



Microbial diversity analysis of two full-scale seawater desalination treatment trains provides insights into detrimental biofilm formation

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ABSTRACT

Detrimental biofilms on RO membranes remain a crucial challenge for seawater desalination. Comparative analysis of 16S rRNA gene amplicon sequencing data revealed differences and commonalities of biofilm communities associated with unit operations in the two largest seawater desalination facilities in the U.S., the Claude "Bud" Lewis Carlsbad Desalination Plant and the Tampa Bay Seater Desalination facility. At both plants, feedwater collected at a single time point was a poor indicator of the RO membrane communities, which showed far greater taxa diversity. The analysis of prefilter cartridges from the Carlsbad plant revealed similarly high taxon diversity as the RO module biofilms, with relevant differences. Algal sequences were enriched on the prefilter cartridges as were sequences representing *Bdellovibrio*, which are predatory bacteria. Sequences representing opportunistic Gammaproteobacteria (i.e., *Shewanella*, *Woesia*) were present in significantly higher relative abundance on the RO membranes than in the prefilter cartridges, suggesting growth of certain taxa in the RO modules. Untargeted metabolomics distinguished intra- and inter-desalination plant biofilm samples, highlighting the potential value of this tool for biofilm monitoring. These findings underscore the value of omics tools for effective microbial monitoring, to understand biofouling dynamics within RO desalination plants, and to provide insight for the development of ecologically-informed biofilm control measures.

Statement of Novelty

This study provides an unprecedented baseline analysis of the microbial community structure and associated metabolomes in end-of-life reverse osmosis (RO) modules from the two major seawater desalination facilities in the U.S., thus providing valuable insight into biofouling and opportunities for higher precision biofilm control measures.

Introduction

Reverse osmosis (RO) membrane seawater desalination is an increasingly relevant technology to augment water supplies in water-stressed coastal regions (Amy et al., 2017). Although effective in generating high-quality drinking water (Elimelech and Phillip, 2011), RO is costly due to the high energy demand and the reoccurring cleaning and replacement of membrane modules. Thus, operational efficiency is paramount to the sustainable operation of these plants and generation of drinking water at reasonable cost. One major challenge to efficient operation is biofouling, where detrimental biofilms grow on the RO membrane and lead to performance decline. Biofouling has been referred to as the "Achilles heel" of membrane water treatment facil-

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ities (Flemming et al., 1997), and substantially inflates the operating costs (Jafari et al., 2021). Current approaches to alleviate biofouling are chemical-intensive and disruptive with the potential to damage membranes (Subramani and Hoek, 2010). Alternative approaches include nutrient balancing (Huang et al., 2019) and hypertonic solutions to inflict osmotic stress on biofilm bacteria (Katebani and Jiang, 2013), periodic osmotic backwashing (Lieberman, 2018), or frequent chemical cleaning. These efforts lead to additional costs, operational downtimes and productivity loss. Although these non-specific microbial control approaches have been implemented with some success, biofilm formation remains problematic for many desalination plants. Opportunities exist for technological innovation to enhance the cost-effectiveness and sustainability of biofouling mitigation.

Insufficient understanding of the ecophysiology and dynamics of microorganisms causing RO membrane biofouling has precluded the rational development of ecologically-informed and more effective proactive control approaches. However, recent advances in omics technologies, ecological network modeling and data sciences generate opportunities to overcome existing hurdles and inform more precise and sustainable solutions (Cho, 2021). Such tools could be used to discern keystone bacterial species and/or functions that could be preferentially, and therefore more efficiently targeted, based on their specific characteristics (e.g., nutritional requirements, susceptibility to bacteriophages and/or specific chemical agents).

A first step to achieve sustainable biofouling control is to understand the diversity and dynamics of RO membrane biofilms, and to reveal the triggers for the transition of benign to detrimental biofilms. Two important questions need to be addressed. Are similar types of microorganisms responsible for detrimental biofilms in desalination plants that use intake water from different sources? If this is not the case and the biofouling culprits are taxonomically distinct, could shared metabolic features be identified and used as indicator for the onset of detrimental biofilm formation? Several phenomenological studies have described microbial communities associated with intake water, prefiltration units, and RO membranes of seawater desalination plants, but there are only a few comprehensive studies providing relevant ecological insights, with some of them reporting contradictory results. Dominant bacteria in RO biofilms were generally found to be distinct from those in inlet seawater in most studies (Zhang et al., 2011; Rehman et al., 2019; Manes et al., 2011). One study reported a decrease in microbial diversity along the treatment train from the pretreatment filter to the RO membrane (Levi et al., 2016), while another study found increased microbial diversity along the treatment train (Kim et al., 2020). Temporal shifts in microbial composition of RO biofilms were also reported (Nagaraj et al., 2017).

