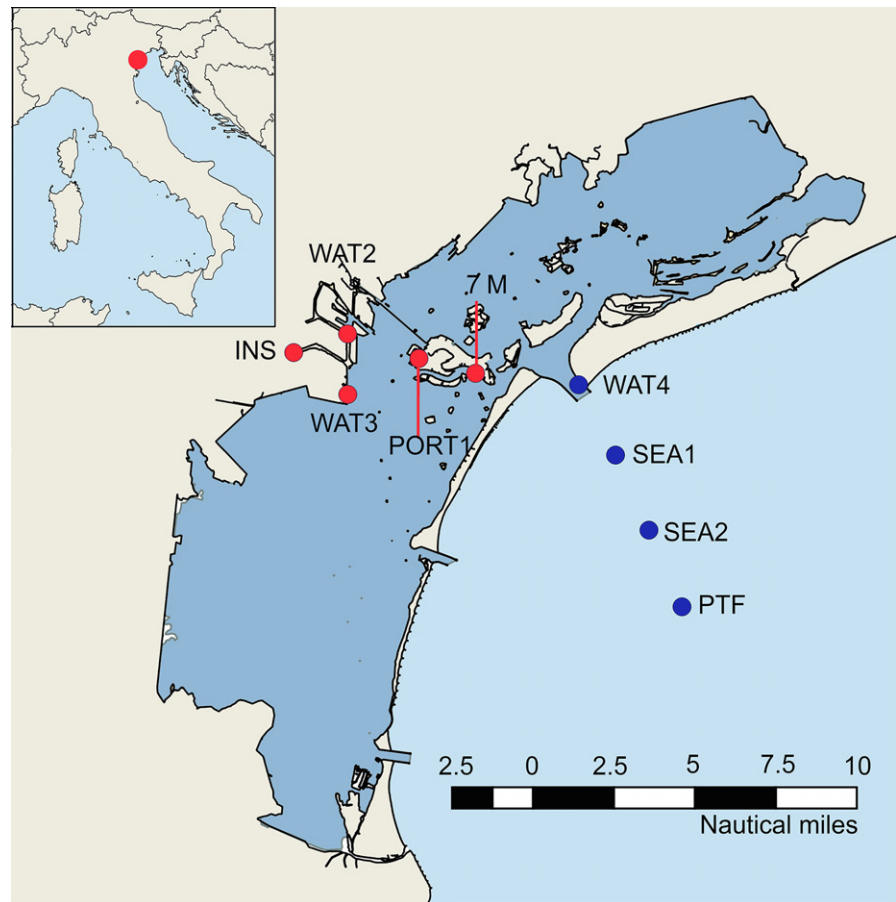
and 0.80 m during neap and spring tides, respectively (Molinarioli et al., 2009). A network of navigation channels connects the three inlets with the mainland and the city of Venice, situated on a group of islands in its northern part. Around 415 km<sup>2</sup> of the lagoon are subject to tidal excursion, with an exchange of water, in each tidal cycle, close to a third of the lagoon volume (Gačić et al., 2004). The freshwater input into the lagoon is around 30–35 m<sup>3</sup>/s from twelve small tributaries (Zuliani, Zaggia, Collavini, & Zonta, 2005). The lagoon represents a complex and human-impacted environment, due to the historical presence of a multitude of stressors such as industrial plants on the mainland, touristic and commercial harbours, agricultural and municipal wastes (Perini, Quero, García, & Luna, 2015). The depths of the sampling stations ranged from 5 to 16 m. Sampling activities were performed on 25th March, 20th–21st May, 21th–22th October 2014, and 11th–12th February 2015 (hereafter referred as "spring", "summer", "autumn" and "winter", respectively). More details are in the Appendix S1.

### 2.2 | Environmental variables

At all stations, temperature, salinity, dissolved oxygen (DO) and turbidity were measured using a CTD probe (Seabird 911, Sea-Bird Electronics, Washington, USA). The concentration of dissolved inorganic nitrogen (DIN, as the sum of ammonia, nitrates and nitrites), silicates, phosphates and chlorophyll-a was also measured. Details on the instruments and laboratory procedures are in Appendix S1. During the study period, the river inputs into the lagoon corresponded to 9 m<sup>3</sup>/s (spring), 8 m<sup>3</sup>/s (summer), 37 m<sup>3</sup>/s (autumn) and 20 m<sup>3</sup>/s (winter), calculated as the average discharge of the Dese river (the closest river to the sampling stations) in the 3 days that preceded each sampling event.

### 2.3 | Bacterial diversity

Seawater samples (1 L) were filtered onto 0.22 µm cellulose nitrate membrane filters (Sartorius) and stored at –20°C until processing. DNA was extracted from each filter using the PowerWater<sup>®</sup> DNA Isolation Kit and from one gram of each sediment sample using the PowerSoil<sup>®</sup> DNA Isolation Kit (both kits from MoBio Laboratories, California) for the analysis of water and sediment, respectively. We followed the manufacturer's instructions with few modifications to increase the DNA yield and quality (see Appendix S1). The DNA concentration was determined using a Qubit Fluorometer (ThermoFisher), and the DNA stored at –80°C until PCR. For HTS library preparation, the Illumina Nextera protocol was used (details in the Appendix S1) to obtain amplicon libraries of the V3-V4 regions of the 16S rRNA gene, which were sequenced on the Illumina MiSeq platform. The 341F and 785R universal bacterial primers (Table S2; Eiler, Heinrich, & Bertilsson, 2012) were used. Once the sequences were obtained, low-quality regions (Q < 20) of the paired-end reads were trimmed from the 5' end of sequences. The paired ends sequences were then joined with fastq-join (<https://expressionanalysis.github.io/ea-utils/>) with a minimum overlap of 10 bases, and



**FIGURE 1** Map showing the study site and the location of the sampling stations in the Venice lagoon and the adjacent Adriatic Sea. The red dots indicate the stations located within the lagoon, while the blue dots those located in the open sea. The map has been produced with the QGIS software (QGIS Development Team, 2016) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

analysed with QIIME v1.8.0 software package (Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2010). Reads were clustered into OTUs (Operational Taxonomic Units) using UCLUST v1.2.22 (Edgar, 2010) with a > 97% similarity threshold with an open-reference OTU picking strategy and default settings. Chimeras were detected using USEARCH v6.1 (Edgar, 2010). Chimera checking and taxonomy assignment were performed using Greengenes 13.8 as reference database (DeSantis et al., 2006). The OTU abundance table was rarefied to an even number of sequences per sample to ensure an equal sampling depth for all samples ( $n = 23,737$ ). Archaeal sequences (previously known to be amplified with the set of primers used) accounted for a low fraction of sequences (on average <0.2% in each sample) and were excluded from further analyses, as well as chloroplasts and eukaryotic sequences. To test for the reproducibility of our protocol, duplicate analyses were performed on selected samples, as described in detail (see Appendix S1, Fig. S1) and analysed as in Masana et al. (2015). To identify typical freshwater OTUs, representative sequences of freshwater taxa (Newton et al., 2011; Ruiz-González, Niño-García, & Giorgio, 2015; Savio et al., 2015) from our data set were extracted and used in a local Basic Local Alignment Search Tool (BLAST) manual search against the NCBI nr/nt database. Their freshwater origin was confirmed when BLAST hits showed at least 99% identity with a query sequence belonging to a freshwater bacteria according to NCBI information. All sequences have been submitted to the SRA Sequence Read Archive (BioProject Accession no. PRJNA342950, Study SRP107850, Submission ID SUB1473540).

