ARTICLE IN PRESS

Egyptian Journal of Aquatic Research xxx (xxxx) xxx

Contents lists available at ScienceDirect

Egyptian Journal of Aquatic Research

journal homepage: www.elsevier.com/locate/ejar

Full length article

Response of seaweed associated microbiome to environmental disturbances from the Gulf of Aqaba, Jordan

Abdul-Salam Juhmani [a,*], Alessandro Vezzi [b], Mohammed Wedyan [a], Alessandro Buosi [c], Mohammad Wahsha [d], Fabio De Pascale [b], Baker Al-Shara [e], Riccardo Schiavon [f], Adriano Sfriso [c], Andrea A. Sfriso [g]

[a] Department of Biology and Biotechnology, Faculty of Science, The Hashemite University, Zarqa 13133, Jordan
[b] Department of Biology, University of Padua, Via U. Bassi 58/B, 35131 Padua, Italy
[c] Department of Environmental Sciences, Informatics and Statistics, Ca'Foscari University of Venice, Via Torino 155, 30172 Venezia, Italy
[d] Marine Science Station, The University of Jordan, Aqaba Branch, 77110 Aqaba, Jordan
[e] Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Jadara University, Irbid, Jordan
[f] BMR Genomics, Via Redipuglia 21a, 35131 Padua, Italy
[g] Department of Life Sciences and Biotechnology, University of Ferrara, Via Fossato di Mortara 17, 44121 Ferrara, Italy

ARTICLE INFO

Keywords:
Microbial Community
Ulva, Illumina
Gulf of Aqaba

ABSTRACT

The seaweed surface mediates interactions between the alga and its environment. Associated microbial communities can influence host morphology, organic matter consumption, and defence. Environmental drivers interact with these communities, affecting host fitness. This study investigated the influence of anthropogenic disturbances on prokaryotic communities associated with the green seaweed *Ulva lactuca* Linnaeus in the Gulf of Aqaba, Jordan. Seaweed samples were collected from three impacted sites during winter and summer. The composition of seaweed-associated microbial communities (SAMCs) was determined using 16S rRNA gene amplicon sequencing targeting the V4 hypervariable region using the Illumina NextSeq 500 platform. The results revealed significant seasonal shifts in SAMC composition, with Firmicutes dominating in winter and Bacteroidetes and Proteobacteria being more prevalent in summer. Higher alpha diversity was observed in summer samples, possibly due to increased nutrients availability. Environmental parameters, notably pH and dissolved oxygen, strongly influenced the distribution of algae-associated bacterial groups, accounting for 77.3 % of the observed variation in SAMCs between seasons. This study provides valuable insights into the dynamic nature of SAMCs in response to diverse environmental disturbances. Our findings underscore the ecological significance of SAMCs and their potential utility as sensitive indicators of environmental perturbations within marine ecosystems. Furthermore, this research contributes to the development of strategies aimed at the conservation and sustainable management of vulnerable coastal ecosystems.

Introduction

The seaweed surface (SS) constitutes a critical interface between the alga and its environment. Seaweeds perceive and respond to environmental and anthropogenic disturbances through their SS (Wahl et al., 2012). This interface is also central to major exchange processes, including the uptake and release of essential nutrients and metabolic compounds (Mancuso et al., 2016). Interactions between seaweeds and bacteria regulate a wide range of functions, from morphological development (Marshall et al., 2006; Nakanishi et al., 1996; Singh et al., 2011) and utilization of organic matter and nitrogen sources (de Oliveira et al., 2012) to defence mechanisms (Campbell et al., 2011; Paul et al., 2006) and the provision of vitamins (Croft et al., 2005). Although research on seaweed-microbiota interactions has intensified (e.g., Bengtsson et al., 2012; Campbell et al., 2015; Hollants et al., 2013; Wahl et al., 2012), the precise mechanisms by which seaweeds tolerate and adapt to biotic and abiotic stressors remain largely elusive.

Recent studies have highlighted the disruption and spatiotemporal dynamics of seaweed-associated microbial communities (SAMCs) in response to diverse environmental and anthropogenic stressors (e.g.,

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* Corresponding author.
E-mail address: A.juhmani@hu.edu.jo (A.-S. Juhmani).

https://doi.org/10.1016/j.ejar.2025.02.005
Received 20 May 2024; Received in revised form 14 February 2025; Accepted 16 February 2025

Please cite this article as: Abdul-Salam Juhmani et al., *Egyptian Journal of Aquatic Research*, https://doi.org/10.1016/j.ejar.2025.02.005

Burke et al., 2011; Goecke et al., 2010; Lachnit et al., 2011; Mancuso et al., 2016; Tujula et al., 2010). Spatial variations in SAMCs have been observed, such as the high species variability among microbial communities associated with *Ulva australis* Areschoug sampled from different rock pools (Burke et al., 2011). However, many studies have focused on seasonal variations in SAMC composition, identifying bacterial taxa characteristic of specific seasons (Lachnit et al., 2011; Mancuso et al., 2016; Serebryakova et al., 2018). For example, bacterial communities associated with *Fucus vesiculosus* Bory exhibited persistent seasonal variation at the phylum level (Lachnit et al., 2010). Recent research on the Mediterranean seaweed *Cystoseira compressa* (Esper) Gerloff & Nizamuddin also revealed notable SAMC dynamics (Mancuso et al., 2016). Specifically, epiphytic bacterial communities on *C. compressa* thalli changed over time, with a dominance of pathogenic bacteria observed during thallus degradation at the end of its annual life cycle (Mancuso et al., 2016).

