

# The influence of invasive jellyfish blooms on the aquatic microbiome in a coastal lagoon (Varano, SE Italy) detected by an Illumina-based deep sequencing strategy

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**Abstract** The rapid expansion of multicellular native and alien species outbreaks in aquatic and terrestrial ecosystems (*bioinvasions*) may produce significant impacts on bacterial community dynamics and nutrient pathways with major ecological implications. In aquatic ecosystems, bioinvasions may cause adverse effects on the water quality resulting from changes in biological, chemical and physical properties linked to significant transformations of the

microbial taxonomic and functional diversity. Here we used an effective and highly sensitive experimental strategy, bypassing the efficiency bottleneck of the traditional bacterial isolation and culturing method, to identify changes of the planktonic microbial community inhabiting a marine coastal lagoon (Varano, Adriatic Sea) under the influence of an outbreak-forming alien jellyfish species. Water samples were collected from two areas that differed in their level of confinement inside in the lagoon and jellyfish densities (W, up to 12.4 medusae m<sup>-3</sup>; E, up to 0.03 medusae m<sup>-3</sup>) to conduct a snapshot microbiome analysis by a metagenomic approach. After extraction of the genetic material in the environmental water samples, we deep-sequenced metagenomic amplicons of the V5–V6 region of the 16S rRNA bacterial gene by an Illumina MiSeq platform. Experiments were carried out in

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triplicates, so six libraries of dual indexed amplicons of 420 bp were successfully sequenced on the MiSeq platform using a  $2 \times 250$  bp paired-end sequencing strategy. Approximately 7.5 million paired-end reads (i.e. 15 million total reads) were generated, with an average of 2.5 million reads (1.25 M pairs) per sample replicate. The sequence data, analyzed through a novel bioinformatics pipeline (BioMaS), showed that the structure of the resident bacterial community was significantly affected by the occurrence of jellyfish outbreaks. Clear qualitative and quantitative differences were found between the western and eastern areas (characterized by many or few jellyfish), with 84 families, 153 genera and 324 species in the W samples, and 104 families, 199 genera and 331 species in the E samples. Significant differences between the two sampling areas were particularly detected in the occurrence of 16 families, 22 genera and 61 species of microbial taxa. This is the first time that a NGS platform has been used to screen the impact of jellyfish bioinvasions on the aquatic microbiome, providing a preliminary assessment of jellyfish-driven changes of the functional and structural microbial biodiversity.

**Keywords** Metagenomics · Next-generation sequencing platforms · Marine microbial ecology · Jellyfish biomass

## Introduction

The recent advent of next generation sequencing technologies has revolutionized microbiome research, allowing unprecedented depth and resolution at affordable costs and, thus, enabling large-scale studies of microorganisms living in taxonomically and physically complex habitats. Because microbes have lived on Earth for more than 3 billion years, they are ubiquitous and play important roles in the Earth's ecosystems (Kowalchuk et al. 2008). In particular,

they participate in essential biogeochemical processes such as the carbon, nitrogen and sulphur cycles in terrestrial and aquatic ecosystems, are producers and decomposers in food webs (Dorigo et al. 2005; Kisand et al. 2012; Zehr 2010), and are primarily responsible for degradation of a large variety of natural organic compounds (Ogawa et al. 2001).

Aquatic environments harbor abundant and diverse microbial communities that ensure their functioning and sustainability (Azam and Malfatti 2007; Zinger et al. 2012). In recent decades, the importance of bacterioplankton has been persuasively demonstrated in nutrient cycling and food-web structure in the marine environment (Azam 1998; Cho 1990) and a multitude of marine bacteria have been isolated in ocean sites and different coastal areas of temperate, tropical and polar zones (Pommier et al. 2005; Rusch et al. 2007; Stabili and Cavallo 2011). Aquatic microbes are genetically, physiologically and ecologically diverse, and exhibit many different physiological responses, adaptation and evolutionary patterns (Bahgat 2011; Kemp and Aller 2004). The microbiota composition varies as a function of water class (e.g. oceans, lakes, rivers, springs, ponds and ground water) and it is affected by different factors such as water salinity, organic compound concentration, turbidity, temperature, and contamination sources (Bahgat 2011).

Study of the overall biodiversity is crucial for assessing and monitoring aquatic ecosystems; however, the composition, abundance, and distribution patterns of microbial communities usually remain poorly explored (Kisand et al. 2012; Zinger et al. 2012).

Previously, difficulties in collecting representative samples in particular habitats (e.g. deep sea) and experimental limitations related to the characterization of the uncultivable microbes have been the two main limitations for comprehensive assessments of the microbial communities living in certain environments (Kisand et al. 2012). This view recently changed with the development of culture-independent approaches, such as metagenomics. As defined by Thomas et al. (2012), metagenomics represent the direct genetic analysis of genomes contained in an environmental sample, without isolation or culture of individual organisms. The metagenomic approach is experiencing an explosive improvement since the advent of high-throughput Next-Generation Sequencing (NGS) technologies, which allow an unprecedented large-scale identification of organisms and communities

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through the production of an enormous amount of genetic data (Bourlat et al. 2013). Either of two experimental strategies can be applied starting from the total extracted DNA: (1) the entire metagenome is sequenced by a shotgun approach, or (2) gene markers specific for a given taxon are selectively sequenced after their amplification by PCR primer pairs able to function over a broad taxonomic range (amplicon-based approach). The second approach is the most common strategy applied in various ecological settings and is used to investigate prokaryotic diversity through analysis of hypervariable regions of the 16S rRNA gene (Hajibabaei et al. 2011; Klindworth et al. 2013). Illumina/Solexa represents the NGS sequencing system most widely used for amplicon-based metagenomic studies (Luo et al. 2012), providing optimal performances in terms of time, costs and coverage (Caporaso et al. 2012; Kozich et al. 2013; Liu et al. 2012; Loman et al. 2012; Quail et al. 2012). In particular, the Illumina MiSeq benchtop sequencer is very suitable for amplicon-based metagenomic studies, considering its overall features including the obtainable read length up to 300 m. Nevertheless, few available experimental protocols for the amplicon-based metagenomic approach on the MiSeq platform are available involving the application of the TruSeq (Caporaso et al. 2012; Gilbert et al. 2010; Rubin et al. 2013) or a custom designed approach (Kozich et al. 2013).

We used a flexible strategy including both a NGS library preparation based on the Illumina-Nextera protocol and a user-friendly data analysis workflow (BioMaS, Bioinformatic analysis of Metagenomic AmpliconS) for monitoring changes in the planktonic microbiome potentially related to the invasion and proliferation of a non-indigenous jellyfish population (the moon jelly *Aurelia* sp.) in a lagoonal habitat, the Varano lagoon (South Adriatic). This strategy may allow the production of a large number of high quality sequencing reads, minimizing the cost per run and simplifying the experimental procedure.