## 2.4 | Data handling and statistical analyses

Neighbor Joining (NJ) was performed to explore and visualize similarities among the different sites and domains, basing on Jensen-Shannon divergence (JSD) distance metric (Koren et al., 2013) in MEGAN6 (<http://www-ab.informatik.uni-tuebingen.de/software/megan6/>). Linear mixed effect model (LMM) (Baayen, Davidson, & Bates, 2008) was used to assess the presence of significant differences in OTU richness between lagoon and open sea, in both domains (pelagic, benthic). “Richness” and “environment” were used as fixed factors, and “time” (season) was used as random factor. Model fit was assessed using chi-square tests based on LMM results. Linear model (LM) was used to assess the significance of differences in OTU richness, using “time” (season) as predictor variable and “OTU richness” as response variable. LMM was also used to assess differences in the patterns of relative abundance of the dominant phyla between the two domains, using “relative abundance” and “environment” as fixed factors, and “time” (season) as random factor, and the model fit assessed using chi-square tests based on LMM results. Lastly, within each domain, LMM was used to test for significant differences in the patterns of relative abundance of each dominant phylum between lagoon and open sea, using “relative abundance” and “environment” as fixed factors, and “time” (season) as random factor, and the model fit assessed using chi-square tests based on LMM results. LM and LMM analyses were carried out in the R programming language and environment (R Core Team, 2017) using the *stats* and

*lme4* (Bates, Maechler, Bolker, & Walker, 2015) software package, respectively. The number of shared OTUs was calculated by Venn diagrams, which were plotted in the free online tool available at the following website: <http://genoweb.toulouse.inra.fr:8091/app/examp.html>. The significance of the differences in community composition was assessed, on the Illumina data set, using the Analysis of Similarity (ANOSIM) tool based on a Bray–Curtis similarity matrix. The same similarity matrix was used to carry out a nonmetric Multidimensional Scaling ordination (nMDS) to visualize similarities in community composition between samples, sampling areas and times (Ramette, 2007). Multivariate, multiple regression analyses were performed to identify the drivers of planktonic and benthic community composition, using the Distance-based Linear Modelling (DistLM) analysis, and including the environmental variables as predictor variables. A distance-based Redundancy Analysis (dbRDA) plot was prepared using all the tested variables to illustrate the DISTLM model. The ANOSIM, nMDS, DistLM and dbRDA were performed using PRIMER 6+ ([www.primers-e.com](http://www.primers-e.com)). To define the statistical relationships between the environmental variables and bacterial taxa, we used the approach adopted in Jeffries et al. (2015) based on the Maximal Information-based Nonparametric Exploration (MINE) algorithm (Reshef et al., 2011). As MINE analysis does not accept non-numerical variables, the variable “Season” was converted into numbers. The results were visualized using Cytoscape (Shannon et al., 2003). More details are reported in Appendix S1.

### 3 | RESULTS

#### 3.1 | Spatio-temporal dynamics of the environmental variables

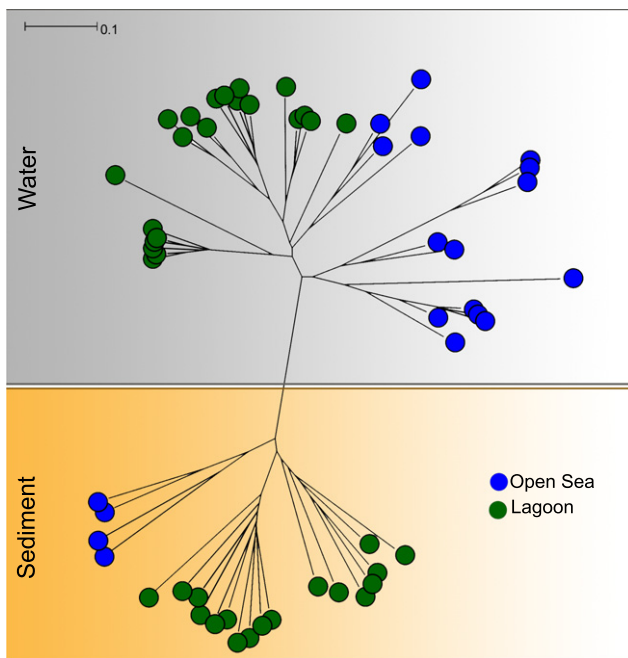
The data of the environmental variables in water are reported in Table S3. Temperature was typically higher within the lagoon, with one exception in winter, when an opposite trend was observed. Salinity, as expected, was higher in the open sea (29.13–36.90 psu) than in the lagoon (24.58–33.28 psu). Despite a slight decrease in summer, turbidity appeared homogenous across the study period, but increased in winter within the lagoon (up to 156.11 NTU). Here, DO increased from spring to summer (until 101.5%), while decreased from winter to spring, when the lowest values were observed (80%). In the open sea, DO concentration was rather constant from spring to autumn (ca. 100%), while decreased to 75.5% during winter. Nutrients were typically higher in the lagoon. DIN was highest in summer in both environments. Silicates were relatively homogeneous in the open sea throughout the year, and higher (up to fourfold) in the lagoon. Similarly, higher concentrations of phosphates were observed in the lagoon in comparison with the sea, with peaks in the lagoon in autumn (up to 1.73  $\mu\text{M}$ ). Within the lagoon, chlorophyll-*a* peaked during summer (9.50  $\mu\text{g/L}$ ) while showed much lower values in winter (0.23  $\mu\text{g/L}$ ). Conversely, in the marine stations, a peak in chlorophyll-*a* (8.88  $\mu\text{g/L}$ ) was observed in autumn.

#### 3.2 | Diversity and community composition in the pelagic and benthic domain

Bacterial OTU richness (Table S4) ranged from 696 (PTF in summer) to 3,003 OTUs (WAT3 in winter) in water and from 2,045 (7M in autumn) to 6,832 OTUs (7M in winter) in the sediments. Within the lagoon, richness averaged  $1,818 \pm 91$  in water and  $4,860 \pm 239$  in sediment while, in the open sea, the average richness was  $1,254 \pm 119$  (water) and  $5,576 \pm 129$  (sediments). As evidenced by the Venn diagrams (Fig. S2a–c), lagoon and open sea shared 17.6% of the total OTUs (Fig. S2a), sediment and water shared 16.4% (Fig. S2b), while only 10.1% of the total OTUs, corresponding to 5,260 OTUs, was shared among all seasons and all domains (Fig. S2c).