While seasonal variations in SAMCs have been studied, a comprehensive understanding of how these variations interact with both environmental and anthropogenic pressures remain limited. SAMC composition can be influenced by biotic factors, such as nutrient availability (Hahn, 2006), and abiotic factors, including seawater temperature (Tujula et al., 2010) and metal concentrations (Jordaan et al., 2019). For example, elevated summer temperatures can alter microbial communities, as seen in *Laminaria hyperborea* (Gunnerus) Foslie, which exhibited high microbial diversity (Bengtsson et al., 2010). Interestingly, some bacteria associated with *L. hyperborea*, such as Betaproteobacteria and Verrucomicrobia, preferred cooler temperatures (below 10°C) (Bengtsson et al., 2010). These changes are likely to be exacerbated by climate change and ocean acidification (Aires et al., 2018; Liu et al., 2010; O'Brien et al., 2016). This study addresses this gap by investigating the seasonal dynamics of *Ulva*-associated microbial communities in the Gulf of Aqaba, Jordan, considering the combined influence of environmental parameters and human-induced disturbances for the first time in this specific ecosystem.

The development of high-throughput sequencing technologies, coupled with comprehensive taxonomic reference databases and sophisticated bioinformatics tools, has revolutionized the study of microbial communities in aquatic ecosystems, allowing for detailed analyses of their responses to various stressors (Michelou et al., 2013; Wahl et al., 2012). The present study investigates the influence of environmental disturbances, particularly anthropogenic pressures, on the dynamics and composition of SAMCs, and explores the impact of seasonal variations on SAMC structure. Using the green seaweed *Ulva* as a model system, we examined SAMCs in the Gulf of Aqaba, Jordan (hereafter GA). We hypothesize that the composition of the *Ulva*-associated microbial community will exhibit significant variation among sites subjected to differing degrees of environmental disturbance. We predict a substantial shift in microbial community composition across seasons, linked to fluctuations in primary productivity. Furthermore, we hypothesize that specific environmental parameters will correlate with observed shifts in microbial community composition.

Study area

The Jordanian coastline along the GA represents the country's only marine access. The GA coastline is characterized by a mix of rocky shores and sandy bottoms, supporting diverse ecosystems, including coral reefs and seagrass meadows. This region is recognized for its exceptional marine biodiversity, harbouring several rare and endemic species of seaweed. Increasing coastal development, driven by tourism, industrial activities, port construction, recreational activities, and intensive shipping and transportation, has placed growing pressure on the marine ecosystems of the GA (Al-rousan et al., 2005). Anthropogenic stressors, notably fishing and industrial activities, have been documented to adversely affect the health and resilience of these ecosystems (Wahsha et al., 2017). Based on the varying intensity of anthropogenic disturbances, three distinct sites were selected along the GA coast to represent the major threats to this area (Fig. 1).

The Marine Science Station (MSS) (29° 27.518 N; 34° 58.563 E) is a designated marine reserve characterized by thriving coral reef ecosystems and extensive seagrass beds (Al-Rousan et al., 2016; Wahsha et al., 2017). Public Beach (PB) (29° 52.611 N; 35° 00.080 E), situated at the northernmost point of the GA coast, features sandy substrates and patchy seagrass meadows. The coastal waters of PB are heavily utilized for recreational activities, resulting in significant anthropogenic pressure from solid waste discharge and exhaust emissions from boat traffic (Wahsha et al., 2017). The Industrial Complex (IC) (29° 45.845 N; 34° 97.631 E), located in the southern region of the Jordanian coast, is also characterized by coral reef ecosystems. This area hosts a cluster of major industrial facilities, including a timber plant, a thermal power station, a phosphate fertilizer complex, a potash export terminal, and a mixed fertilizer plant. These intensive industrial activities represent a substantial threat to the health and integrity of the adjacent coastal environment (Zibdeh & Damhoureyeh, 2006).

Sample collection

Water sampling and environmental parameter monitoring

Duplicate water samples were collected from each sampling site during the summer (July 2017) and winter (January 2018) seasons. *In situ* measurements of key physicochemical parameters were conducted, including pH (measured with an accuracy of ± 0.015 units using a portable pH meter, model PH25+, (CRISON instruments, Spain)), surface water temperature, and dissolved oxygen concentration (expressed in mg L^{-1}, measured using a portable oximeter, model OXI45+, (CRISON instruments, Spain)). The percentage of oxygen saturation was calculated according to the formula described by(Weiss, 1970). Nutrient parameters, specifically reactive phosphorus (RP), dissolved inorganic nitrogen (DIN), and silicates (SiO_4), were quantified spectrophotometrically in triplicate following the methods outlined by Strickland and Persons (1972). Chlorophyll-*a* (Chl-*a*) concentrations were determined using a spectrophotometric method after extraction with 90 % acetone, as described by Lorenzen (1967). Salinity was measured using the chlorine titration method with 20 mL water samples (Oxner, 1962).

Seaweed sampling

Seaweeds of the genus *Ulva* are commonly found in the intertidal zone of sheltered harbours (Sfriso, 2010). In the study area, *Ulva* spp. reach peak abundance between December and January, followed by a decline until the end of July (Zibdeh & Damhoureyeh, 2006). To investigate associated microbial communities, several thalli of *U. lactuca* attached to the hard substratum were collected from each sampling site. Collected thalli were carefully washed with seawater to remove or

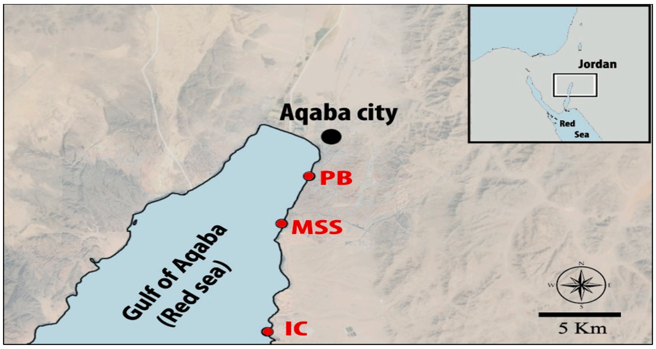

Fig. 1. Map of the sampling sites across the GA (Jordan). (MSS: Marine Science Station, IC: Industrial Complex, PB: Public Beach).