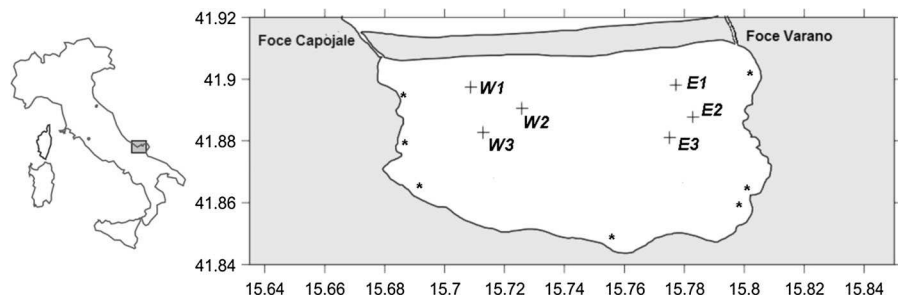
Jellyfish blooms are occurring with increasing frequency and magnitude in many estuarine and coastal habitats (Attrill et al. 2007; Boero et al. 2008; Mills 2001; Purcell 2012). In the Mediterranean Sea, *Aurelia* sp. invasions are widely recorded, in the Mljet lakes, Croatia (Malej et al. 2007), in Etang de Thau, France (Bonnet et al. 2012), in the Gulf of Trieste, Italy and Slovenia (Malej et al. 2012). This is

also the case for the Varano lagoon, a coastal lagoonal habitat with a strong aquaculture vocation and tradition. The Varano ecosystem was invaded by the moon jellyfish *Aurelia* sp. more than 10 years ago and now has a persistent population with high densities in some parts (Belmonte et al. 2011; Manini et al. 2005; Scorrano 2014).

The invasion of *Aurelia* jellyfish in coastal lagoons often can be related to the introduction of mussel culture rafts bearing the polyp stages, which asexually release great numbers of medusae into the water column (Lo et al. 2008; Purcell, 2012). In Varano lagoon, eventually a year-round resident population was established with high densities of jellyfish (mean 4.5 ind) in spring and summer months. Molecular COI barcoding assigned the Varano jellyfish population to none of the presently known *Aurelia* species in the Mediterranean Sea (*Aurelia* sp. 5 and *Aurelia* sp. 8 sensu Dawson), suggesting a new jellyfish bioinvasion event (Scorrano, 2014). Mussel rafts are concentrated in the western part of Varano lagoon and may have introduced attached jellyfish polyps to the lagoon. Active swimming of the jellyfish against the main W–E water flow may contribute to maintaining a spatial segregation of jellyfish in the western part of the lagoon.

Jellyfish may acquire key roles in planktonic food webs by converting large quantities of carbon (C) into gelatinous biomass available only to a few higher consumers (Arai, 2005). Furthermore, the biomass of jellyfish generates a large amount of carbon-rich colloidal and dissolved organic matter (jelly-DOM) (Hansson and Norrman 1995) that is released into the water and easily available to the bacterioplankton (Carlson et al. 2002; Condon and Steinberg 2008), with consequences for bacterial growth and enzymatic activities (Riemann et al. 2006; Titelman et al. 2006). The protein-rich decay jellyfish biomass and the mucous excretion of living jellyfish also are readily assimilated by jellyfish-associated and free-living bacteria and can cause rapid shifts in microbiome structure and organization (Tinta et al. 2010, 2012). The bacterial use of jelly-DOM could lead to the repackaging of gelatinous organic carbon and its reincorporation into the food web (Condon et al. 2011). In addition, the decay of gelatinous biomass (jellyfalls) following bloom events leads to the rapid and massive release in the water column of dissolved inorganic and organic compounds (Pitt et al. 2009).

**Fig. 1** Map of Varano lagoon (geographical coordinates on the axes) with West (W) and East (E) replicate sampling points (*plus symbol*) and fresh water springs (*asterisk*)



The present investigation focused on assessing the potential of NGS-based methods for rapid detection of structural and functional changes in the microbial community associated with jellyfish blooms in a marine coastal lagoon.

## Materials and methods

### Sampling

The Varano lagoon is a shallow enclosed coastal basin (Fig. 1) located on the southern Adriatic coast (Apulia region, Italy) on the north side of the Gargano promontory. The lagoon has a 33 km perimeter and covers an area of over 60.5 km<sup>2</sup>. The lagoon represents a typical eurythermal habitat (low 7 °C, high up to 30 °C), with a maximum depth of 5 m and high inter-seasonal physico-chemical variability. Freshwater springs are distributed all around the lagoon perimeter (Fig. 1) and two artificial channels (foce Capojale, NW; foce Varano, NE) regulate lagoon-sea water exchanges (Specchiulli et al. 2008), facilitating the hydrodynamic balance of sea water masses at reduced salinity (25–29 ppt) through tidal and wind forces. Prevailing N–NE winds make these exchanges much more effective in autumn and winter (Spagnoli et al. 2002), with the main sea water inflow through the NW channel. Due to the small tidal excursion and reduced exchange with the adjacent coastal area, water residence time is very long (about 1.5 years; Specchiulli et al. 2008).

To reveal changes in the planktonic bacterial communities in the lagoon relative to the direct or indirect impact of the jellyfish biomass, a metagenomic approach was applied to triplicate water samples collected on 17 July 2013 in two areas of the lagoon (Fig. 1), designated West (W) and East (E), and differing in their level of confinement (Melaku Canu et al. 2012) and jellyfish densities (W: up to

12.4 ind m<sup>-3</sup>; E: up to 0.03 ind m<sup>-3</sup>; Scorrano 2014). Samples were collected in pre-sterilized bottles at –0.50 m depth and stored at +4 °C until filtration through a 0.2-µm pore size filter (47 mm diameter) MO BIO (MoBio Laboratories, Inc. CA, USA). Sample membranes then were stored at +4 °C until DNA extraction, which was carried out within 4 h from the sampling. Vertical profiles of temperature, salinity, pH, depth, and dissolved oxygen were recorded every 6 weeks by a multiparametric CTD probe Ocean Seven 401 (IDRONAUT; Scorrano 2014). From that dataset, we obtained the means ± standard deviations of temperature, salinity, and oxygen saturation at 0.5 m depth in triplicate samples collected at W and E areas on the day of water sample collection.

### DNA extraction

Microbial DNA was extracted from each filter (3 replicates/area) using the MO BIO PowerWater<sup>®</sup> DNA Isolation Kit (MoBio Laboratories) following the manufacturer's instructions. The bead-beating step was performed in the FastPrep Instrument (BIO 101, Carlsbad, Canada) for 40 s at speed 6. The homogenized samples were centrifuged (4,000×g for 3 min at room temperature) and the supernatant subjected to the subsequent protein and inhibitor removal steps as recommended by the manufacturer. DNA was eluted in 100 µl of the PW6 solution. The quantity and quality of the extracted DNA were assessed by spectrophotometric quantification using NanoDrop 2000c (Thermo Fisher Scientific, Inc., DE, USA) and agarose gel (1.2 %) electrophoresis, respectively. The extracted DNA was stored at –20 °C.