The values of OTU richness, separated by environment and domain, are shown in Fig. S3. The analysis of the richness variability revealed that the environment significantly affected bacterioplankton richness (LMM,  $\chi^2(1) = 17.251$ ,  $p = 3.275e-05$ ), which was higher within the lagoon than the open sea, while the same comparison did not reveal significant differences between lagoon and open sea sediments (LMM, n.s.). At the same time, in the two environments and the two domains, richness did not show any significant seasonal pattern, exception made for the lagoon sediments only (LM,  $F = 4.4, 921$ ,  $df = 3$ ,  $p = .02, 259$ ). The comparison of community composition, performed at the OTU taxonomic resolution, showed two clusters of samples, corresponding to the pelagic and benthic domain and, within each cluster, lagoon and the open sea samples grouped separately (Figure 2).

The analysis of community composition revealed the cumulative number of 51,826 bacterial OTUs, affiliated to 71 phyla and 242 classes. Bacterioplankton assemblages were dominated by five phyla (Bacteroidetes, Proteobacteria, Cyanobacteria, Verrucomicrobia and Actinobacteria) accounting, cumulatively, for an average 98% of the sequences in each sample in both the lagoon and the open sea. The same phyla accounted for a considerable fraction of benthic assemblages (average contribution 82% and 73%, lagoon and open sea, respectively). At the same time, in the benthic domain, assemblages included an important fraction of Chloroflexi (on average 3.84% in the lagoon and 1.95% in the open sea), Planctomycetes (2.59% and 6.66%, respectively) and Acidobacteria (2.35% and 5.49%, respectively). At the class level, the results for the most abundant ( $\geq 1\%$ ) taxa are summarized in Figure 3. The most relatively abundant bacterioplankton classes (Figure 3, upper part) were Alphaproteobacteria and Flavobacteria, while the dominant classes in sediments (Figure 3, lower part) were Gammaproteobacteria and Deltaproteobacteria. Planktonic and benthic assemblages shared a number of relatively abundant classes. The percentage of unassigned OTUs, as well as the percentage of the OTUs that were less abundant (cumulatively grouped as “Other”), was much higher in the benthic (on average 2.5% and 19.9%, respectively) than in the pelagic domain (on average 0.7% and 3.6%, respectively). More details about community composition and the identity of the dominant OTUs are in the SI results.



**FIGURE 2** Bacterial communities clustered using Neighbor-Joining algorithm based on the Jensen–Shannon divergence (JSD) values calculated at the OTU level. The scale bar indicates distance. Each circle is coloured according to the environment (lagoon, open sea) [Colour figure can be viewed at wileyonlinelibrary.com]

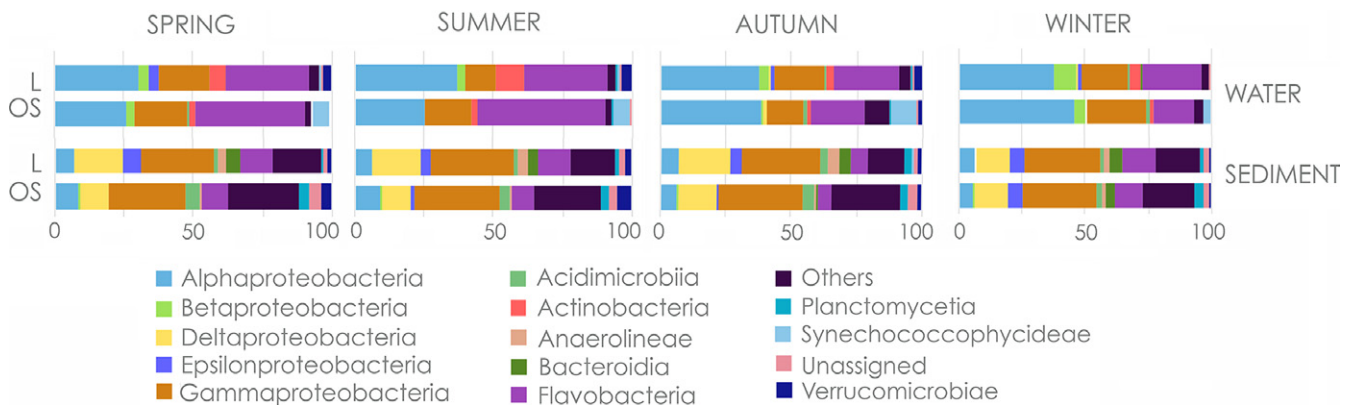
### 3.3 | Spatio-temporal patterns of the assemblages

When all the assemblages were compared, the nMDS ordination of community composition showed a clear clustering of bacterioplankton according to the season in both the lagoon and the open sea (Fig. S4a-b). This seasonal clustering was significant (ANOSIM, global  $R = 0.929$ ,  $p < .01$  for the lagoon; global  $R = 0.932$ ,  $p < .01$  for the open sea). All the pairwise comparisons between seasons were significant (ANOSIM,  $p < .01$  for all comparisons in the lagoon;  $p < .05$  in the open sea). Similarly, in the sediments, a significant grouping of assemblages according to season was observed in the lagoon