minimize the presence of attached invertebrates and settled sediment particles. The washed thalli were then immediately preserved in bottles containing pre-sterilized seawater to prevent any inappropriate contamination. These bottles were stored on ice until they were transported to the laboratory for analysis. Microbiological analyses were conducted in duplicate for each site, with each replicate sample comprising ten equally sized thalli of *U. lactuca.*

DNA isolation and PCR amplification

Total DNA associated with the surface of *Ulva* thalli was extracted in the laboratory following a filtration step (using 0.20 μm nitrocellulose filters) to collect thalli-detached microorganisms. The *Ulva* thalli were gently sonicated (three cycles of 30 s each, at a frequency of 35 KHz) with ultrasonic path (Bendelin, Germany) in sterile seawater, as previously described by Juhmani et al. (2020). Genomic DNA was then extracted from the collected microorganisms on the filters using the DNeasy PowerSoil Kit (Qiagen, USA) according to the manufacturer's specifications. The quality and quantity of the isolated nucleic acids were assessed using agarose gel electrophoresis and a Qubit Fluorometer 2.0 (Invitrogen, USA). The extracted DNA samples were stored at –20°C until further analysis. For each sample, two extracted DNA replicates were combined to provide a more representative assessment of the resident prokaryotic communities.

The V4 hypervariable region of the 16S rRNA gene was amplified using the prokaryotic-specific primers pair 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACNVGGGTWTCTAAT-3). PCR amplifications were performed under the following conditions: initial denaturation at 98 °C for 4 min, 25 cycles of denaturation at 98 °C for 20 s, annealing at 57 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 5 min. Phusion High-fidelity DNA Polymerase (NEB Inc, USA) was used for amplification. The resulting PCR products were purified using Agencourt Ampure XP beads (Agencourt Bioscience Corporation, MA, USA) and quantified utilizing a Qubit system (Invitrogen). The Nextera XT DNA Sample Preparation Kit (Illumina, Inc., San Diego, USA) was employed for tagmentation and dual indexing of the amplicons. A subsequent PCR with 12 cycles was performed to attach the index sequences to the purified amplicons. Sequencing was carried out on the Illumina NextSeq 500 platform using the Nextseq 500/550 High-Output Kit v2 (Illumina, Inc., USA). Purified, tagged amplicons were pooled in equimolar amounts and sequenced according to the manufacturer's instructions.

Sequence data were analysed using the DADA2 pipeline (Callahan et al., 2016) with the dada2 R package (v. 1.8.0). Raw sequences were quality trimmed, chimeras were removed, and singletons were discarded. High-quality sequences were assigned to Amplicon Sequence Variants (ASVs). Rarefaction curves were generated to assess sequencing depth. Taxonomic classification of ASVs (phylum to genus) was performed using the dada2 classifier against the SILVA SSU database (v. 128) (Quast et al., 2012). Eukaryotic, unassigned, chloroplast, and mitochondrial sequences were excluded. Feature counts were normalized by relative abundance (raw counts divided by the total counts per sample and subsequently multiplied by the median total count). ASVs with relative abundances above 3×10^{-5} were retained for further analysis. The resulting features were used to characterize bacterial communities. 16S amplicon sequences are available in the NCBI Sequence Read Archive (SRA) under accession number PRJNA1103428.

Statistical analysis

Statistical analyses of environmental parameters were performed using one-way ANOVA (Statistica, v. 10). Significant differences between sites or seasons (summer vs. winter) were determined at $p < 0.05$.

Statistical analyses of the SAMCs data were conducted using a rarefaction approach, normalizing sequence counts to the sample with the lowest sequencing depth. Rarefaction curves were generated using the ggrare function implemented in the anacapae R package (http://www.r-project.org) (Kandlikar et al., 2018). A suite of alpha diversity indices, including richness (Chao1), the Shannon index, the Simpson index, and the Fisher index, were calculated using the MicrobiomeAnalyst tool (Dhariwal et al., 2017). Beta diversity analyses were performed by clustering samples based on Bray–Curtis distances. The resulting distance matrices were visualized using Principal Coordinate Analysis (PCoA) with the MicrobiomeAnalyst tool. To assess the statistical significance of clustering patterns in the ordination plot, Permutational ANOVA (PERMANOVA) was employed to test for differences in community composition between seasons (summer vs. winter). Samples were considered statistically distinct at a significance level of $p < 0.05$.

Differences in the relative abundance of bacterial classes between sampling seasons were assessed using independent samples t-tests. Statistical significance was determined using a threshold of $p < 0.05$. Prior to these analyses, Welch's *t*-test was employed to confirm the normality and homogeneity of variance for each variable's distribution. To identify the bacterial families that were most representative of SAMC composition at each sampling site, as well as those that contributed most substantially to the observed differences between sites, a similarity percentage analysis (SIMPER) was conducted using PRIMER v.6 software (Clarke & Gorley, 2006). A cut-off value of 60 % was applied. The relationships between SAMCs and the measured environmental parameters were investigated using principal component analysis (PCA) implemented in CANOCO v. 5.0 software. Log_{10}-transformed sequence data were used to explore the environmental parameters that most strongly influenced the distribution of the different classes of SAMCs. Prior to PCA, sample data were centered and standardized to achieve a normal distribution.