### Library preparation and sequencing

Among the nine hypervariable regions present in the 16S rRNA gene, the V5–V6 regions were chosen as

the target for prokaryotic identification (Chakravorty et al. 2007). The microbial DNA extracted from each sample, named respectively W1, W2, W3 and E1, E2, E3, was used as templates for the amplicon preparation. The strategy adopted for the amplicon library preparation consisted of two successive rounds of PCR. In the first round, the V5–V6 regions were amplified from the extracted DNA using the overhang primer pairs, named V5–V6 NextFor/Rev (5' TCGTCG GCAGCGTCAGATGTGTATAAGAGACAG [ATTAGA TACCCYGGTAGTCC] 3'/5' GTCTCGTGGGCTCGGAG ATGTGTATAAGAGACAG [ACGAGCTGACGACARC CATG] 3') and designed to contain (from 5' to 3' ends) the transposon Nextera's sequences (Nextera DNA sample preparation guide, Illumina) and the universal BV5 (Next For) and AV6 (Next Rev; Stecher et al. 2010) priming sequences (underlined nucleotides).

Amplification was performed using the Phusion<sup>®</sup> High-Fidelity DNA polymerase (Thermo Fisher Scientific, Inc., New England Biolabs) in a Mastercycler Thermal Cycler (Eppendorf, Hamburg, Germany). Each reaction mixture contained 0.25 ng of extracted DNA, 1X Buffer HF, 0.2 mM dNTPs, 0.5  $\mu$ M of each primer, and 1U Phusion DNA Polymerase in a final volume of 50  $\mu$ l. The cycling parameters for PCR were standardized as follows: initial denaturation 98 °C for 30 s, followed by 10 cycles of denaturation at 98 °C for 10 s, annealing at 58 °C for 30 s, extension at 72 °C for 15 s, and subsequently 15 cycles of denaturation at 98 °C for 10 s, annealing at 62 °C for 30 s, extension at 72 °C for 15 s, with a final extension step of 7 min at 72 °C. All PCRs were performed in the presence of a negative (Molecular Biology Grade Water, RNase/DNase-free water) and a positive (genomic DNA from pure culture of *Oenococcus oeni*) control. The PCR products (around 354 bp long) were visualized on 1.2 % agarose gel and purified using the AMPure XP Beads at a concentration of 0.8 $\times$  vol/vol (Agencourt Bioscience Corporation, Beverly, Massachusetts).

The purified amplicons were used as templates in the second PCR round, which was performed with the Nextera indices priming sequences as required by the dual index approach reported in the Nextera DNA sample preparation guide (Illumina). The dual index strategy consists of incorporating unique indices into both ends of the library molecules to allow sample identification for the subsequent bioinformatics analysis (Kozich et al. 2013).

The 50  $\mu$ l reaction mixture was made up of the following reagents: template DNA (40 ng), 1X Buffer HF, dNTPs (0.1 mM), Nextera index primers (index 1 and 2) and 1U Phusion DNA Polymerase system. The cycling parameters were those suggested by the Illumina Nextera protocol. The dual indexed amplicons obtained ( $\sim$ 420 bp) were purified using magnetic beads AMPure XP (0.6X, vol/vol), checked for quality control on Bioanalyzer 2100 (Agilent Technologies, Santa Clara, California) and quantified by fluorimetry using the Quant-iT<sup>™</sup> PicoGreen<sup>®</sup> dsDNA Assay Kit (Invitrogen, Carlsbad, California). Finally, equimolar ratios of the purified amplicons (six in total) were pooled and subjected to 2  $\times$  250 bp paired-end sequencing on the MiSeq platform. In order to increase the genetic diversity, as required by the MiSeq platform, the phage PhiX genomic DNA library was added to the mix and co-sequenced (Kozich et al. 2013).

#### Taxonomic binning

A modular pipeline, called BioMaS developed in our laboratory, was applied to analyze the paired-end (PE) reads obtained by the six samples analyzed (3 replicates/area) in order to characterize the bacterial composition. Before BioMaS analysis, a statistical and quality check of the reads was carried out by FastQC and denoising performed by trim-galore ([http://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)) setting a quality-score threshold equal to 25.

The BioMaS pipeline consists mainly of three steps. Because most of the generated reads were overlapping due to the amplicon length ( $\sim$ 420 bp) being shorter than the total sequenced PE length (2  $\times$  250 bp), in the first step, the overlapping PE reads were merged into contiguous consensus sequences using *Flash* (Magoc and Salzberg 2011). These were subsequently dereplicated by *Usearch* (Edgar 2010), but retaining the information about the total number of original consensus sequences. A small proportion of PE reads (generally about 5 %), which did not overlap to form a consensus contig probably due to the sequencing of amplicon subfragments. These were further cleaned through the removal of low quality regions and retained for further analysis as paired-end if both sequenced reads were  $\geq$ 50 bp long; otherwise, they were discarded. Then, a similarity-based analysis was

performed in order to infer the taxonomic origin of the generated metagenomic sequences. In particular, the consensus sequences and the non-overlapping pairs were mapped against the RDP database (Ribosomal Database Project; release 11.2; Cole et al. 2009) by means of Bowtie2 (Langmead and Salzberg 2012) and the mapping data were filtered using a Python script according to two parameters: identity % ( $\geq 97$  %) and query coverage ( $\geq 95$  %). The choice of this stringent % identity threshold allows classifying most of the reads up the species level (Mende et al. 2013). Finally, all mapped reads fulfilling the filters set were taxonomically annotated using the Tango tool (Clemente et al. 2011). All the residual unclassified reads were clustered in UTU (*unknown taxonomic units*) at  $\geq 97$  % similarity by *Usearch* and those represented by fewer than 5 sequences in all samples were discarded. Statistically significant differences of the bacterial population in different samples were determined at family, genus and species levels using the DESeq, an R/Bioconductor package (Anders and Huber 2010).

## Results

The water samples among the low-jellyfish (E1, E2, E3) and high-jellyfish (W1, W2, W3) areas did not differ significantly in the measured abiotic parameters (T, S, oxygen saturation; Table 1), merely showing a slight intrusion of cold freshwater in the E zone. Differences in oxygen saturation were not significant between the two sectors. From the membrane filters, microbial DNA samples (W1, W2, W3, E1, E2, E3) were extracted with average DNA yields of 2.64 and 1.97  $\mu\text{g}$  for W and E samples, respectively.

Six libraries of dual indexed amplicons of 420 bp (Fig. 2) were successfully sequenced on the MiSeq platform using a  $2 \times 250$  bp paired-end sequencing strategy. Approximately 7.5 million paired-end (PE) reads (i.e. 15 million total reads) were generated,

**Table 1** Abiotic parameters measured at E (low-jellyfish) and W (high-jellyfish) areas

	Temperature ( $^{\circ}\text{C}$ )	O <sub>2</sub> saturation (%)	Salinity (‰)
E	28.71 $\pm$ 0.40	95.48 $\pm$ 1.4	25.05 $\pm$ 0.45
W	28.12 $\pm$ 0.04	94.78 $\pm$ 1.4	26.22 $\pm$ 1.6