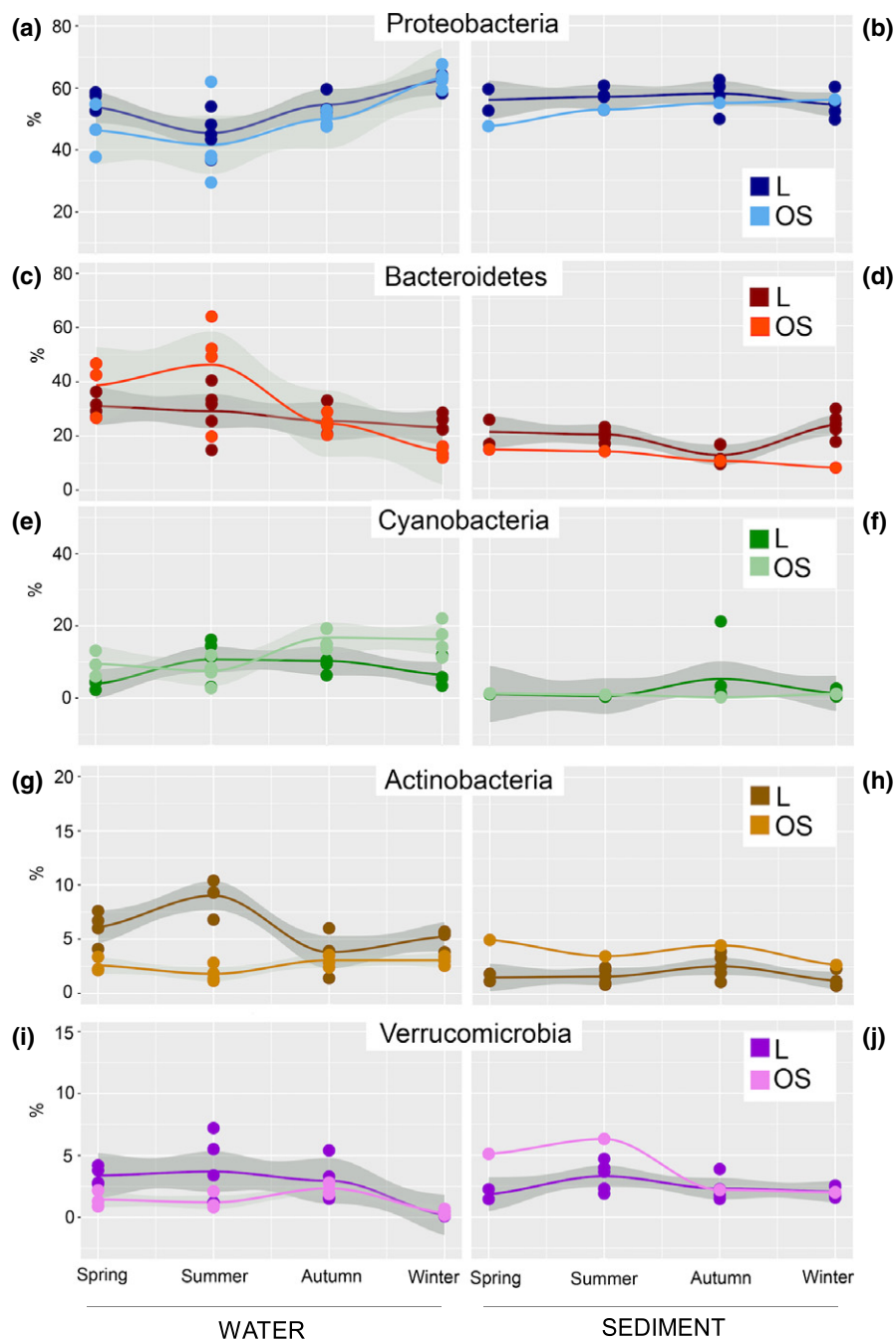
(Fig. S4c; ANOSIM, global  $R = 0.422$ ,  $p < .01$ ). However, the pairwise tests between seasons were not always significant (such as the comparisons spring vs. summer and spring vs. winter, for both ANOSIM n.s.) indicating that seasonal fluctuations were less pronounced in the sediment than the overlying water. A similar seasonal separation was observed for the open sea benthic assemblages (Fig. S4d), but assemblages appeared similar between spring and summer. As opposed to the seasonal clustering, ANOSIM did not reveal spatial differences between stations in water (ANOSIM, n.s. for both lagoon and open sea). A similar spatial homogeneity was observed in the sediments (ANOSIM, n.s.), where significant differences between stations were observed in only one instance (pairwise comparison 7M vs. INS,  $p < .05$ ). More detailed information about the ANOSIM results is reported in Table S5.

Given that assemblages showed the dominance of some phyla, we also analysed their patterns, in terms of relative abundance, across the four seasons in the two environments (lagoon, open sea) and domains (pelagic, benthic). Members of these phyla followed marked temporal patterns, and these patterns were significantly different between the benthic and pelagic domain for most of the dominant phyla: Proteobacteria (LMM,  $\chi^2(1) = 4.4,382$ ,  $p = .03,514$ ), Cyanobacteria (LMM,  $\chi^2(1) = 9.3,546$ ,  $p = .00,224$ ) and Actinobacteria (LMM,  $\chi^2(1) = 2.9,308$ ,  $p = .0,869$ ). Conversely, the patterns were not significantly different for the phyla Bacteroidetes and Verrucomicrobia.

In the water (Figure 4, left panels), Proteobacteria showed an increase in winter, consistently observed in the lagoon and the open sea (LMM, n.s.). This pattern was opposite to Bacteroidetes, which showed the lowest relative abundance in winter, and the highest in summer (both lagoon and open sea; LMM, n.s.). Cyanobacteria (which, only here, also includes the microalgal contribution) showed significant differences in the seasonal patterns between the lagoon (a peak in summer) and the open sea (a peak in winter) (LMM,  $\chi^2(1) = 8.8,504$ ,  $p = .00,293$ ). Similarly, the seasonal patterns of planktonic Actinobacteria and Verrucomicrobia were different between the lagoon and the open sea (LMM,  $\chi^2(1) = 34.773$ ,  $p = 3.705e-06$  for Actinobacteria; LMM,  $\chi^2(1) = 5.8,385$ ,  $p = .01,568$  for Verrucomicrobia).



**FIGURE 3** Bacterial community composition at the class level. Classes with an average relative abundance across all samples  $<1\%$  were aggregated into the group reported as “Others.” “Unassigned” includes all those reads that did not match any known taxonomy. In each environment (lagoon, open sea) or domain (pelagic, benthic), data from multiple stations have been averaged. L = lagoon. OS = open sea [Colour figure can be viewed at wileyonlinelibrary.com]