Results

Physicochemical water analysis

The physicochemical characteristics of the surface water (top 50 cm) at the sampling sites are summarized in Table 1. Across the sampling periods, higher water column temperatures and dissolved oxygen (DO) percentages were recorded during the summer months, whereas relatively higher pH values and Chl-*a* concentrations were observed during the winter season. The maximum water temperature (26.8 °C) and DO percentage (104.3 %) were measured at the IC site during the summer. Statistically significant variations between seasonal measurements were observed for pH ($p = 0.007$), water temperature ($p = 0.011$), and Chl-*a* concentration ($p < 0.001$). No statistically significant differences in salinity were detected between sites or between seasons.

In the surface water, concentrations of DIN and SiO_4 generally exhibited an increase during the winter season compared to the summer months, while RP levels were generally higher during the summer. The lowest RP concentration (0.05 μM) was measured at the MSS during the winter, while this same site recorded the highest SiO_4 level (2.31 μM) during the same period. Statistically significant differences in the water column concentrations of DIN and RP were observed between seasons ($p = 0.047$ and $p = 0.024$, respectively). No statistically significant differences were observed in the levels of DIN and RP among the different sampling sites within a given sampling season.

Sequencing output overview

Following the removal of low-quality, chimeric, chloroplast, and mitochondrial sequences, a total of 626,793 reads were retained for subsequent analysis of the normalized abundance data. The average number of utilized reads per sample was 104,465, with a standard deviation of 1102 (minimum = 102,917; maximum = 105,679). Analysis based on the SILVA rRNA gene database revealed a total of 742 ASVs across all samples. Of these ASVs, approximately 45 % could not be assigned to a specific genus. Rarefaction curve analysis indicated good

Table 1
Water column physical–chemical parameters of GA sampling sites over sampling seasons (summer and winter) (MSS: Marine Science Station, IC: Industrial complex, PB: Public Beach).

Site	Season	pH	Temp. (°C)	DO (mg/L)	Chl-*a* (µg/L)	Sal. PSU	DIN (µM)	RP (µM)	SiO_4 (µM)
MSS	Summer	8.12	23.9	6.2	0.21	40.82	0.73	0.05	1.24
PB		7.89	26.6	6.54	0.18	40.84	0.72	0.07	1.80
IC		7.91	26.8	6.63	0.18	40.79	0.78	0.09	1.82
MSS	Winter	8.38	21.5	7.4	0.29	40.40	1.11	0.08	2.31
PB		8.32	21.6	7.2	0.32	40.48	1.74	0.12	1.38
IC		8.43	21.6	7.13	0.31	40.42	1.14	0.11	2.00

(DO: dissolved oxygen saturation; Sal: Salinity; Temp: water column temperature; Chl-*a*: Chlorophyll-*a* concentration; reactive phosphorus (RP), silicates ($SiO_{4)}$ and dissolved inorganic nitrogen (DIN)).

diversity coverage and saturation for all samples, suggesting that sequencing depth was sufficient to capture the majority of the microbial diversity (**Fig. S1**).

Alpha and beta diversity of SAMCs

Alpha diversity indices, including community richness (Chao1), the Shannon index, the Simpson index, and Fisher evenness, are presented in Fig. 2 **(a-d)**. Overall, the alpha diversity of the summer season samples was characterized by greater richness, higher diversity, and increased evenness compared to the winter samples (**Table S2**). Statistically significant differences in the various diversity indices were observed between sampling seasons (ANOVA, *t*-test). For instance, a highly significant difference was measured between the Shannon diversity index values recorded in summer and winter (*p*-value: 0.003; t-statistic: 6.3496). Remarkably, the highest levels of richness, diversity, and evenness were observed at the PB site during the summer season (Table S1). Specifically, the PB summer sample was characterized by the highest number of ASVs (407 ASVs) and the highest species diversity (Shannon index = 5.27 and Simpson index = 0.988). Conversely, the lowest Shannon and Simpson index values were recorded for the IC winter sample (2.52 and 0.815, respectively). The beta diversity (PCoA) ordination plot generated using Principal Coordinate Analysis (PCoA) based on Bray–Curtis distances (Fig. 2**e**) indicated that the microbial communities at the summer and winter sampling sites formed distinct clusters, with particularly strong independent clustering observed for the Bray–Curtis metric similarities reflecting community structure. The first principal coordinate (x-axis) explained 56.9 % of the variation, while the second principal coordinate (y-axis) explained 22.7 % of the variation. PERMANOVA analysis confirmed a statistically significant difference between the seasonal sampling factor (summer vs. winter) (F-statistic: 4.787; R^2: 0.545; *p*-value < 0.1).

SAMC distribution

Phylum level SAMCs composition in GA samples is shown in Fig. 3**a**. Phylogenetic classification revealed 11 prokaryotic phyla across all sites; three phyla (Bacteroidetes [24.0 %], Firmicutes [48.9 %], and Proteobacteria [24.7 %]) comprised ~ 97.5 % of total sequences. Less abundant phyla (Chloroflexi, Epsilonbacteraeota, Tenericutes, and Thaumarchaeota) constituted 0.07 % of sequences. Relative phylum abundance varied notably between seasons. For example, Firmicutes