Data are reported as mean values  $\pm$  standard errors

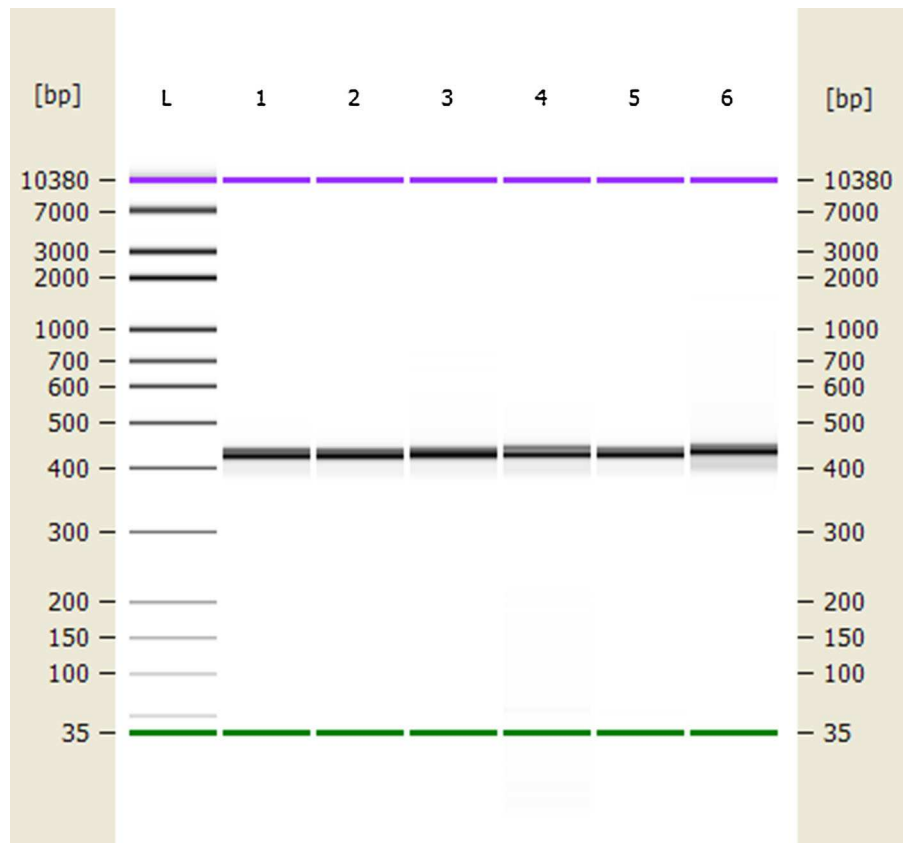
averaging 1.25 million PE reads (2.5 M reads) per sample replicate (see Sup. Table 1 in Supplementary Material). All sequenced samples generated reads of high quality, mostly having the expected length of 250 bp. The paired-end reads produced for the six samples (3 replicates/area) were subjected to *BioMaS* computation. First, the paired-end reads were merged and low quality regions removed from the non-overlapping reads. About 94 % of the paired-end reads were merged into a consensus sequence, while 2 % were treated as paired-end and the remaining 4 % were discarded. The sequences passing the quality control were then compared to the RDP database content. About 86.6 % of the analyzed sequences had at least one match in RDP above the adopted similarity cut-off (97 %). Finally, the taxonomic binning of the matching reads was carried out by using TANGO (Clemente et al. 2011). The PE reads matching against RDP with a percent identity above the threshold (86.6 %) were classified at the family (74.2 %), genus (38.2 %) and species (8.6 %) levels (Supplementary Tables). Remarkably, rarefaction curves (Fig. S1) indicated that species richness was comprehensively represented in all samples.

A total of 109 families, 213 genera and 353 species were represented in the samples, with clear differences between the W samples with high jellyfish density (84 families, 153 genera and 324 species) and the E samples with low-no jellyfish (Scorrano 2014; 103 families, 199 genera and 331 species; Supplementary Material).

The heat map showed clear-cut clustering of the bacterial populations in W and E samples (Fig. 3). Furthermore, the beta-diversity values calculated for all nine pairwise comparisons involving the W and E samples supported very good reproducibility between microbial communities among the three replicates ( $0.151 \pm 0.016$ ).

Statistically significant differences between the aquatic bacterial communities in the W and E zones were determined at family, genus and species levels using *DESeq* (Table 2 and Supplementary Tables 3, 5, 7). In particular, we observed significant differences in the occurrence of 16 families (Halomonadaceae, Saprospiraceae, Shewanellaceae, Flavobacteriaceae, Chloroplast, Oceanospirillaceae, Cryomorphaceae, Comamonadaceae, Flammeovirgaceae, Rhodobacteraceae, Family\_II (Cyanobacteria), Acidimicrobiaceae, Simkaniaceae, Acholeplasmataceae, Rhodospirillaceae,

**Fig. 2** Agilent Bioanalyzer chip profile of purified amplicons (420 bp), relative to V5–V6 hypervariable region of the bacterial 16S rRNA gene, obtained applying the modified Nextera's protocol for the six samples (W1, W2, W3 and E1, E2, E3). L, molecular weight marker. Lanes 1–3, West samples (W1, W2, W3); lanes 4–6, East samples (E1, E2, E3). Bands at 35 and 10,380 bp represent internal size standards and not DNA from the sample



Microbacteriaceae), 22 genera and 61 species in the W and E samples (Table 2).

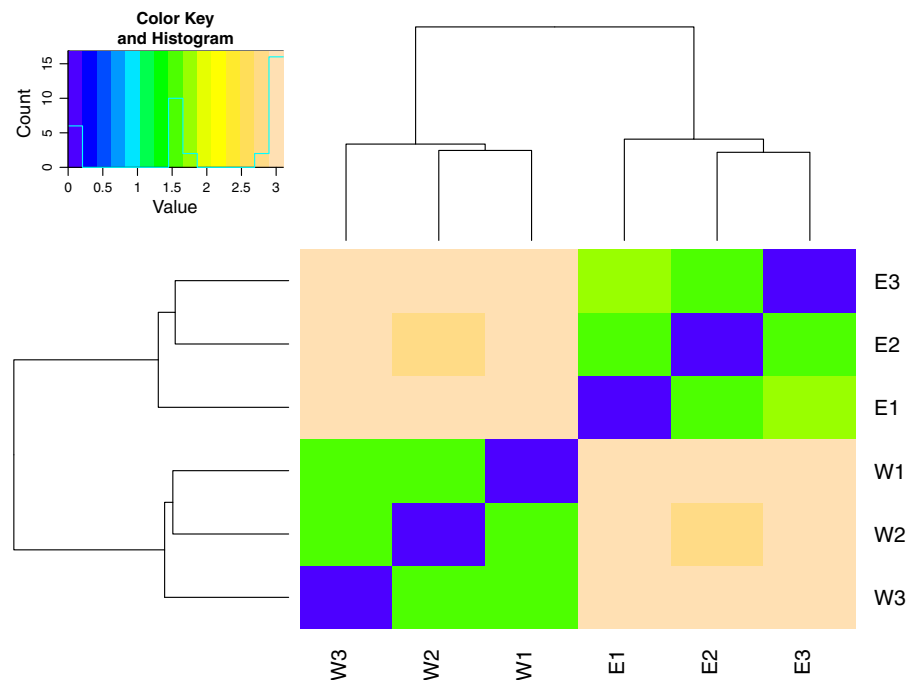
The families Halomonadaceae, Saprospiraceae, Shewanellaceae, Chloroplast, Flammeovirgaceae, Family\_II (Cyanobacteria), and Simkaniaceae were significantly enriched in the jellyfish-rich (W) zone with an over-representation of several genera including *Halomonas*, *Haliscomenobacter*, *Shewanella*, *Fabibacter*, *Roseivirga* and *Owenweeksia* (see the relevant normalized counts in Supplementary Material). Interestingly, the amplicon based approach detected also the chloroplast rRNA of *Bacillariophyta* and an over-representation of diatoms the W zone was significantly supported. Conversely, the families Oceanospirillaceae, Comamonadaceae, Acholeplasmataceae, Acidimicrobiaceae, Cryomorphaceae, Flavobacteriaceae, Rhodobacteraceae, Rhodospirillaceae, Microbacteriaceae were significantly enriched in the jellyfish-poor (E) zone with several over-represented genera including *Cellulophaga*, *Crocinitomix*, *Ilumatobacter*, *Diaphorobacter*. The tree in Fig. S2 graphically illustrates significantly

over- and under- represented families and genera in the jellyfish-rich zones (all six sample-specific trees generated by BioMaS tool are available in Supplementary Figs. 3–8).