This present baseline study provides a detailed analysis of the microbial community structure and metabolome profiles of end-of-life filtration units, including RO modules from the two major seawater desalination facilities in the U.S. We collected and analyzed samples from the treatment train and applied 16S rRNA gene amplicon sequence and untargeted metabolome analyses to assess differences and commonalities in biofilm communities within the one desalination plant and between two plants as a first step towards informing novel, ecologically-informed biofilm mitigation and control measures.

Materials and methods

Sampling Locations and Collection. Fig. 1 illustrates the sampling locations at the two desalination plants considered in this study: the Claude "Bud" Lewis Carlsbad Desalination Plant in Carlsbad, CA and the Tampa Bay Seawater Desalination plant in Tampa Bay, Florida. Samples were collected at the Carlsbad facility on March 24th, 2021 and May 13th, 2021. The plant operates in accordance with all state and federal regulations, and at full capacity produces the plant produces 54 million gallons of drinking water per day. A single sampling event occurred at the Tampa Bay facility on May 27th, 2021. Samples collected included

feedwater (intake), prefilter cartridges (Carlsbad only), and end of life RO modules (lead modules at the time of sampling). The RO modules obtained from the Tampa Bay facility were Hydranautics SWC4 LD modules that had been in operation for 524 days. The overall average operational flux for modules at the plant was ~14 Liters per squared meters per hour (LMH) with the lead module operating at a flux of ~31 LMH. Total organic carbon of influent water at Tampa was between 3 and 6.8 mg/L for the duration of module operation. The RO modules obtained from the Carlsbad desalination facility were Dupont seawater (SWRO) modules operated for between 3 and 5 years. The membranes in the facility are operated at an average flux of 12-17 LMH. Details of procedures and samples collected are summarized in the Supporting Information and Table S1.

DNA extraction and 16S rRNA gene amplification, sequencing, and analysis. DNA extraction, 16S rRNA gene amplification and sequencing, and untargeted metabolome workflows followed established procedures and analysis pipelines. Procedural details are presented in the Supporting Information.

Results

High microbial diversity but uneven taxa distribution across samples at the Carlsbad plant. The analysis of feedwater samples reflected microbial communities that are characteristic of coastal environments. Between the two collection dates, we observed shifts in the community structure, likely reflecting seasonal changes and fresh water runoff into the lagoon is typically higher during the January to March versus the April to May time periods (Tetratech 2007), in addition to frequent algal blooms occurring in spring (Anderson and Hepner-Medina, 2020). The most abundant taxa changed from *Arcobacteraceae* (*Poseidonibacter*, 19% of sequences), *Rhodobacteraceae* (*Amylibacter*, 9%), various Gammaproteobacteria (*Vibrio*, *Colwellia*, *Marinomonas*) and Alphaproteobacteria (SAR11, 6%) in March to a dominance of SAR11 sequences (18%), SAR86 (Gammaproteobacteria, 9%) and *Flavobacteriaceae* (NS5, 6%) in May (Fig. 2). Some lineages remained relatively constant between sampling dates (e.g., *Planctomarina*, Marine group II Archaea). AAlgae, detected based on chloroplast sequences (5-6% of the amplicons), shifted somewhat in composition but were still dominated by *Ochrophyta* (brown seaweeds) and *Haptophyta* (*Thalassiosira*, *Haslea*). Both the prefilter cartridges and the RO modules harbored diverse microbial taxa, indicating that the prefilter cartridges (20- μ m cutoff) do not act as a strict barrier (Fig. 2). The observed community differences between prefilter cartridges and the RO modules are therefore not caused by the filtration (i.e., retention) per se, but by the establishment of somewhat distinct communities on the RO membranes. The prefilter does, nevertheless, appear to exert some separation, as indicated by the relatively higher abundance of SAR11 (very small vibrioid-shaped cells with a length of < 1 μ m and a cell diameter of < 0.2 μ m (Rappé et al., 2002)) in the water collected past the prefilter cartridges. The prefilter cartridges retained larger algal cells and chloroplast sequences contributed no more than 0.5% of sequences in the RO module samples. Oligotrophic SAR11 were not enriched in the RO biofilm and photosynthetic algae did not accumulate in the prefilter cartridge, suggesting these organisms do not grow and their biomass is likely consumed by heterotrophic bacteria. An interesting observation was the increased relative abundance of *Bdellovibrionota*, a group of bacteria with predatory behavior (Shilo and Bruff, 1965), in the prefilter cartridges, which was observed at both sampling events and suggests active growth. Multiple members of the Bacteroidia and the Gammaproteobacteria, *Shewanella* and *Woesia* that generally opportunistic r-strategists, were barely detected in the intake water and the prefilter cartridges but were enriched in the RO biofilms.