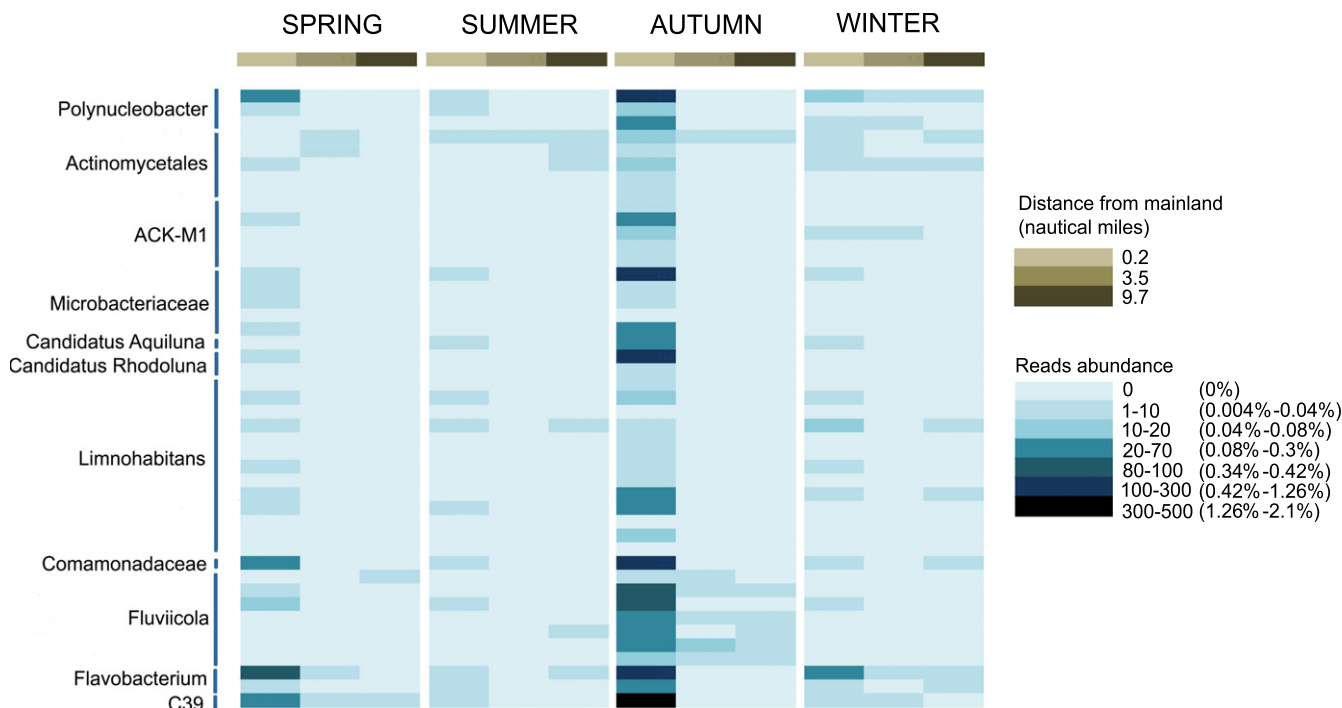


**FIGURE 4** Temporal patterns of the five dominant bacterial Phyla in the Lagoon (L) and the adjacent open sea (OS) across the study period. a, c, e, g and i: water samples. b, d, f, h and j: sediment samples. Dark (lagoon) and light (open sea) grey shadows indicate the standard error. The y-axes are at different scales [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

In the sediments (Figure 4, right panels), patterns of Proteobacteria differed between the two environments (LMM,  $\chi^2(1) = 2.7, 225$ ,  $p = .09, 894$ ), as well as the patterns of Bacteroidetes (LMM,  $\chi^2(1) = 10.718$ ,  $p = .001, 061$ ), which increased considerably during winter only in the lagoon. Benthic cyanobacteria (including microalgae) were relatively constant throughout the year (exception made for one outlier in the lagoon set of samples) and their patterns did not change between lagoon and open sea (LMM, n.s.), while the patterns of benthic Actinobacteria were significantly different between the two environments (LMM,  $\chi^2(1) = 22.291$ ,  $p = 2.343e-06$ ). Lastly, the temporal patterns of benthic Verrucomicrobia also significantly differed between lagoon and open sea (LMM,  $\chi^2(1) = 6.2, 373$ ,  $p = .01, 251$ ).

### 3.4 | Freshwater influence on planktonic assemblages

Lagoon planktonic assemblages were characterized by the presence of typical freshwater taxa. These included several OTUs within the classes Betaproteobacteria (genera *Polynucleobacter* and *Limnohabitans*), Actinobacteria (family Microbacteriaceae, genera *Candidatus Aquiluna* and *Candidatus Rhodoluna*) and Flavobacteriia (genera *Fluviicola* and *Flavobacterium*). These taxa appeared more relevant in those stations located in the inner part of the lagoon and closer to the river effluents (e.g., INS and WAT2), while decreased in importance in the stations more close to the sea (e.g., 7M). The analysis of the temporal dynamics of these freshwater bacterioplankton taxa



**FIGURE 5** A heat map which summarizes the dynamics of the most abundant freshwater OTUs in the lagoon. The colour gradient from light to dark blue indicates the contribution of the respective bacterial OTU in terms of reads number in the respective sample, with a maximum of 500 reads, and percentage (%) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

indicated that they increased in relevance, especially in the inner stations, during the periods of higher river run-off (autumn and winter; Figure 5) as compared to spring and summer, characterized by lower freshwater input. The cumulative percentage of reads affiliated to freshwater taxa within the assemblages ranged from being close to 0% (value observed in spring and summer at more than 3.5 nm distance from mainland) to a maximum of 8.8% (observed in autumn, in the more inner stations located at 0.2 nm distance from mainland).

### 3.5 | Drivers of community composition

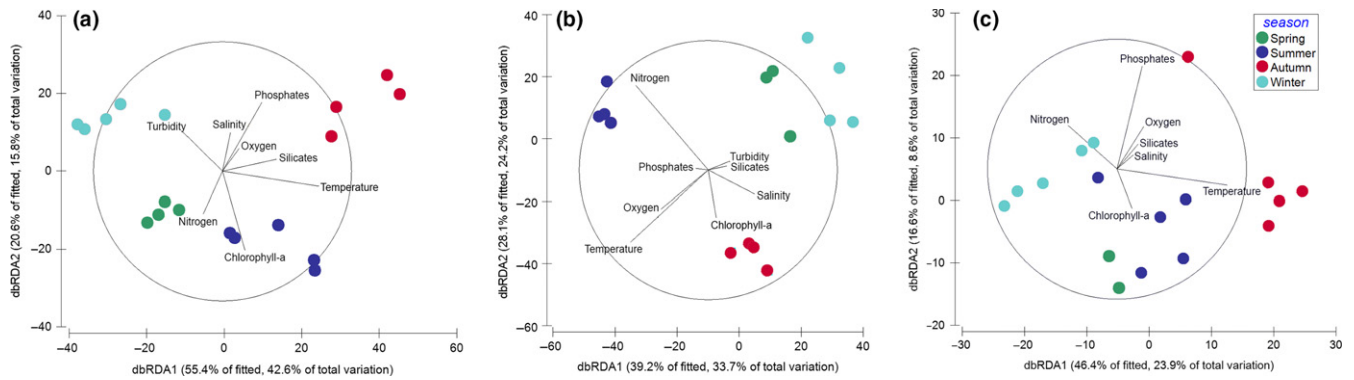
DistLM revealed that different environmental variables were associated with shifts in community composition, at the OTU level, in the different environments and domains. Of the eight variables tested, six of them (all variables with the exception of DO and silicate) were significantly associated with the variation in bacterioplankton community composition at the significance level of  $p < .01$ , and explained the cumulative percentage of 68% and 75% of variation (lagoon and open sea, respectively). Temperature alone was associated with the largest variance of bacterioplankton community composition (36% and 29%, lagoon and open sea, respectively). The variation in benthic community composition was mainly associated with temperature, which alone explained up to 19% of the variance within the lagoon and the open sea. A dbRDA analysis was used for the graphical visualization of the DistLM outputs (Figure 6a-c). The dbRDA plots showed that the environmental variables divided the stations into separate clusters that corresponded to the different seasons.