About 12.8 % of the reads did not show any significant match ( $\geq 97$  % identity) against the RDB. Those reads were probably derived from novel species not represented in the RDB and were classified as UTU. In total, we detected 44 UTUs, showing significantly different abundances in the W and E zones (Supplementary Tables 8–9).

## Discussion

Molecular tools are increasingly used for screening biodiversity in terrestrial and aquatic ecosystems to develop appropriate management programs (e.g. Bourlat et al. 2013; Darling and Mahon 2011; Geller et al. 2010; Karp et al. 1997). In the marine environment, molecular methods can reveal patterns of



**Fig. 3** Heat map visualization at the family level of the six samples (W1, W2, W3 and E1, E2, E3), obtained applying the DESeq package on the taxonomic data produced by BioMaS, which show the clustering of samples from jellyfish-rich and jellyfish-poor sites

community dynamics and ecosystem functioning processes by rapid detection of the identities of a vast majority of taxa in a community and, therefore, are used for environmental monitoring programs, directly contributing to development of management strategies of coastal areas. Molecular methods can play an important role in several fields of aquatic bioinvasions, including identification of non-indigenous species and their origins, historical reconstruction of invasive routes and the underlying pathways, estimation of genetic connectivity among populations, as well as detection of overall changes in the residential native community induced by new invaders (e.g. Darling and Blum 2007; Darling and Mahon 2011; Estoup and Guillemaud 2010; Geller et al. 2010; Ghabooli et al. 2013; Hänfling 2007; Miura 2007; Muirhead et al. 2008; Piraino et al. 2014). In particular, NGS platforms are known as rapid and sensitive means of characterizing complex microbial communities, and are supplanting traditional surveys of microbial diversity based on initial cultivation screenings followed by PCR amplification, cloning and sequencing of universally conserved molecules, such as the 16S rRNA gene (reviewed by Degnan and Ochman 2012).

We investigated the changes in the planktonic microbiome diversity of a lagoon habitat probably driven by the invasion and proliferation of a non-indigenous jellyfish (*Aurelia* sp.). In parallel, this study offered the opportunity to verify the effectiveness of coupling the Illumina protocol with the BioMaS pipeline. Significant differences in microbiota abundance were determined at family, genus and species levels (Table 2 and Supplementary Material) and interpreted as related to changes in the nutrient availability. Indeed, jellyfish carcasses and mucous exudates possibly serve as important carbon sources for these bacteria. The total organic content of jellyfish, in fact, generally consists of carbohydrates ( $7 \pm 5\%$ ), intermediate lipids ( $22 \pm 12\%$ ), and especially proteins ( $72 \pm 14\%$ ; Billett et al. 2006; Doyle et al. 2006; Pitt et al. 2009; Yamamoto et al. 2008).

Interestingly, some of these results are confirmed by a study on the microbial diversity in the Varano lagoon conducted by traditional analysis of the cultivable heterotrophic bacterial community, followed by the standard PCR-based amplification of the 16S rRNA gene (Caprioli 2014; Stabili et al. 2013).



**Table 2** Statistically significant differences of the bacterial population in East (E) and West (W) areas at family (A), genus (B) and species (C) level

ID	E nCount	W nCount	FoldChange	Pval	Padj
<b>(A) Family</b>					
Halomonadaceae	4.59E+04	9.04E+04	1.97	2.51E−38	2.74E−36
Saprosiraceae	1.89E+04	3.92E+04	2.08	3.52E−30	1.92E−28
Shewanellaceae	2.15E+04	3.89E+04	1.81	4.10E−21	1.49E−19
Flavobacteriaceae	9.84E+04	6.13E+04	0.62	2.80E−20	7.63E−19
Chloroplast	2.01E+03	6.47E+03	3.21	3.94E−18	8.58E−17
Oceanospirillaceae	3.48E+03	8.25E+02	0.24	1.30E−14	2.36E−13
Cryomorphaceae	3.28E+04	2.01E+04	0.61	4.34E−14	5.92E−13
Comamonadaceae	6.91E+03	2.65E+03	0.38	4.06E−14	5.92E−13
Flammeovirgaceae	5.63E+04	7.81E+04	1.39	3.67E−10	4.45E−09
Rhodobacteraceae	4.90E+04	3.42E+04	0.70	7.31E−10	7.97E−09
Family_II	1.68E+05	2.48E+05	1.48	7.65E−09	7.58E−08
Acidimicrobiaceae	6.84E+03	3.95E+03	0.58	3.56E−06	3.23E−05
Simkaniaceae	1.13E+02	8.92E+02	7.87	5.35E−06	4.49E−05
Acholeplasmataceae	2.77E+03	1.47E+03	0.53	0.00	0.02
Rhodospirillaceae	3.27E+04	2.75E+04	0.84	0.01	0.06
Microbacteriaceae	2.53E+05	2.19E+05	0.86	0.01	0.08
<b>(B) Genus</b>					
<i>Halomonas</i>	8.73E+04	1.83E+05	2.09	8.49E−64	1.81E−61
<i>Haliscomenobacter</i>	3.58E+04	7.89E+04	2.20	1.75E−50	1.86E−48
<i>Shewanella</i>	4.10E+04	7.86E+04	1.92	3.39E−36	2.41E−34
<i>GpIIa</i>	3.19E+05	5.01E+05	1.57	3.88E−23	2.06E−21
<i>Crocinitomix</i>	3.63E+04	1.81E+04	0.50	1.82E−22	7.74E−21
<i>Fabibacter</i>	6.83E+04	1.01E+05	1.47	2.34E−17	8.32E−16
<i>Diaphorobacter</i>	1.00E+04	3.67E+03	0.37	1.58E−13	4.80E−12
<i>Bacillariophyta</i>	1.30E+03	5.15E+03	3.96	5.40E−12	1.44E−10
<i>Roseivirga</i>	3.34E+04	4.98E+04	1.49	1.65E−11	3.90E−10
<i>Paracoccus</i>	3.21E+01	1.88E+03	58.51	2.81E−11	5.99E−10
<i>Nitrincola</i>	4.19E+03	9.72E+02	0.23	6.31E−11	1.22E−09
<i>Cellulophaga</i>	6.58E+03	2.55E+03	0.39	9.21E−09	1.64E−07
<i>Owenweeksia</i>	6.65E+03	1.25E+04	1.87	4.56E−08	7.47E−07
<i>Ilumatobacter</i>	1.30E+04	7.97E+03	0.61	8.13E−06	0.00
<i>Simkania</i>	2.20E+02	1.81E+03	8.24	1.16E−05	0.00
<i>Haloferula</i>	3.63E+02	1.71E+03	4.71	1.24E−05	0.00
<i>Loktanella</i>	2.70E+01	5.41E+02	20.05	7.48E−05	0.00
<i>Micrococcus</i>	0.00E+00	2.38E+02	Inf	8.47E−05	0.00
<i>Umboniibacter</i>	1.88E+02	0.00E+00	0.00	0.00	0.00
<i>Acholeplasma</i>	5.23E+03	2.95E+03	0.56	0.00	0.01
<i>Arenibacter</i>	1.97E+03	7.94E+02	0.40	0.00	0.02
<i>Legionella</i>	1.07E+02	0.00E+00	0.00	0.01	0.09
<b>(C) Species</b>					
<i>Paracoccus</i> sp. <i>TDMA-8</i>	0.00E+00	1.21E+03	Inf	2.61E−26	9.20E−24
<i>Paracoccus</i> sp. <i>KD58-7</i>	0.00E+00	1.15E+03	Inf	2.64E−25	4.66E−23
<i>Nitrincola</i> <i>lakisaponensis</i>	4.56E+03	6.47E+02	0.14	2.20E−21	2.59E−19