In addition to the bacterial and archaeal sequences that represented the top 25-30 most dominant lineages (Fig. 2) and (depending on samples) accounted for 40-60% of all sequences, we also analyzed the overall community diversity taking into consideration the hundreds of lower

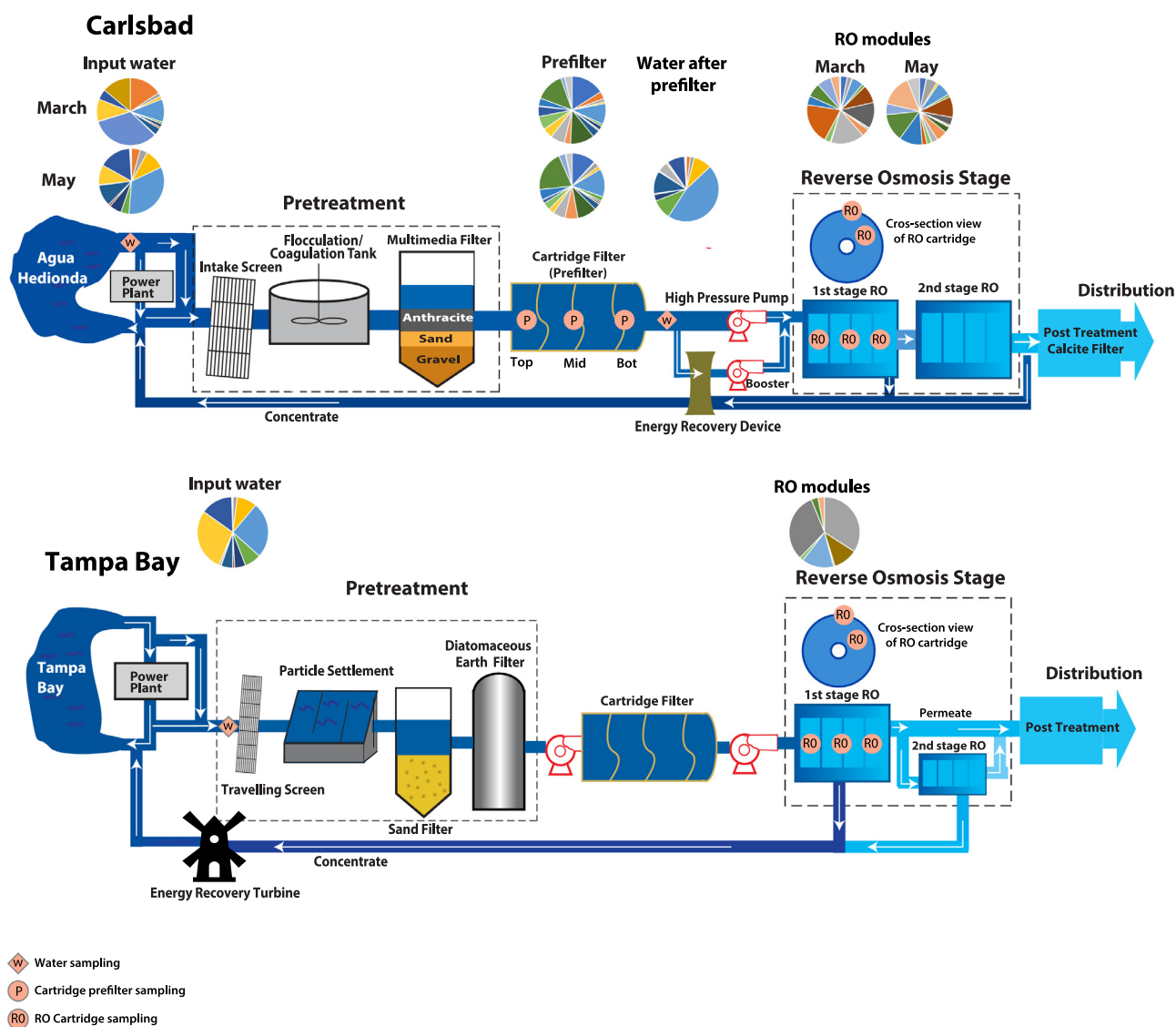


Fig. 1. Plant layouts and sampling locations at the Claude “Bud” Lewis Carlsbad Desalination Plant in Carlsbad, CA, and the Tampa Bay desalination plant in Tampa Bay, FL. The red diamond and the red circles indicate the sampling locations. The pie charts represent the relative abundance of the top 25 microbial taxa and illustrate the changes in microbial community composition along the water treatment process, full details of the charts are provided in the Supporting Information.

abundance taxa. In this analysis, as is common in microbial ecology literature, Shannon entropy (Shanon and Weaver, 1963) was used as a measure of diversity. Alpha diversity, which accounts for diversity within individual samples, was calculated based on the phylogenetic distance between microbial taxa, considering both the abundance of individual taxa and the evenness of their distribution within the community (Shanon and Weaver, 1963) (Fig. 3).

The highest diversity was observed in the prefilter cartridge samples. The lagoon intake water samples showed a lower diversity; however, these samples represent snapshots of an open environment, and time-series sampling would be needed to capture microbial community dynamics, and thus overall diversity, in the feedwater. The prefilter cartridge community is influenced by inflowing pretreated feedwater over extended periods of time, allowing lagoon water microorganisms that