### 3.6 | Network analysis

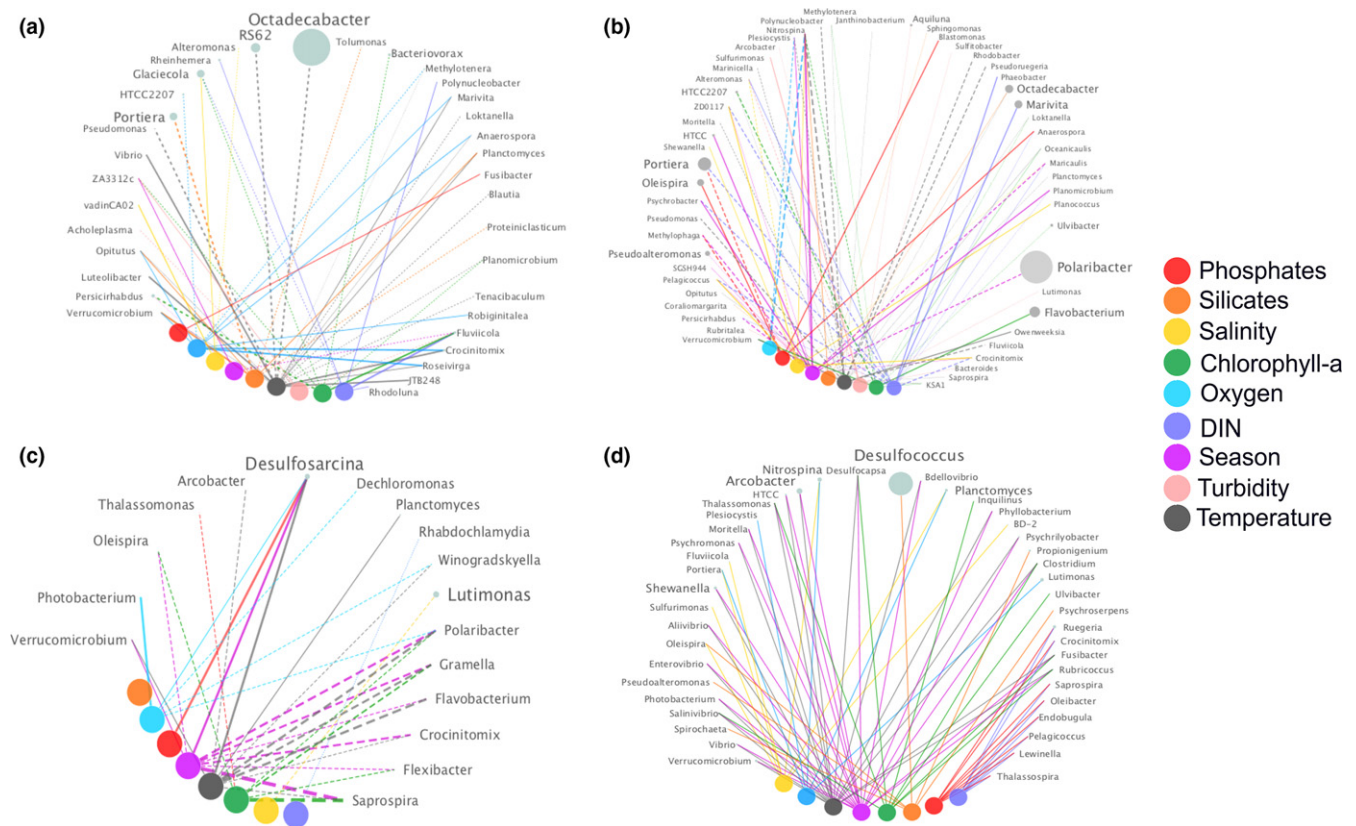
The network analysis was used to identify statistical links between the environmental variables and the relative abundance of bacterial genera in water (Figure 7a-b, lagoon and open sea, respectively) and sediments (Figure 7c-d, lagoon and open sea, respectively). Overall, these analyses revealed more complexity of positive and negative interactions in the open sea than the lagoon. In lagoon water, there were a number of 70 significant correlations (at  $p < .05$ ) between 35 genera and the environmental variables (Figure 7a), as opposed to 117 significant correlations found in the open sea water (Figure 7b). Temperature was the most connected node (33 nodes in the lagoon, 26 nodes in the open sea). In the lagoon sediments, 17 genera displayed significant relationships with the environmental variables, leading to 41 significant correlations (Figure 7c), while a much higher number of significant relationships (210) were observed in the open sea sediments between the environmental variables and 44 genera (Figure 7d). The most connected nodes in the sediments were, both in lagoon and open sea, temperature (13 and 10 nodes, respectively) and season (32 nodes in both instances).

## 4 | DISCUSSION

Coastal lagoons are highly productive ecosystems characterized by chemical and physical gradients that make these systems unstable and subjected to fluctuating conditions (Manini et al., 2003). They provide diverse ecosystem services, such as flood and erosion



**FIGURE 6** dbRDA ordinations of the distLM model which describes the relationship between the environmental variables and the taxonomic composition at the OTU level in lagoon water (a), open sea water (b) and lagoon sediments (c). The different colours indicate the four seasons [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**FIGURE 7** Network analysis of the associations between relative abundance of bacterial taxa (at the genus level) and the main environmental variables in the water (a lagoon, b open sea) and sediment samples (c lagoon, d open sea). The size of each node is proportional to the taxon's relative abundance. Positive interactions are solid lines, while negative are dashed, with the edge width being proportional to the strength of the relationship (MIC score). In the network, the term "candidatus" is omitted for the taxa *Rhabdochlamydia*, *Aquiluna* and *Portiera*. Only those genera with a recognized affiliation are reported in the network. As MINE analysis does not accept non-numerical variables, the variable "Season" was converted into numbers [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

control, shoreline stabilization, sediment and nutrient retention, local mitigation of climate change effects and water purification, and they represent a reservoir of biodiversity and biomass (Danovaro & Pusceddu, 2007). At the same time, coastal lagoons, especially those located in the Mediterranean Sea, are vulnerable to a number of anthropogenic disturbances such as agricultural, industrial, mining

and touristic activities (Ferrarin et al., 2014; Ghai et al., 2012). They are transition zone between terrestrial, marine and freshwater interfaces (Newton et al., 2014) and act either as sinks for organic matter accumulation (Pusceddu et al., 2003), or reservoirs able to fertilize the adjacent sea by exporting organic and inorganic nutrients (Marini & Frapiccini, 2014). The balance between export and accumulation



depends, besides physical and hydrological factors, on the degradation and utilization processes by the planktonic and benthic microbes, which highlights the importance of understanding the spatial and temporal dynamics of lagoon microbes. Here we show the results of a seasonal study that was carried out to investigate the magnitude, patterns and drivers of bacterial diversity in a large coastal lagoon and its adjacent sea. The analysis of the environmental variables confirm the complexity of transitional areas like the Venice lagoon, characterized by large fluctuations in the hydrological and chemical variables, as a function of river inputs, water exchanges with the sea and water-sediment interactions due to the shallow depth (Gaćić et al., 2004). The seasonal trends in the physical-chemical conditions were those expected in this lagoon (Bandelj et al., 2008; Pugnetti et al., 2010) as well the adjacent sea (Solidoro et al., 2009).