Table 2 continued

ID	E nCount	W nCount	FoldChange	Pval	Padj
<i>Paracoccus</i> sp. <i>PaH2.06a</i>	0.00E+00	1.16E+03	Inf	1.09E−18	9.64E−17
<i>Nitrincola</i> sp. <i>LAR05R3</i>	4.49E+03	6.53E+02	0.15	2.47E−18	1.74E−16
<i>Nitrincola</i> sp. <i>E-038</i>	4.48E+03	7.66E+02	0.17	3.27E−18	1.92E−16
<i>Nitrincola</i> sp. <i>LAR05R4</i>	4.16E+03	7.24E+02	0.17	6.95E−17	3.50E−15
<i>Umboniibacter marinipunicus</i>	1.01E+03	0.00E+00	0.00	1.72E−15	7.57E−14
<i>Nitrincola</i> sp. <i>LAR05R7</i>	3.90E+03	7.33E+02	0.19	6.56E−15	2.57E−13
<i>Diaphorobacter</i> sp. enrichment culture clone <i>NFL-7</i>	3.77E+03	8.48E+02	0.22	2.96E−12	1.05E−10
<i>Diaphorobacter</i> sp. <i>TPD-1</i>	3.70E+03	8.32E+02	0.22	4.24E−12	1.36E−10
<i>Diaphorobacter</i> sp. <i>F2</i>	3.72E+03	8.49E+02	0.23	5.13E−12	1.51E−10
<i>Loktanella cinnabarina</i>	0.00E+00	3.89E+02	Inf	8.37E−12	2.27E−10
<i>Diaphorobacter</i> sp. <i>GS-1</i>	3.74E+03	8.80E+02	0.24	1.05E−11	2.65E−10
<i>Ilumatobacter fluminis</i>	3.85E+04	1.57E+04	0.41	3.39E−11	7.98E−10
<i>Legionella</i> sp. <i>H052920586</i>	3.78E+02	0.00E+00	0.00	3.63E−11	8.00E−10
<i>Diaphorobacter</i> sp. <i>KOTLB</i>	3.67E+03	8.99E+02	0.25	5.48E−11	1.09E−09
<i>Diaphorobacter</i> sp. <i>PD-7</i>	3.57E+03	8.60E+02	0.24	5.58E−11	1.09E−09
<i>Diaphorobacter</i> sp. <i>bk_62</i>	3.64E+03	9.13E+02	0.25	8.53E−11	1.58E−09
<i>Ilumatobacter nonamiensis</i> <i>YM16-303</i>	2.89E+04	1.22E+04	0.42	9.78E−11	1.73E−09
<i>Diaphorobacter</i> sp. <i>Phe82</i>	3.60E+03	8.89E+02	0.25	1.08E−10	1.81E−09
<i>Diaphorobacter</i> sp. <i>P2</i>	3.65E+03	9.40E+02	0.26	1.83E−10	2.94E−09
<i>Diaphorobacter</i> sp. <i>MC-pb1</i>	3.34E+03	8.41E+02	0.25	9.25E−10	1.42E−08
<i>Diaphorobacter</i> sp. <i>R9</i>	3.49E+03	9.31E+02	0.27	1.05E−09	1.55E−08
<i>Diaphorobacter</i> sp. <i>DS3</i>	3.72E+03	9.19E+02	0.25	1.49E−09	2.10E−08
<i>Diaphorobacter</i> sp. <i>R-41385</i>	3.36E+03	9.02E+02	0.27	3.16E−09	4.28E−08
<i>Diaphorobacter</i> sp. <i>PD-12</i>	2.95E+03	7.08E+02	0.24	3.41E−09	4.46E−08
<i>Diaphorobacter</i> sp. <i>NA5</i>	3.45E+03	9.61E+02	0.28	4.13E−09	5.21E−08
<i>Haloferula phyci</i>	1.54E+03	5.03E+03	3.26	8.63E−09	1.05E−07
<i>Nitrincola</i> sp. <i>E-048</i>	4.32E+02	0.00E+00	0.00	6.46E−08	7.61E−07
<i>Loktanella</i> sp. <i>K4B-4</i>	3.97E+00	2.86E+02	72.10	1.36E−05	0.00
<i>Loktanella</i> sp. <i>DSSK1-5</i>	0.00E+00	1.88E+02	Inf	2.53E−05	0.00
<i>Loktanella</i> sp. <i>SS16.2</i>	7.93E+00	3.08E+02	38.76	4.21E−05	0.00
<i>Halomonas rifensis</i>	2.05E+05	2.94E+05	1.43	5.39E−05	0.00
<i>Oceanicola pacificus</i>	2.72E+03	1.06E+03	0.39	6.09E−05	0.00
<i>Halomonas phoceae</i>	1.49E+05	2.09E+05	1.40	6.58E−05	0.00
<i>Halomonas</i> sp. <i>Sa4-2I</i>	6.73E+04	9.50E+04	1.41	0.00	0.00
<i>Loktanella</i> sp. <i>IAJ4</i>	0.00E+00	1.61E+02	Inf	0.00	0.00
<i>Halomonas</i> sp. <i>A-7</i>	1.90E+04	2.62E+04	1.38	0.00	0.00
<i>Nisaea</i> sp. <i>MOLA 131</i>	1.52E+02	0.00E+00	0.00	0.00	0.00
<i>Roseovarius</i> sp. <i>kmb21</i>	6.24E+03	3.51E+03	0.56	0.00	0.00
<i>Loktanella</i> sp. <i>SS16.12</i>	1.62E+01	2.98E+02	18.44	0.00	0.01
<i>Shewanella</i> sp. <i>AM9</i>	2.99E+04	3.97E+04	1.33	0.00	0.01
<i>Marivita cryptomonadis</i>	1.18E+03	3.06E+02	0.26	0.00	0.01
<i>Shewanella</i> sp. <i>SG133</i>	1.36E+04	1.85E+04	1.36	0.00	0.01
<i>Polaribacter</i> sp. <i>p1s1</i>	3.50E+02	5.10E+01	0.15	0.01	0.04
<i>Loktanella</i> sp. <i>USW116</i>	0.00E+00	1.16E+02	Inf	0.01	0.04