pass through the sand/multimedia filtration step to establish biofilms, which explains the highly diverse communities associated with prefilter cartridges. We observed somewhat higher diversity in the samples collected in May, in intake water, prefilter cartridges and the RO elements, suggesting a dynamic composition of biofilms in prefilter cartridges and RO elements.

Spatially, the individual prefilter cartridge and RO membrane microbial communities were overall quite similar, as indicated by the lack of statistically significant differences (Kruskal–Wallis ANOVA test) between the subsamples taken along the length of the prefilter cartridges or at different depths of the RO modules (Fig. 3, panels B and C). These are relevant observations suggesting that single samples of prefilter cartridges and RO membranes are representative for the entire cartridge or module, respectively, and should simplify biofouling diagnostics.

High microbial diversity in the Tampa Bay intake water, while distinct microbes in the Tampa Bay RO modules, reflect the intermittent operation and storage regime. The Tampa Bay intake water microbial community structure was distinct from that recovered from the feedwater at Carlsbad though some commonalities were observed (Fig. 2). The bacterial populations were dominated by several SAR11 clades (~23% of the amplicons), SAR86, and the AEGEAN-169 marine group ‘*Candidatus Actinomarina*’ (Alphaproteobacteria) (Ghai et al., 2013). Algae constituted a larger fraction (17% of the amplicons) than in the Carlsbad samples and were primarily represented by a diatom related to *Cylindrotheca closterium*, known to produce mucilage (Alcoverro et al., 2000). Prefilter cartridges were not available at the