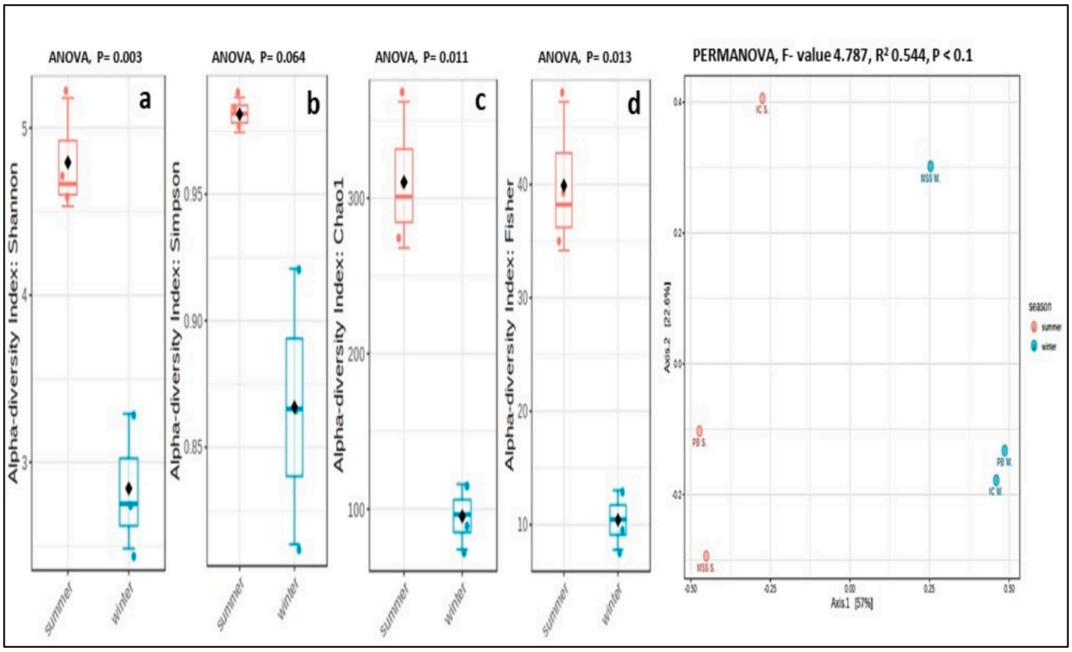

Fig. 2. (a-d) Boxplots of the bacterial alpha-diversity measure using: a: Shannon's Index, b: Simpson's Index, c: Chao1 Index, d: Fisher index at ASVs level across all the samples. The samples are represented on X-axis and their estimated diversity on Y-axis. Each sample is coloured based on season class (Red: summer, Blue: winter). (e) Principal coordinate analysis plot (PCoA) based on a Bray-Curtis distance matrix calculated from the square-root transformed ASVs abundance data of the bacterial community on *U. lactuca* among sampling periods. The explained variances are shown in brackets. (MSS: Marine Science Station, IC: Industrial Complex, PB: Public Beach, S: summer season, W: winter season).

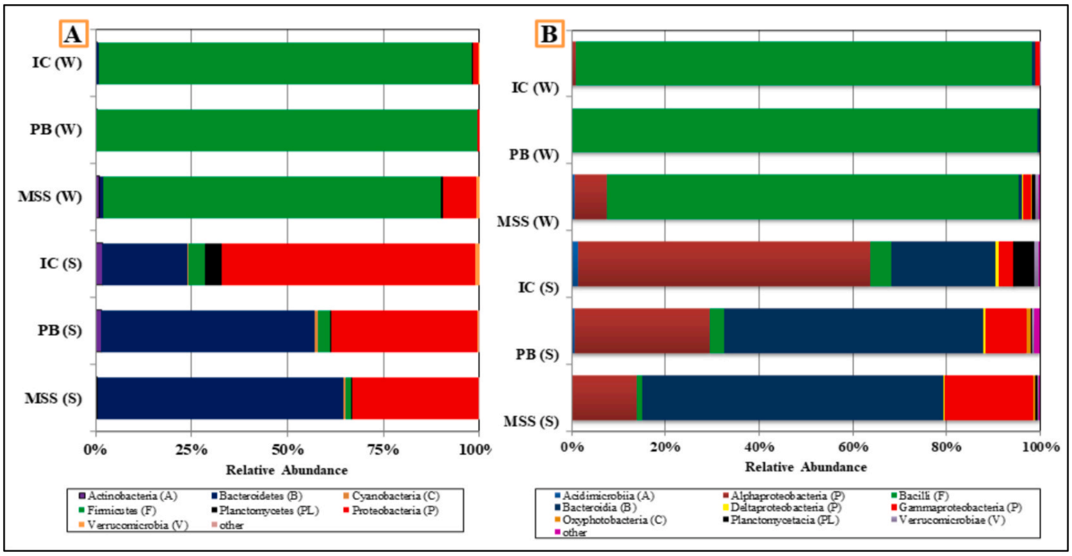

Fig. 3. SAMCs at the A: phylum level B: class level across GA sampling sites. The phyla with relative abundance lower than 1.0% were grouped as [other]. (MSS: Marine Science Station, IC: Industrial Complex, PB: Public Beach) (W: Winter, S: Summer).

comprised 95 % of sequences in winter. The average relative abundance of Bacteroidetes decreased from 47.4 % in summer to 0.6 % in winter, and Proteobacteria decreased from 45.7 % to 3.5 %.

Seventeen bacterial classes were identified across all sites during both seasons. Within Proteobacteria, Alphaprotebacteria had the highest average relative abundance (18.8 %), followed by Gammaproteobacteria (5.6 %) and Deltaprotebacteria (0.3 %) (Fig. 3**b**). Bacteroidia (Bacteroidetes) comprised 47 % of summer sequences, while Bacilli (Firmicutes) comprised 95 % of winter sequences (**Table S3**). Campylobacteria (Epsilonbacteraeota) had the lowest abundance (< 0.04 %). Seasonal abundance differed significantly for Deltaproteobacteria, Bacilli, and Bacteroidia (*t*-test, $p = 0.013$, 3×10^{-5}, and 0.022, respectively) (**Table S4**).

Family-level taxonomic classification of SAMCs revealed 52 families across all samples. Planococcaceae and Rhodobacteraceae were the most abundant families (**Table S5**). Bacillaceae was most abundant in BP and IC samples (14.2 and 15.9 %, respectively). Saprospiraceae and Hyphomonadaceae were notably abundant at PB.