**Table 2** continued

ID	E nCount	W nCount	FoldChange	Pval	Padj
<i>Roseovarius</i> sp. <i>RP8</i>	6.18E+03	3.54E+03	0.57	0.01	0.04
<i>Corynebacterium kroppenstedtii</i>	0.00E+00	2.17E+02	Inf	0.01	0.06
<i>Tenacibaculum</i> sp. <i>35</i>	1.10E+03	3.61E+02	0.33	0.01	0.06
<i>Shewanella</i> sp. <i>UAM38</i>	2.36E+04	3.07E+04	1.30	0.01	0.06
<i>Shewanella</i> sp. <i>W3</i>	1.37E+04	1.77E+04	1.29	0.01	0.06
<i>Shewanella</i> sp. <i>enrichment culture clone HS-shanghai2011-1</i>	1.55E+04	1.99E+04	1.28	0.01	0.07
<i>Haloferula rosea</i>	3.12E+02	9.67E+02	3.10	0.01	0.07
<i>Lactococcus</i> sp. <i>TP1MK</i>	0.00E+00	1.07E+02	Inf	0.01	0.07
<i>Tenacibaculum</i> sp. <i>MGP-74/AN6</i>	1.04E+03	3.19E+02	0.31	0.01	0.07
<i>Tenacibaculum</i> sp. <i>MAR_2010_175</i>	9.85E+02	3.19E+02	0.32	0.01	0.07
<i>Shewanella</i> sp. <i>YASM-24</i>	8.17E+03	1.08E+04	1.33	0.01	0.08
<i>Halomonas</i> sp. <i>A-9</i>	1.01E+04	1.48E+04	1.47	0.01	0.08
<i>Shewanella</i> sp. <i>BJGMM-B28</i>	1.46E+04	1.86E+04	1.27	0.02	0.09
<i>Hydrogenophaga</i> sp. <i>GPTSA100-12</i>	1.03E+02	0.00E+00	0.00	0.02	0.09

The following items are shown: the taxonomic name (ID); the normalized reads count for the E condition and W Condition (nCount); the W/E foldChange; the *p* value for the statistical significance of the change (Pval); the *p* value adjusted for multiple testing with the Benjamini-Hochberg procedure (Padj), which controls the False Discovery Rate (FDR < 0.1)

This study from cultivable bacteria corroborated a significant difference between low-jellyfish and high-jellyfish areas of the lagoon, and showed a prevalence of Shewanellaceae in the presence of jellyfish, as also detected by our metagenomic approach. Overall, jellyfish may influence bacterial abundance and composition by shifting the native microbial community towards more opportunistic consortia with higher metabolic potential for exploitation of mucopolysaccharides and glycoproteins from jellyfish tissues. In spite of considerable evidence for predation on jellyfish tissues (e.g. Arai 2005; Milisenda et al. 2014), jellyfish are usually considered to be a low-value biomass not easily available to higher trophic levels and dependent on the microbial loop for recycling (Condon et al. 2011). Overall, the observed differences in the bacterial community might be interpreted as an ecosystem response to allow adaptation of metabolic pathways to the jellyfish-derived organic carbon substrate. The biochemical and ecological characteristics of the heterotrophic bacterial taxa that are selectively promoted in the Varano lagoon in the areas under massive jellyfish blooms reveal some adaptive features favoring microbial growth and selection on jellyfish tissues or on decaying jellyfall organic matter, as follows.

The phenotypically diverse family Halomonadaceae consists mostly of marine and moderately halophilic bacteria (Arahal et al. 2008) adapted to a wide range of salinities. Members of this family are Gram-negative, either slight or moderate halophiles with straight or curved, rod-shaped cells (Baumann et al. 1983; Bouchotroch et al. 2001; Duckworth et al. 2000; Franzmann et al. 1987; Mellado et al. 1995; Mormile et al. 1999; Quesada et al. 1984, 1990; Valderrama et al. 1991; Vreeland et al. 1980). This bacterial group is characterized by the production of extracellular polymers including exopolysaccharides (EPS), which has been described as a strategy for growth (Costerton 1999). Studies of bacteria growing in aquatic systems on marine sediments, aggregates and detrital particles show that nearly all of the cells are surrounded by EPS (Costerton 1999; Decho 1990) and many of the cells are enclosed with adherent biofilms. Thus, the significantly higher abundance of Halomonadaceae in the W zone might be related to their ability to produce EPS for adhesion to rich particulate organic matter derived from jellyfall aggregates. As an example, *Halomonas rifensis* is a recently described species of the genus (Amjres et al. 2011). It is an EPS-producing, halophilic bacterium capable of growth in salt concentrations (mixture of

sea salts) of 0.5–20 % (weight/volume, optimum 5–7.5 % w/v), incapable of growing without NaCl, grows at 25–45 °C (optimum 32 °C) and at pH 5–10 (optimum pH 6–9). Nevertheless, the jellyfish-associated growth of Halomonadaceae might be an indirect consequence due to release from competition with less tolerant prokaryotic groups. Interestingly, also in Jellyfish Lake, Palau known for the large resident population of *Mastigias papua*, members of Halomonadaceae dominated among cultivable heterotrophic bacteria (Venkateswaran et al. 1993).

The family Shewanellaceae was established from the amended description of a group of marine Alteromonas-like bacteria (Ivanova et al. 2004a, b, c). The Shewanellaceae comprises Gram-negative, straight or curved rod-shaped, aerobic or facultative anaerobic and readily cultivated gammaproteobacteria isolated frequently in the marine environment from diverse sources, including red algae (Simidu et al. 1990), a tidal flat (Yoon et al. 2004a), seawater (Ivanova et al. 2001, 2004b; Yoon et al. 2004b), sediments (Venkateswaran et al. 1998) and marine invertebrates (Ivanova et al. 2004c). In particular, *Shewanella* spp., significantly abundant in the W zone, have been recovered from red algae and are occasionally human pathogens (Sharma and Kalawat 2010).

Although bacteria of the genus *Shewanella* belong to one of the readily cultivable groups of microorganisms, little is known about their occurrence and abundance in marine ecosystems. Members of *Shewanella* usually are found in marine environments in warm climates or during summer in temperate climates. One of the striking features of these bacteria is the ability of some recently described species to produce polyunsaturated fatty acids (PUFAs) at relatively high incubation temperatures (25–30 °C; Ivanova et al. 2001, 2003; Skerratt et al. 2002), which contradicts the idea that only barophilic or cold-adapted species are able to produce significant levels of PUFAs. Notably, most of these microorganisms, including several *Shewanella* species particularly abundant in the W zone, are metabolically active and produce a range of hydrolytic enzymes, i.e. proteinases (gelatinases and caseinases), lipases, amylases, agarases, and alginases. It is most likely that bacteria of this genus can actively degrade organic matter, including the gelatinous material from jellyfalls. It is noteworthy that the genus *Shewanella* is part of the normal bacterial symbiotic flora in cnidarians,

including the moon jellyfish *Aurelia aurita* in the North Sea (Schuett and Doepke 2010; LaDouceur et al. 2013).