Our results provide the largest inventory so far on the diversity of planktonic and benthic bacterial assemblages in the Venice lagoon and the nearby marine area. Previous microbiological studies in this and other lagoons were only restricted to water (Celussi et al., 2009; Piccini et al., 2006; Simonato et al., 2010) or sediment alone (Borin, Brusetti, Daffonchio, Delaney, & Baldi, 2009; Mohit et al., 2015; Pavloudi et al., 2016), without addressing the possible benthic-pelagic interactions. In addition, most of them were performed using community fingerprinting or cloning, known to insufficiently describe diversity compared to the 16S rRNA gene HTS we used. As such, our inventory of >50,000 bacterial OTUs expands the limited body of knowledge available about the magnitude of bacterial diversity in closed and semi-closed lagoons (Ghai et al., 2012; Mohit et al., 2015) that are largely unexplored if compared with other coastal ecosystems, such as estuaries (Campbell & Kirchman, 2013). Improving our understanding on the biodiversity of prokaryotes in transitional systems is crucial, on light of the valuable ecosystem goods and services these ecosystems provide to the society (Newton et al., 2014).

We report that assemblages separated into groups defined by the domain (pelagic and benthic) and the environment (lagoon and open sea). This finding was evident in the low number of shared OTUs, which resulted from the Venn diagrams. It is known that bacterial community structure differs between coastal marine environments and domains (Feng et al., 2009; Rappé, Vergin, & Giovannoni, 2000; Zinger et al., 2011). However, this is, to our knowledge, the first study that investigates differences in bacterial composition across multiple aquatic ecosystems (lagoon, coastal sea) and domains (pelagic, benthic) explored at the same time and over a seasonal cycle. The highest OTU richness (alpha diversity) was observed in sediments than in the overlying water, confirming shallow marine sediments as highly diversified systems (Torsvik, Øvreås, & Thingstad, 2002; Zinger et al., 2011). At the same time, bacterioplankton alpha diversity is higher in the lagoon than the nearby sea, supporting the hypothesis that highly productive transitional environments, compared to the less productive coastal sea, host more microbial species (Danovaro & Pusceddu, 2007). Similarly, in a study that compared lagoons differing in primary productivity, higher bacterial richness was found in the more productive lagoon, suggesting that

richness increases with primary productivity (Benlloch et al., 1995). Horner-Devine, Leibold, Smith, and Bohannon (2003) confirmed primary productivity as a key determinant of richness, but showed that different bacterial taxa exhibit different response. We hypothesize that the higher bacterioplankton richness in lagoons is also the consequence of the high terrestrial inputs, given that lagoons, being ecotones at the land-sea interface, receive contributions of microbes from both the watershed and coastal waters (LaMontagne & Holden, 2003). This hypothesis is supported by our evidences for a notable contribution of freshwater taxa in the lagoon, especially during the periods of higher run-off. It is worth noting that the same pattern of decreasing richness moving from the lagoon to the sea was not observed in sediments, which suggests that constraints other than productivity and terrestrial inputs shape benthic bacterial richness, deserving further investigations.

Unveiling the mechanisms and drivers of community assembly is key to understand the processes of microbial variation and maintenance (Wang et al., 2015), especially in coastal lagoons because of their socio-economic and ecological importance (Gonenc & Wolflin, 2005). Factors controlling the spatio-temporal distribution of bacterial diversity have been studied in marine waters and sediments (Böer et al., 2009; Fortunato et al., 2012; Gobet et al., 2012; Hatosy et al., 2013; Quero & Luna, 2014) while lagoons, and especially lagoon sediments (Mohit et al., 2015), have been less explored. Here, we show that, within each environment and domain investigated, temporal variations are more important than spatial variations in structuring the assemblages. Our results corroborate previous findings highlighting the fundamental role played by seasonality in structuring coastal bacterioplankton (Alonso-Sáez et al., 2007; García et al., 2015; Gilbert et al., 2012). However, we also provide among the first evidences for similar seasonal patterns of bacterioplankton variation in transitional systems, only sporadically reported in the past and only using low-resolutions methods, such as fingerprinting (Celussi et al., 2009; LaMontagne & Holden, 2003), known to only identify the dominant taxa.

We also observed a seasonally driven variability in the benthic domain, pointing to lagoon sediments as a highly dynamic component, as shown recently in a Canadian lagoon (Mohit et al., 2015). Temporal variations in transitional and coastal sediments have been poorly explored (Feng et al., 2009), and contrasting results were reported. Feng et al. (2009) reported seasonally driven changes in the sediment populations in the Changjiang estuary and the coastal area of the East China Sea, related with the hydrological regime. Böer et al. (2009) identified time as the most important factor affecting bacterial diversity in coastal sands but, given that turnover was not related to any environmental parameter, suggested that temporal variations were due to unknown environmental drivers and/or stochastic events. It is worth noting that we did not observe a seasonal pattern in the sediments of the adjacent sea, but the low number of samples analysed does not allow testing the robustness of this pattern, deserving future studies.

We hypothesize that the seasonal variability observed in lagoon community composition is driven by seasonal changes in the

environmental and trophic conditions. This seasonal variability depends, especially in shallow lagoons, on changes in the environmental conditions triggered by the interplay of external inputs of water and nutrients, internal hydrological processes and sedimentation (Laque, Farjalla, Rosado, & Esteves, 2010). Our hypothesis is supported by the multivariate analyses, showing that different environmental variables significantly explain the variance in community composition across the different seasons. The dbRDA plot of lagoon bacterioplankton revealed a separation among the four seasons, which appear to be related with different environmental drivers. The observed successional pattern of bacterioplankton fits with the ecological functioning of the Venice lagoon, where a bloom of planktonic phototrophs typically occurs in summer (Bernardi Aubry, Acri, Bianchi, & Pugnetti, 2013), followed by an increase in river run-off, which causes nutrients to be delivered into the lagoon in autumn (Bandelj et al., 2008). In winter, the temperature decreases and the lagoon is mostly physically homogeneous, and is controlled by run-off and winds, that cause typically bottom disturbances leading to sediment resuspension and increases in turbidity. The significant correlations reported here suggest that all these physical processes shape lagoon bacterioplankton. Given that phytoplankton is an important source of fresh organic carbon, it can be hypothesized that the summer bloom of phytoplankton may have sustained populations specialized for the decomposition of algal-derived organic matter (Teeling et al., 2012). Temperature contributes to separate spring/summer versus autumn/winter bacterioplankton communities, and, similarly, turbidity and phosphates act as key environmental drivers of temporal shifts over the seasonal scale. It must be pointed out that, in our study, the values of temperature appeared similar between the summer and autumn sampling. This is likely the result of the fact that the summer sampling was performed early, before the warming of temperature usually observed in the lagoon in July and August (where the water can reach temperatures >25°C). In lagoon sediments, the seasonal variation in benthic assemblages is strongly related with some of the same drivers, such as temperature and nutrients, suggesting the existence, in this shallow ecosystem, of tight pelagic-benthic interactions. A similar influence of temperature on sediment bacteria has been reported in coastal lagoons in Japan (Tsuboi et al., 2013). However, we report here that the fraction of unexplained variance is higher in sediments than in the overlying waters, suggesting that other sediment variables, which were not included in our study (e.g., sediment organic matter availability or contaminants), may act as important drivers in structuring the benthic microbiota, as described in previous studies (Hewson & Fuhrman, 2007; Korlević et al., 2015; Luna, Dell'Anno, Giuliano, & Danovaro, 2004).