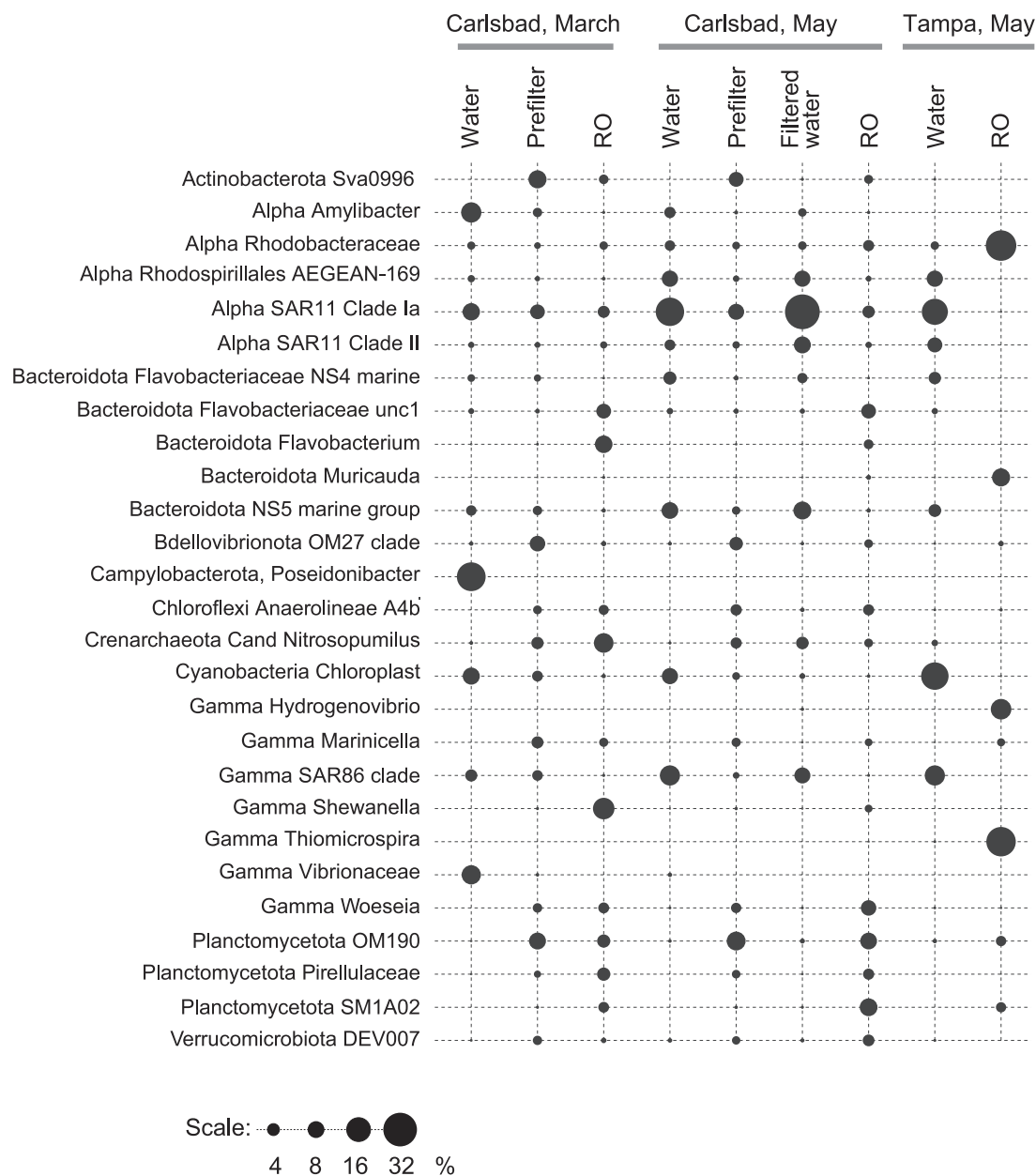


Fig. 2. Bubble plot presenting the relative taxonomic abundance of the most abundant microbial taxa across samples collected at the two major seawater desalination plants in the U.S. The size (area) of the circle is scaled to the relative abundance of that taxon. For each sample type, we used an average of the abundance detected across all samples/replicates from that sample point.

Tampa plant. The RO modules sampled had been taken off regular operation 3-4 days before the sampling event and were being flushed with a solution of sodium bisulfite (SBS). The amplicon sequence variant (ASV) analysis of biofilms recovered from the Tampa Bay RO modules presented a very distinct microbial community of much lower diversity than that recovered from the RO modules at the Carlsbad desalination plants (Fig. 3). The ASV analysis indicated that bacteria involved in sulfur metabolism, including *Thiomicrospira*, *Hydrogenovibrio*, *Muricauda* and a member of *Rhodobacteraceae* were abundant, possibly reflecting the re-occurring SBS flushing and storage (2-3 months per year) of RO modules at the Tampa Bay plant.