At the ASV level, SAMC composition was more distinct between sites. Forty ASVs comprised ~ 60 % of total SAMC reads. The distribution and relative abundance of these abundant ASVs varied considerably between sites (Table 2). The ten most abundant ASVs at each GA site were assigned to Bacillaceae, Planococcaceae, Rhodobacteraceae, and Flavobacteriaceae. Bacillaceae ASVs (1, 5, and 7) were abundant at PB and IC, while Planococcaceae ASVs (9, 30, 37, and 47) were remarkably abundant at SMM. Rhodobacteraceae (ASV 21) dominated at IC.

Shared and unique ASV distribution in SAMCs

Fig. 4 presents a Venn diagram depicting the distribution of shared and unique ASVs between seasons. A total of 93 unique ASVs were observed in winter. Moreover, 106 ASVs (14.3 % of total ASVs) were shared between seasons. The mean similarity between summer and winter SAMCs was 24.44 % (SIMPER analysis). Bacillaceae, Flavobacteriaceae, Saprospiraceae, Rhodobacteraceae, and Planococcaceae comprised 52.5 % of this similarity.

Environmental effects on SAMCs

The relationships between SAMCs across the different sampling periods and the measured environmental parameters were visualized using PCA (Fig. 5). The first two principal components accounted for 96.87 % of the total variance in the samples, with all explanatory variables contributing to the overall variance. The SAMC samples collected during the summer months formed a distinct cluster on the opposite side of the ordination plot from the cluster formed by the winter SAMC samples. PCA revealed that the relative abundance of the Bacilli class during the winter season was strongly and positively correlated with pH, Chl-*a*, DO, RP, and DIN, but exhibited a negative correlation with both temperature and salinity. Remarkably, DIN contributed to 87.6 % of the total sample variations (*pseudo-F* = 28.1, $p = 0.006$), suggesting that this nutrient plays a critical role in driving the dynamics of the microbial community. The relative abundance of Gammaproteobacteria and Bacteroidia classes during the summer season was observed to be strongly and positively correlated with salinity and temperature.

Table 2
The 10 most abundant asvs in all sampling sites during the two sampling periods and their relative abundances (as percentage of the total sequences of the same site [sum of the sequences of the two seasons]).

PB (%)	IC (%)	MSS (%)	ASV #	Phylum	Class	Order	Family
15.2	18.9	2.0	1	Firmicutes	Bacilli	Bacillales	Bacillaceae
2.6	0.1	5.2	4	Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae
6.8	7.2	0.8	5	Firmicutes	Bacilli	Bacillales	Bacillaceae
5.4	6.5	0.7	7	Firmicutes	Bacilli	Bacillales	Bacillaceae
1.8	2.3	10.0	9	Firmicutes	Bacilli	Bacillales	Planococcaceae
0.3	2.5	0.1	21	Proteobacteria	Alpha-proteobacteria	Rhodobacterales	Rhodobacteraceae
3.1	0.6	2.6	27	Firmicutes	Bacilli	Bacillales	Planococcaceae
1.6	0.7	4.2	30	Firmicutes	Bacilli	Bacillales	Planococcaceae
0.8	0.9	3.7	37	Firmicutes	Bacilli	Bacillales	Planococcaceae
0.6	0.8	3.4	47	Firmicutes	Bacilli	Bacillales	Planococcaceae

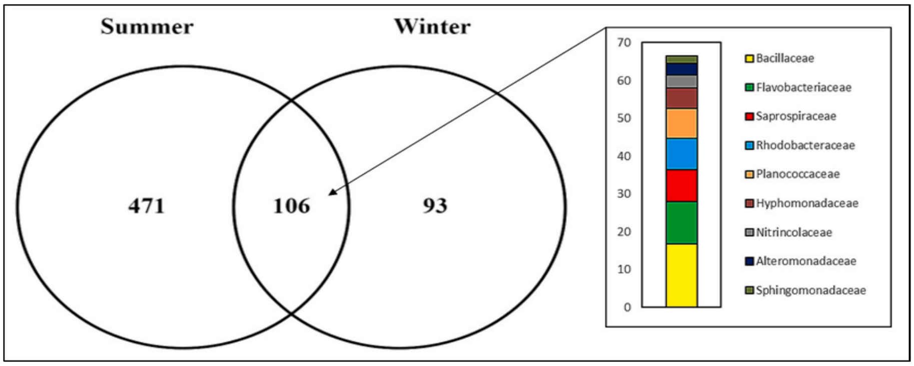

Fig. 4. Distribution of shared and unique ASVs from SAMCs during sampling season (winter vs. summer). Bar graph represent the percentage of bacterial families contributing to similarity (using SIMPER software) between sampling seasons.

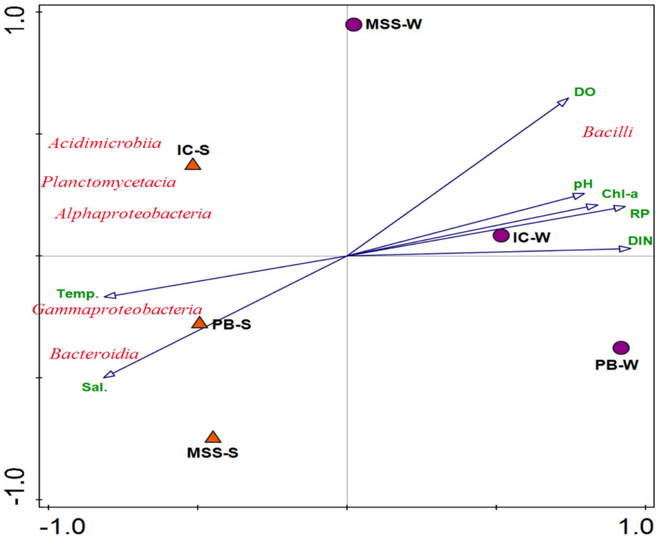

Fig. 5. PCA with supplementary variables analysis for *U. lactuca* associated bacterial communities in response to environmental parameters. Sites: MSS: Marine Science Station; IC: Industrial Complex; PB: Public Beach; Seasons: S: summer, W: winter; Environmental parameters: DO = dissolved oxygen, DIN = dissolved Inorganic Nitrogen, Temp. = Temperature, Sal. = Salinity, RP = reactive phosphorus, Chl-*a* = chlorophyll-*a*.