Among the environmental factors that shape lagoon bacterioplankton, we highlighted the role of riverine inputs. The signature of freshwater taxa described a spatial gradient from the stations close to the mainland, under higher influence of inland waters, to those closer to sea. The inner stations showed the highest proportion of taxa of freshwater origin, within the classes Betaproteobacteria and Flavobacteria and the phylum Actinobacteria (Newton et al., 2011;

Ruiz-González et al., 2015, Savio et al., 2015). The increased relevance of the freshwater microbial signature observed in autumn, when the largest freshwater input was recorded, suggests that the flow of terrestrially derived bacteria is tightly linked to the river run-off. As the contribution of riverine OTUs declines with increasing distance from land, we hypothesize that riverine bacteria are readily dispersed due to the hydrological forcing, and/or that a fraction of them do not tolerate changes in salinity after entering in the lagoon. The fate of newly introduced allochthonous bacteria in aquatic systems depends on the complex balance of local extinction and dispersal rates. The ability to survive under different settings (marine, freshwater) ultimately depends on the genetic background. Eiler et al. (2016) recently demonstrated that freshwater and marine ecotypes of the same species (SAR11) differ profoundly in their genome, providing functions that confer selective advantages under the different setting. Information regarding mechanisms of entering, transiting and survival of freshwater bacteria in lagoons and the coastal sea are still scarce. Our results point out at lagoon bacterioplankton as a dynamic mosaic of bacterial species, characterized by the transient coexistence of taxa originating from different sources (lagoon, river and sea). The finding that the freshwater signature varies according with variations in the hydrological conditions, suggests that this dynamic microbial landscape is expression of the network metacommunity across the whole hydrological continuum, from rivers to the sea. Disentangling the role that the diverse microbial components play in the lagoon over different timescales, and how anthropogenic changes in the hydrological regime may influence community assembly, is a challenge to improve the preservation of coastal lagoons and their functioning, and to define appropriate management priorities.

Network analyses are used increasingly to explore large data sets generated by DNA HTS technologies, and to elucidate potential interactions between microbial taxa (co-occurrence patterns), or between taxa and environmental variables across spatial or temporal scales (Barberán, Bates, Casamayor, & Fierer, 2012; Jeffries et al., 2015). Results from our network analyses showed that, in water, temperature was the most connected node in both the environments, confirming its key role in structuring bacterioplankton assemblages. The analysis of the network topological parameters indicated that temperature showed positive and negative correlations with many bacterioplankton genera. In the lagoon, positive correlations were found with *Vibrio*, confirming previous knowledge about its temperature dependence in aquatic environments (Vezzulli, Colwell, & Pruzzo, 2013). We found temperature interactions with a variety of taxa, including genera that are dominant in the lagoon (such as *Octadecabater*) but also several minor members, suggesting that the rarest taxa are also influenced by thermal fluctuations. The negative correlation of temperature with many bacterioplankton taxa reflects their preference for colder waters. In the open sea, beside temperature and season that were, as expected, the more connected nodes, numerous taxa were positively or negatively correlated with nitrogen, as observed in a large temperate estuary (Jeffries et al., 2015), suggesting different life and nutrient utilization strategies, by the

different taxa that form the complex assemblage, to the seasonally changing trophic conditions. In the sediments, lagoon and open sea networks differed profoundly, with only few, mostly negative, interactions observed in the first, and much more complexity of interactions, all positive, in the latter. These differences in the network complexity and the type of correlations, indeed, reflect differences in taxonomy and function of lagoon and open sea benthic assemblages. It is worth mentioning that, although based on a limited number of samples, the network analysis in the open sea sediments showed the highest number of interactions which, moreover, were all positive. Lagoon sediments showed a reduced complexity of interactions than the open sea sediments, which may reflect the high level of sediment contamination due to recent industrial activities (Zonta et al., 2007), known to influence benthic microbial diversity (Quero et al., 2015) and to perturb community associations and functions (Sun, Dafforn, Johnston, & Brown, 2013). Our understanding of the complex network of microbial interactions with the surrounding aquatic environment is still at the beginning. In this first attempt to compare lagoon planktonic and benthic assemblages, we document the potential of network analysis to start disentangling those interactions, also at the level of individual taxa, with potential usefulness to investigate the auto-ecology of aquatic microbes.

To conclude, this study highlights the importance of seasonality in modulating planktonic and benthic bacterial assemblages in coastal lagoons and the adjacent sea. This knowledge expands the limited information available so far on bacterial diversity and its patterns in aquatic transitional environments, which lie at the forefront of land and sea. Furthermore, this study provides a framework for future studies aiming at understanding and predicting how lagoon microbes and the ecosystem services that microorganisms contribute to provide will be impaired by the anthropogenic pressure, which increasingly affects coastal lagoons and the nearby coastal ocean.

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## DATA ACCESSIBILITY

Raw sequences have been deposited at to the NCBI's Sequence Read Archive (SRA) under BioProject Accession no. PRJNA342950, Study SRP107850, Submission ID SUB1473540.

## CONFLICT OF INTEREST

All the authors have not any financial or personal competing interests to disclose.

## AUTHOR CONTRIBUTION

G.M.Q. and G.M.L. designed the research. G.M.Q., L.P. and M.B. performed the fieldwork. L.P. performed most of the laboratory analyses and the DNA extraction from all samples. G.P., C.M. and C.L. performed the NGS sequencing. G.M.L. and G.M.Q. analysed the sequence data and performed the statistical analyses. M.M. and M.B. provided data and interpretation on the environmental variables and their patterns. G.M.L. and G.M.Q. wrote the manuscript, and all the authors provided constructive comments, revised and edited the manuscript.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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