Microbial diversity comparisons across all samples reveals site specific and temporal influences. To determine how the microbial communities from all sampling locations relate, a beta diversity comparison was performed using the unifracs distance (Lozupone and Knight, 2005), a metric that considers phylogenetic diversity of indi-

vidual samples. The more similar the communities, the closer they are in a three-dimensional projection based on principal coordinates analysis (PCoA) (Fig. 4 a). The PCoA illustrates that the RO membrane microbial diversity is quite distinct between the RO modules from Carlsbad versus Tampa Bay, being clearly separated in the first principal coordinate. The prefilter cartridges and the RO modules from Carlsbad show distinct community structures, separation between the two time points (March versus May) was evident, within filtration units, indicating dynamic microbial communities in both the prefilter cartridges and the RO modules. It is possible that such variation is linked to temporal changes of microbes in the intake water, which was apparent between the March and the May samples. During the May sampling event at Carlsbad, an RO feedwater sample was collected post cartridge filter (Fig. 1). The microbial community associated with these water samples was clearly distinct from that in the lagoon intake water (Fig. 1) and in the prefilter cartridges, indicating that some microbes

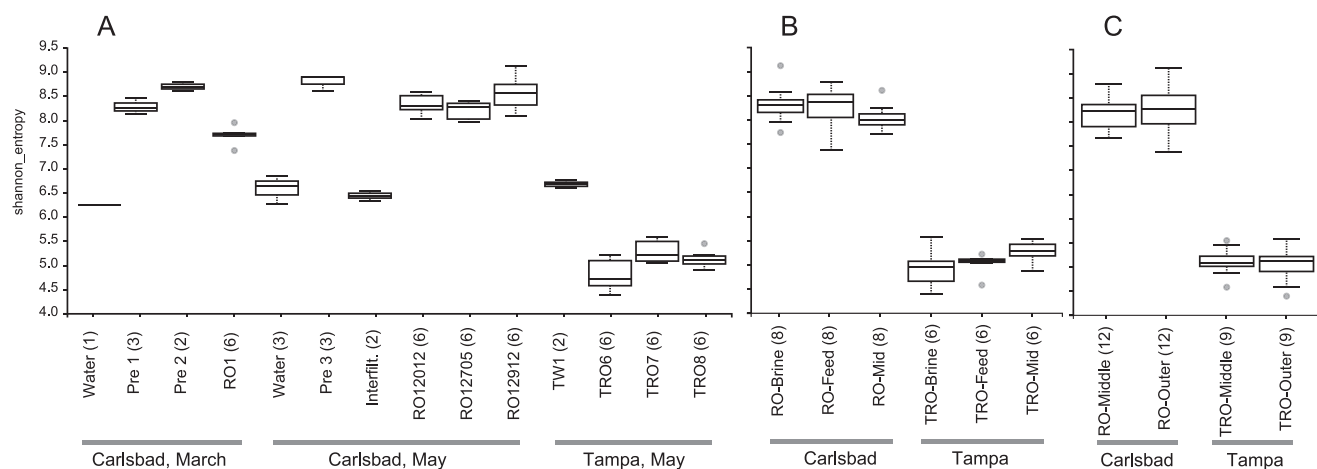


Fig. 3. Box and whisker plots showing the Shannon's entropy for water samples and filtration cartridges. The numbers of samples for each category are indicated in parentheses. A larger Shannon entropy (y-axis) corresponds to higher taxon diversity. Panels B and C show grouped subsamples that were collected along the length and the depth of RO modules. RO12012, RO12705, and RO12912 are three replicate RO modules (for biofilm samples) collected from Carlsbad and TRO7, TRO8, and TRO9 are three replicate RO modules (for biofilm samples) from Tampa Bay.