Discussion

The green seaweed *U. lactuca* is a common and widely distributed species in coastal marine ecosystems. However, the composition and dynamics of the bacterial biofilm communities residing on its surface remain poorly understood. In this study, we investigated the compositional dynamics of the microbial communities associated with *U. lactuca* in the Gulf of Aqa (Jordan), specifically targeting areas subject to varying degrees of environmental disturbances. To our knowledge, this work represents one of the first comprehensive characterizations of the SAMCs in the Jordanian sector of the Gulf of Aqaba and aims to contribute to a deeper understanding of the complex interactions between this seaweed host and its surrounding environment.

Our study demonstrated a significant shift in the composition of SAMCs across the sampling seasons, correlating with key abiotic environmental variables. The temporal dynamics of the SAMCs were clearly illustrated by the pronounced seasonal variation in the relative abundance of dominant bacterial phyla. Specifically, Firmicutes was highly abundant during the winter period, while Bacteroidetes and Proteobacteria were predominant during the summer months.

Ulva lactuca-associated community alpha diversity (ASVs richness) varied significantly between seasons in response to local environmental disturbances. Consistent with (Mensch et al., 2016) on *F. mytili* (Wadden Sea), biofilm alpha diversity was higher during spring/summer, coinciding with maximum seaweed size and physiological activity. This likely reflects the nutrient-rich macroalgal surface providing habitat for microbial colonization and increased species richness (Dang & Lovell, 2016; Egan et al., 2013). We hypothesize that lower ASV richness, correlating with Bacilli dominance, results from the natural colonization process during minimal seaweed size and physiological activity.

The surfaces of seaweeds provide a nutrient-rich biofilm environment that supports the growth and development of distinct bacterial communities (Barott et al., 2011; Glasl et al., 2020). In the present study, the bacterial communities associated with *U. lactuca* were found to be dominated by Bacteroidetes (24.0 %), Firmicutes (48.9 %), and Proteobacteria (24.6 %). Consistent with observations in the Mediterranean macroalga *Cystoseira compressa* (Mancuso et al., 2016), seasonal shifts in the relative abundances of bacterial families belonging to the phyla Firmicutes and Bacteroidetes (specifically Bacillaceae and Flavobacteraceae, respectively) were observed throughout the sampling period. Furthermore, these phyla are frequently among the most dominant components of seaweed microbiomes associated with various species, including *Ulva laetevirens* (Juhmani et al., 2020), *Sargassum* spp. (Glasl et al., 2021), *and Sargassum muticum* (Serebryakova et al., 2018). The observed dominance of bacterial sequences assigned to Flavobacteriaceae (32.3 %), Rhodobacteraceae (25.7 %), and Saprospiraceae (14.5 %) exhibited similarities to the core microbiome reported for *Sargassum* spp. samples collected from Magnetic Island, Australia (Glasl et al., 2020). Such high microbial abundance is also consistent with the dominant bacterial families identified on the surface of the green macroalga *Ulva australis* collected from Bare Island, La Perouse (Burke et al., 2011). The lower relative abundance of Planctomycetes (1.5 %) and Actinobacteria (1.1 %) observed in this study, which is inconsistent with findings from other seaweed studies (Bondoso et al., 2014), may reflect the influence of the host species on the composition of the SAMCs. Verrucomicrobiae, Planctomycetacia, and Oxyphotobacteria were either absent or under-represented on both the Australian and the Baltic Sea algal samples (Longford et al., 2007). The most pronounced seasonal changes observed in the bacterial community during the winter months were the significantly increased abundances of Bacillaceae (65.9 %) and Planococcaceae (28 %). Glasl et al. (2021) have hypothesized that the observed shift in the relative abundance of these two dominant families is closely related to the annual growth, reproduction, and senescence cycle of the seaweed host.

Seaweed-associated bacterial communities play a vital role in the physiology and development of their hosts. Cross-kingdom chemical signals essential for *Ulva mutabilis* development have been identified from *Roseobacter* (Rhodobacteraceae) and *Sulftobacter* (Flavobacteriaceae) (Spoerner et al., 2012). Dimethylsulfoniopropionate (DMSP) is crucial for macroalgal-bacterial interactions (e.g., Kessler et al., 2018).

Alphaproteobacteria, due to their morphological and metabolic diversity, are crucial in DMSP assimilation and global sulphur cycling (Malmstrom et al., 2004). Macroalgal-produced DMSP (e.g., *Ulva* sp.) attracts specific bacteria (Kessler et al., 2018); Hyphomonadaceae, abundant in summer, may play a role in dissolved organic matter mineralization in oligotrophic waters. Bacteroidetes, abundant in organic particle-rich coastal waters, possess numerous genes for exopolysaccharide, protein, protease, peptidase, and lipase adhesion (Glasl et al., 2020), suggesting their specialization as seaweed surface colonizers and key roles in dissolved organic matter degradation and utilization.