The families Flammeovirgaceae and Saprospiraceae, which were more abundant in the W zone, belong to the phylum Bacteroidetes also known as the Bacteroides–Cytophaga–Flexibacter group. Members of the phylum Bacteroidetes are the most abundant bacteria in the ocean after Proteobacteria and Cyanobacteria (Amaral-Zettler et al. 2011; Glockner et al. 1999; Kirchman 2002). They account for a significant fraction of marine bacterioplankton especially in coastal areas, where they represent between 10 and 30 % of the total bacterial counts (Alonso-Saez and Gasol 2007). Bacteroidetes are increasingly regarded as specialists in degradation of high molecular weight organic matter, i.e., proteins and carbohydrates (Thomas et al. 2011) and have a preference for growth attached to particles and surfaces, including algal cells. Because of these features, their significant presence in the W zone may be related to a lifestyle as jellyfish-associated bacteria. In particular, the family Flammeovirgaceae currently includes the genera *Aureibacter*, *Csiribacter*, *Fabibacter*, *Flammeovirga*, *Flexithrix*, *Fulvivirga*, *Limibacter*, *Marivirga*, *Nafulsella*, *Perexilibacter*, *Persicobacter*, *Porifericola*, *Rapidithrix*, *Reichenbachiella*, *Roseivirga*, *Sediminitomix* and *Tunicatimonas* (Kim et al. 2013). Interestingly, among the genera determined by DESeq, *Fabibacter* and *Roseivirga* were significantly more abundant in the W zone. These two genera share many phenotypic features and were previously isolated together from the marine sponge *Tedania ignis* in the Bahamas (Lau et al. 2006). The family Saprospiraceae (Yoon et al. 2012) consists of the genera *Aureispira*, *Haliscomenobacter*, *Lewinella*, and *Saprospira* found in various habitats especially as epiphytic protein-hydrolyzing bacteria. Large, filamentous microorganisms are widely distributed in freshwater lakes (Schauer and Hahn 2005) and are believed to play an important roles there. Molecular evidence demonstrates that members of the family Saprospiraceae are also present in hypersaline mats (Ley et al. 2006), and three strains of gliding bacteria belonging to the genus *Saprospira* have been isolated from marine sponges and algae from the southern coastline of Thailand (Hosoya et al. 2006). Of these genera, only the genus *Haliscomenobacter* was detected in the W zone. Members of Bacteroidetes

are generally associated with the degradation of complex organic materials (Cottrell and Kirchman 2000; Reichenbach 1991; Riemann et al. 2000), but other than *Haliscomenobacter*, little is known about the detailed ecophysiology of other Saprospiraceae. Isolates of *Haliscomenobacter* hydrolyze starch and grow aerobically on glucose, N-acetylglucosamine, lactose, and sucrose, but not on glycerol, lactate, acetate, and succinate (van Veen et al. 1973). Therefore, in the Varano lagoon in presence of jellyfish bloom, members of *Haliscomenobacter* may be involved in the hydrolysis of polysaccharides to utilize the hydrolysates as energy and carbon sources for growth. Thus, Bacteroidetes probably have a different life strategy that do other marine bacteria, such as Cyanobacteria (Family\_II GpIIa), which are photoautotrophs and also were more abundant in the W zone.

The Micrococcaceae family is composed of bacteria with either respiratory and/or fermentative metabolism, using carbohydrate and/or amino acid substrates, which are the main components of jellyfish organic matter. In particular, *Micrococcus*, which was significantly more abundant in the W zone, includes ubiquitous Gram-positive bacteria species. Its presence in marine habitats has not been frequently reported, but unspecified micrococci were described from different seawater locations (Sieburth 1979), and a *Micrococcus* sp. was reported as a commensal of the sponge *Tedania ignis* (Stierle et al. 1988).

Few methodologies for Illumina-based analyses of microbial communities have been reported to date (Caporaso et al. 2012; Degnan and Ochman 2012; Kozich et al. 2013). In particular, the strategy of Caporaso et al. (2012) requires the synthesis of a large collection of different primers as a function of the number of samples to be tested; it was applied to analysis of only the variable V4 region of the 16S rRNA gene and referred to a previous single indexed Illumina sequencing system. More recently, Kozich et al. (2013) set up a dual index paired-read approach based on custom primers, employing a lower number of primer pairs during library construction. Nevertheless, in both approaches, the number of custom primers depends on the number of samples and especially on the number of regions to be tested in the same run. The strategy adopted here, based on the Nextera Illumina protocol and a dual-indexing principle, may overcome those issues, because it reduces the number of primer

pairs to be used and, therefore, allows analysis of more samples per run at reduced costs.

The above results show significant differences in the composition of the community of bacteria and a few microalgae, between the samples from the W and E areas, separated by only 5–6 km, but characterized by high numbers or near absence of jellyfish, respectively. The simple experimental design of this pilot study specifically addressed the issue of the usefulness and reproducibility of NGS methods to rapidly obtain point information on ecological phenomena, such as jellyfish outbreaks, occurring within temporally limited windows. For this reason, we cannot rule out the possibility that the observed differences in the microbial structure in the water column might have preceded the jellyfish invasion, depending on area-specific, chemico-physical features and/or some biotic factors (e.g. organic matter, Chl-a content) and processes related to the structure and function of the biological communities (e.g. primary production, predation, competition, decomposition). Nonetheless, in spite of strong seasonal fluctuations of most environmental variables (as typical of confined coastal habitats), the Varano lagoon is apparently a relatively homogeneous basin from spatial, hydrological, and geomorphological standpoints. Previous studies on the water column, sediments, and biological features (organic matter, Chl-a, phyto- and zooplankton compositions) did not show significant differences across the lagoon and, specifically, between the two areas (high-jellyfish vs low-jellyfish) (Caroppo 2000; Spagnoli et al. 2002; Specchiulli et al. 2008), which are marked by a comparable level of confinement (e.g. in terms of relative distance from the open sea). One remarkable difference is the abundance of artificial structures in the western area of the lagoon, where mussel seed farming is common. Artificial substrates are usually known as the preferential site of settlement of planula larvae and their development into the polyp stage (Holst and Jarms 2007; Lo et al. 2008). The jellyfish spatial segregation might also be favoured by the jellyfish aggregative locomotion, actively directed against the water flow (Rakow and Graham 2006). The lack of consistent data corroborating alternative explanations, and the relative spatial homogeneity of the Varano lagoon both indirectly suggest that jellyfish outbreaks can be considered at least one of the main drivers of the microbial community differences between the two areas.

To our knowledge, this is the first time that a NGS platform has been used to screen changes of the aquatic microbiome associated with the proliferation of an invasive alien species. Here we valued the potential of NGS analysis for assessing the impact of jellyfish outbreaks on carbon flow in aquatic food webs and ecosystem functioning, promisingly offering additional perspectives for the use of NGS methods in ecological investigations. Invasive jellyfish species producing large outbreaks in coastal ecosystems may have significant impacts on bacterial population dynamics and nutrient pathways with major ecological implications. The use of fast and sensitive methodologies, such as Illumina-based sequencing surveys, will allow rapidly gaining major insights into the functioning of coastal ecosystems and adopting the best practices for management and valuation of coastal habitats and their living resources.

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