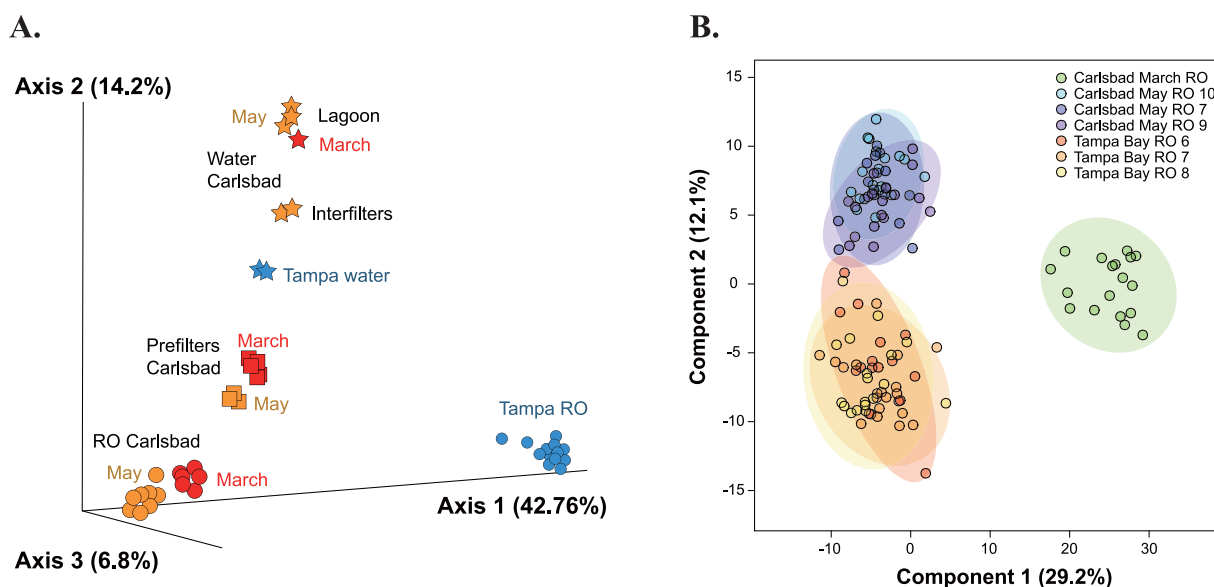


Fig. 4. A. Beta diversity analysis of microbial communities across samples. Visualization of the degree of similarity between samples using a three-dimensional projection of their composition metric. Each symbol represents one sample. The samples are color-coded for location (Tampa Bay, blue) and sampling date (Carlsbad only). The symbol shapes indicate different types of samples collected along the treatment train (Fig. 1). The numbers on the axes indicate the percent of variation explained by each coordinate. B. Partial least squares-discriminant analysis (PLS-DA) of untargeted metabolomics data collected from seven RO membranes (distinguished in legend by numbers 7 through 10 for the May samples) from two desalination plants. RO samples collected from the same plant and time of year clustered together and separated from each other. TRO7, TRO8, and TRO9 are three replicate RO modules (for biofilm samples) from Tampa Bay

that colonize the prefilter cartridges become dislodged and seed the RO elements

Untargeted metabolome analysis generates patterns of metabolites and can distinguish microbial communities based on metabolic activity. The application of this omics tool to prefilter cartridge and RO biofilm samples illustrated unique metabolite patterns that clearly differentiated samples (Fig. 4B). Further work is needed to assess if this approach provides sufficient resolution to discern specific metabolites or metabolite patterns that can serve as indicators when a benign (neutral) biofilm transitions to a detrimental biofilm impacting RO performance.

Discussion

Feedwater and RO membrane biofilm communities are distinct. Microbial communities of the feedwater are very different from the

communities associated with the prefilter cartridges (Carlsbad) and RO membranes (both Carlsbad and Tampa Bay), in line with previous findings at other desalination plants (Zhang et al., 2011; Rehman et al., 2019; Manes et al., 2011; Levi et al., 2016). Distinct microbial communities in the feedwater collected in March versus May suggest a highly dynamic nature of these microbial communities (Needham and Fuhrman, 2016). A year-round sampling campaign would be needed to better capture this dynamic nature in terms of taxa composition and total cell abundances, and its potential impact on biofilm formation and community composition in downstream filtration units.

Despite extensive pretreatment including flocculation/coagulation and multimedia filtration steps to remove suspended solids, highly diverse microbial communities were observed in the prefilter cartridges, indicating that these pretreatment steps are not a strict barrier for microbial cells (Levi et al., 2016; Kim et al., 2020). The prefilter cartridges

at the Carlsbad plant have a 20- μm cutoff indicating that larger cells (i.e., >20 μm) will be retained. Consistently, algal chloroplast sequences had much higher relative abundances in samples of the prefilter cartridges than in the samples collected from the RO membranes. There was strong enrichment of *Shewanella* in RO membrane samples while such sequences were barely detected in prefilter cartridge samples, indicating *Shewanella* grew in biofilms on RO membranes, at least during the March to May period at the Carlsbad plant. Metabolically versatile *Shewanella* spp. are known to form biofilms (Thormann et al., 2004; Bagge et al., 2001) and may play a key role in RO biofouling (Nagaraj et al., 2017; Bereschenko et al., 2010). Other bacterial groups with potential roles in primary attachment and biofilm resiliency include *Caulobacteriales* and glycosphingolipid-producing bacteria, namely *Sphingomonadales*, *Rhizobiales* and *Sphingobacteriia* (Nagaraj et al., 2017).