Our findings suggest that *Ulva's* ability to thrive in oligotrophic waters (GA) may be enhanced by microbial mineralization. Supporting this notion, Holmström et al. (2002) showed that *Pseudoalteromonas* produces bioactive compounds crucial for *U. lactuca*'s chemical defence against biofouling (especially in summer). Winter dominance of Firmicutes (91.4 % of winter SAMCs) may reflect their capacity to produce antibiotics (i.e. bacilysin), hindering colonization by opportunistic microbes on the surface of the macroalgae (Glasl et al., 2020).

While previous studies have largely focused on characterizing the spatiotemporal diversity of macroalgal epiphytic bacteria (Aires et al., 2016; Campbell et al., 2015; Tujula et al., 2010), relatively few reports have explicitly linked variations in the composition of these communities across different geographic locations to environmental selection processes (Lindström & Langenheder, 2012; Liu et al., 2022). In the present study, the shifts observed in SAMCs were strongly correlated with critical environmental conditions present in the surrounding seawater, including temperature, DO, pH, salinity, and DIN concentration (Fig. 5). The observed influence of environmental factors on SAMCs composition is consistent with the findings of a previous study(Juhmani et al., 2020). A positive correlation was measured between seawater temperatures and the abundance of bacterial classes Bacteroidia and Gammaproteobacteria. The increased seawater temperatures are known to augment both the photosynthetic activity of the macroalgae and the related exudation rates of carbohydrates (Abdullah & Fredriksen, 2004; Wada et al., 2007), which can create favourable conditions for the growth of heterotrophic bacteria (Bengtsson et al., 2011, 2012). Saha et al. (2020) reported that the relative abundance of Bacteroidia and Gammaproteobacteria on invasive seaweeds is altered under salinity fluctuation. Similarly, Comba González et al. (2021) observed high variability in the epiphytic bacterial community associated with *U. lactuca*, driven by changes in seawater temperature. Furthermore, members of the phylum Bacteroidota have recently been shown to be enriched in GA coral-associated microbiome and macroalgae-dominated reefs (Hussein et al., 2022; Hussien et al., 2019). Summer Alphaproteobacteria and Planctomycetes prevalence was also observed in *Sargassum muticum* bacteria (Serebryakova et al., 2018).

The observed correlation between Firmicutes (Bacilli) and nutrient concentrations and DO in the seawater during the winter season is consistent with the findings of (Hu et al., 2023), who reported a positive correlation between members of this class and total organic nutrient (carbon, nitrogen, and phosphorus) concentrations during the decomposition of litter from the seaweed *Gracilaria lemaneiformis* (Bory) Greville. In line with this, it has been shown that Proteobacteria and Firmicutes enhance the production of plant growth-promoting substances, quorum-sensing molecules, and bioactive compounds involved in seaweed development and growth (Singh & Reddy, 2014). Winter dominance of Firmicutes (Bacillaceae and Planococcaceae) on growing *U. lactuca* thalli (December-January) resembles bacterial communities of growing *Sargassum* spp. on coral reefs (Glasl et al., 2021). (Glasl et al., 2021) also reported a differential abundance of Bacillaceae ASVs across organic carbon and ammonium treatments.

The physicochemical properties of the seaweed surface play an important role in shaping the composition and dynamics of the epithetic microbial communities. Lachnit et al. (2011) suggested that these properties create selective pressures that favour the settlement and colonization of specific bacteria. Consistent with this, proteins responsible for both the production and excretion of galactoglycans or exopolysaccharides were found to be abundant in the *U. australis* microbial community (Borthakur et al., 1986). This abundance allows the bacteria within the biofilm to utilize these sugars as a source of both carbon and energy, consequently gaining a competitive advantage in the colonization of the host surface, similar to the symbiotic relationship observed in *Rhizobium* strains (Borthakur et al., 1986). For instance, Rhizobiaceae bacteria were detected in the summer samples collected from the GA. Proteins for *Ulva* sp. polysaccharide breakdown may enable bacteria to utilize these sugars, enhancing their competitive edge in colonizing the host surface (Lahaye & Axelos, 1993).

Conclusion

The effects of changing environmental conditions of marine ecosystems on the diversity, composition, and structure of seaweed-associated microbial communities remain poorly understood. This study investigated SAMCs in the Gulf of Aqaba, Jordan, revealing significant seasonal shifts in *Ulva*-associated bacterial community composition: Firmicutes dominance in winter and higher Bacteroidetes and Proteobacteria abundance in summer. These SAMC variations are driven by seasonal changes in nutrient availability and water temperature. Distinct microbial communities were observed at sites with varying anthropogenic disturbance levels, highlighting the effect of such disturbances. Epiphytic bacteria are potential first indicators of environmental perturbations and valuable bioindicators of ecosystem health in the Gulf of Aqaba. Further research should clarify the complex dynamics between environmental disturbances and microbial community shifts through controlled experiments manipulating environmental variables to assess causal effects on SAMC composition and function. Long-term monitoring is crucial to understand SAMC temporal dynamics and responses to seasonal and interannual environmental variations.

Declaration of Generative AI and AI-assisted technologies in the writing process.

During the preparation of this work the authors used ChatGPT in order to rephrase some sentences. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Abdul-Salam Juhmani: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Alessandro Vezzi:** Writing – review & editing, Conceptualization. **Mohammed Wedyan:** Writing – original draft. **Alessandro Buosi:** Writing – review & editing, Conceptualization. **Mohammad Wahsha:** Data curation. **Fabio De Pascale:** Software, Resources, Methodology. **Baker Al-Shara:** Writing – review & editing. **Riccardo Schiavon:** Software, Methodology. **Adriano Sfriso:** Writing – review & editing, Supervision. **Andrea A. Sfriso:** Writing – review & editing, Resources, Conceptualization.

Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejar.2025.02.005.

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