A relative enrichment of *Shewanella* was not observed in the RO modules from the Tampa Bay plant. *Shewanella* are broadly distributed and possibly reach the RO modules of the Tampa Bay plant; however, the nutritional and/or geochemical conditions in the RO modules apparently did not favor this bacterial group. A possible explanation is the annual SBS treatment that occurred before RO module sampling at the Tampa Bay plant and impacted the microbial community composition as well as the continuous dosing of excess SBS (2-3 times that required for dichlorination) at this plant after cartridge filtration. SBS treatment does not preclude the presence of oxygen (Sommariva et al., 2012) and this could explain the abundance of *Thiomicrospira*, which was also dominant in another study where RO membranes were operated intermittently (Kim et al., 2020). *Thiomicrospira* spp. oxidize reduced sulfur compounds (sulfide, thiosulfate, and elemental sulfur) with oxygen as electron acceptor.

SAR11 is a group of heterotrophic bacteria that thrive under oligotrophic conditions and make up approximately 25% of all oceanic plankton (Giovannoni, 2017). While it was not surprising to find SAR11 sequences, we observed a relative abundance increase in the post prefilter cartridge water samples, presumably due to the small size of SAR11 cells. SAR11 was part of the RO biofilm communities albeit present in low relative abundances, likely due to retention rather than growth. SAR 11 has also been found in intake water and RO membranes in other studies (Levi et al., 2016), and SAR11 necromass may play a role in supporting microbial growth on RO membranes.

Amplicon sequencing provides relative abundance information, and data collected from the sampling location over time or from different sampling locations (e.g., different desalination plants) cannot be directly compared. Absolute quantitative information about relevant community members (e.g., *Bdellovibrionota*, *Shewanella*, SAR 11) is desirable to document seasonal dynamics in the intake waters and within the desalination treatment train, and to assess growth within prefilter cartridges and RO module biofilms.

An interesting question revolves around the carbon and energy sources that allow microbial growth in the prefilter cartridges and the RO modules. A major goal of pretreatment is the removal of organic carbon, and the total organic carbon (TOC) of post-filtration water is substantially reduced; however, assimilable organic carbon (AOC) is likely more important (Weinrich et al., 2011, 2016). Biofouling intensity generally increases at the Carlsbad Plant with algal blooms in the lagoon and more algal cells or components thereof reach the prefilter cartridges. Since algal cells are generally larger and retained by prefilter cartridges with a 20- μm cutoff, macromolecules and metabolites released from lysed cells are likely nutritional resources for microbes colonizing the RO modules, including those involved in the formation of biofilms (Caron et al., 2010). Gaining control over nutrients can prevent microbial growth in RO modules but is difficult to achieve in practice. Alternative strategies are needed and the in-depth, time-resolved understanding of microbial communities associated with prefilter cartridges and RO modules can inform innovative control approaches to prevent the transition from benign to detrimental biofilms. For example, our study identified that predatory bacteria, *Bdellovibrio*, are present in pre-

treatment units and could possibly be exploited to control biofouling. Similarly, polysaccharide consuming *Planctomycetacea* that have been found on RO biofilms (Bereschenko et al., 2008) can provide a mechanism to control biofilm formation on RO membranes.

The complexity of dynamic microbial communities across the seawater desalination treatment train highlights the need for more comprehensive, time-resolved sampling campaigns. Integrated meta-omics analyses promise to unravel temporal dynamics of RO biofilm formation and transition to detrimental and uncontrolled biofilm propagation in response to feedwater conditions and pretreatment. Such experimental datasets may reveal potential biomarkers for biofouling, which would support the development of in operando biofouling sensors to inform plant operators of forthcoming biofouling events. Our study illustrates the power of integrated meta-omics analyses and identifies a need for more comprehensive sampling campaigns capture year-round microbial community dynamics.

Author contributions

MK, PA, FEL, and MP designed the study. MK, PA, FEL, MP, LS conducted sampling with assistance from YP, DS, and IB. FEL, MP, CMS and ALM processed samples in the laboratory. ALM conducted the metabolomic analyses with assistance from DAFL. CMS and KP conducted DNA extractions, while DMK and KP performed amplicon preparation and sequencing. MP conducted all of the DNA data analysis. JM and WB assisted in analytical procedures and figure preparation. All authors contributed to data interpretation and writing the paper.

Conflict of interest

The authors declare no conflict of interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.memlet.2021.100001](https://doi.org/10.1016/j.memlet.2021.100001).

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